Doctoral Thesis

Host-guest cyclodextrin chemistry in the gas phase

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Host–Guest Cyclodextrin Chemistry in the Gas Phase

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Doctor of Sciences

Presented by

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2013
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2) Oral presentation: The Intrinsic Non-covalent Interactions within Complexes of \( \alpha \)-Cyclodextrin and Benzoate Derivatives
Presented at the 4th EuCheMS Chemistry Congress, Prague, Czech Republic, August 26–30, 2012.

1) Oral presentation: A Straightforward Approach to Study the Intrinsic Properties of Host–Guest Cyclodextrin Complexes in the Gas Phase
Presented at the Swiss Chemical Society Fall Meeting, EPFL, Lausanne, Switzerland, September 9, 2011.
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## Abbreviations and Acronyms

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<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AA</td>
<td>1-adamantanecarboxylic acid</td>
</tr>
<tr>
<td>A-A</td>
<td>alcohol-alcohol</td>
</tr>
<tr>
<td>A-E</td>
<td>alcohol-ether</td>
</tr>
<tr>
<td>Ala</td>
<td>alanine</td>
</tr>
<tr>
<td>BA</td>
<td>benzoic acid</td>
</tr>
<tr>
<td>BSSE</td>
<td>basis-set superposition error</td>
</tr>
<tr>
<td>cc</td>
<td>counterclockwise</td>
</tr>
<tr>
<td>CCS</td>
<td>collision cross section</td>
</tr>
<tr>
<td>CD</td>
<td>cyclodextrin</td>
</tr>
<tr>
<td>CID</td>
<td>collision-induced dissociation</td>
</tr>
<tr>
<td>cw</td>
<td>clockwise</td>
</tr>
<tr>
<td>DFT</td>
<td>density functional theory</td>
</tr>
<tr>
<td>$E_b$</td>
<td>alternative bonding energy</td>
</tr>
<tr>
<td>$E_{CM}$</td>
<td>energy in the center-of-mass frame</td>
</tr>
<tr>
<td>$E_{int}$</td>
<td>total attractive energy</td>
</tr>
<tr>
<td>$E_p$</td>
<td>preparation energy</td>
</tr>
<tr>
<td>$E_R$</td>
<td>remaining interaction energy</td>
</tr>
<tr>
<td>ESI–MS</td>
<td>electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>FA</td>
<td>formic acid</td>
</tr>
<tr>
<td>FAB–MS</td>
<td>fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>GB</td>
<td>gas-phase basicity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>GB&lt;sup&gt;app&lt;/sup&gt;</td>
<td>apparent gas-phase basicity</td>
</tr>
<tr>
<td>GS</td>
<td>gas-phase stability</td>
</tr>
<tr>
<td>H–G</td>
<td>host–guest</td>
</tr>
<tr>
<td>Ile</td>
<td>isoleucine</td>
</tr>
<tr>
<td>IM–MS</td>
<td>ion mobility mass spectrometry</td>
</tr>
<tr>
<td>&lt;i&gt;m/z&lt;/i&gt;</td>
<td>mass-to-charge ratio</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>NBO</td>
<td>natural bond orbital</td>
</tr>
<tr>
<td>NCI</td>
<td>non-covalent interaction</td>
</tr>
<tr>
<td>PA</td>
<td>pentanoic acid</td>
</tr>
<tr>
<td>Phe</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>R</td>
<td>daughter-ion fraction</td>
</tr>
<tr>
<td>RBA</td>
<td>benzoic acid derivatives</td>
</tr>
<tr>
<td>&lt;i&gt;T&lt;sub&gt;eff&lt;/sub&gt;&lt;/i&gt;</td>
<td>effective temperature</td>
</tr>
<tr>
<td>TS</td>
<td>transition–state</td>
</tr>
<tr>
<td>VdW</td>
<td>van der Waals</td>
</tr>
</tbody>
</table>
Abstract

Diverse weak non-covalent interactions (NCIs) are fundamental to many biological functions and play a vital role in recognition and reversible association processes. Electrospray ionization mass spectrometry (ESI–MS) has been intensively applied as a rapid and powerful analytical tool to study non-covalent complexes, and to achieve deeper insights into intrinsic NCIs without the influence of solvent. However, these observations (of mass-to-charge ratios, \( m/z \)) alone do not provide any structural information. The nature of these non-covalent complexes, such as host–guest cyclodextrin (CD) complexes, remains ambiguous or even controversial in the gas phase. In order to provide a deeper understanding of the properties of NCIs and non-covalent host–guest complexes in the gas phase, this thesis presents a mass-spectrometric and computational study mainly focused on CD complexes.

We observed exclusively 1:1 host–guest CD complexes by ESI–MS, as was confirmed by their \( m/z \) and unit-mass isotope patterns. Our results further revealed that the gas-phase stabilities of these CD complexes are correlated with the gas-phase basicities of the guest anions employed. This was shown by performing qualitative collision-induced dissociation (CID) experiments. Subsequently, for the first time, the non-covalent bond strengths of these CD complexes were quantified by energy-resolved CID reaction cross-section measurements on a customized ESI–MS instrument. As the ESI–MS experiments did not allow detailed structural characterization, complementary density functional theory (DFT) calculations were performed. Extensive DFT calculations at the M06-2X//M06-L/6-31+G(d,p) level of theory indicated that the host–guest inclusion complexes are more stable than non-specifically bound structures. From an energetic point of view, however, the agreement between the experimental and
theoretical results is not consistent. Therefore, it is not possible to draw an unambiguous conclusion about the structure of these CD complexes in the gas phase from the aforementioned results.

A systematic study on the binding specificity of these CD complexes was carried out. Our experiments revealed that the gas-phase stability of these CD complexes was neither affected by the CD’s cavity size, nor by the size or chirality of the guests. These results indicate that it is the hydrogen bonding that primarily stabilizes these non-covalent complexes. The influence of electronic effects on the gas-phase stability was studied, affording linear Hammett plots that confirmed the putative electrostatic nature of the binding. Additionally, the results suggest that all the investigated anionic CD complexes behave similar to electrostatic adducts. An extensive study on the gas-phase basicities of CDs was performed experimentally and theoretically, which afforded similar results for all CD anions examined. The comparison of the CD complexes in the solid state, solution, and the gas phase reveals that not only the magnitudes of the NCI energies change dramatically, but also the dominating NCIs are different. In conclusion, all of our results strongly point to the formation of non-specific complexes, which are stabilized primarily by hydrogen bonding in the gas phase, and highlight the influence of the surrounding medium on the NCIs and the geometries of the related non-covalent complexes. The presented research sheds light on the electrostatic nature of the intrinsic NCIs and on the huge changes in NCIs depending on the physical state. As such, this thesis may provide a benchmark basis for the study of NCIs in non-covalent host–guest complexes.
Zusammenfassung


2X/M06-L/6-31+G(d,p) deuten an, dass Wirt-Gast-Komplexe stabiler sind als unspezifisch gebundene Komplexe. Aus energetischer Sicht ist die Übereinstimmung zwischen experimentellen und theoretischen Resultaten jedoch inkonsistent. Darum ist es nicht möglich, aus den oben erwähnten Resultaten einen klaren Rückschluss auf die wirkliche Struktur von CD-Komplexen in der Gasphase zu ziehen.

Chapter 1

Introduction
1.1 Supramolecular Chemistry

1.1.1 What is Supramolecular Chemistry

“... Just as there is a field of molecular chemistry based on the covalent bond, there is a field of supramolecular chemistry, the chemistry of molecular assemblies and of the intermolecular bond ...”

– Jean-Marie Lehn

Supramolecular chemistry has been defined by Jean-Marie Lehn, Donald J. Cram, and Charles J. Pedersen, who were awarded the Nobel Prize for his work in this field in 1987. It can also be expressed as ‘chemistry beyond the molecule’ and ‘the chemistry of the non-covalent bond’. From the definition of supramolecular chemistry, the obvious differences between supramolecular and molecular chemistry can be derived. The molecular chemistry describes the chemistry of molecules formed through covalent bonding, while supramolecular chemistry deals with molecules that are associated, combined, or aggregated through non-covalent interactions (NCIs) as shown in Scheme 1.1. One of the most important aims of this discipline is to create molecular devices which are able to mimic biomolecular functions.

\[\text{Scheme 1.1 Schematic elucidation of molecular and supramolecular chemistry.}\]
1.1.2 History

The start of supramolecular chemistry dates back to the end of the 19th century. For example, Villiers and Hebd discovered cyclodextrin (CD) inclusion compounds in 1891, Alfred Werner initiated the field of coordination chemistry in 1893, and Emil Fischer introduced the lock-and-key concept in 1894. Much of supramolecular chemistry has emerged from advances in macrocyclic chemistry, particularly from the development of macrocyclic ligands designed for metal cations. For instance, crown ethers (Scheme 1.2), which were discovered by Pedersen in the 1960s, became one of the most important types of ligand in supramolecular complexes with cationic alkali metals. In the late 1960s, another important macrocyclic ligand type, the cryptands (Scheme 1.2), was prepared by Jean-Marie Lehn, who has made a tremendous contribution to the development of modern supramolecular chemistry. The cation-accepting spherands (Scheme 1.2), which are macrocyclic systems featuring fully pre-organized binding sites, were developed by Cram and co-workers in the 1970s.

![Molecular structures of a representative crown ether, a cryptand, and a spherand.](image)

**Scheme 1.2** Molecular structures of a representative crown ether, a cryptand, and a spherand.
In the 1990s, supramolecular chemistry became even more sophisticated. For instance, James Fraser Stoddart and co-workers studied molecular devices and highly complex self-assembled structures.\textsuperscript{7} Itamar Willner and co-workers developed sensors and methods for electronic and biological interfacing.\textsuperscript{8} Since then, electrochemical and photochemical motifs are being integrated into supramolecular systems in order to increase functionality.\textsuperscript{9} These days, supramolecular chemistry has become one of the most vigorous and fast-growing fields in the chemical sciences. Supramolecular chemistry can be considered as an interdisciplinary field, encompassing applications and problems from inorganic chemistry to organic chemistry as well as from biology and physics to materials science and computer science.\textsuperscript{3,10}

1.2 Non-covalent Interactions

“Readily reversible, non-covalent molecular interactions are key steps in the dance of life. Such weak, non-covalent forces play essential roles in the faithful replication of DNA, the folding of proteins into intricate three-dimensional forms, the specific recognition of substrates by enzymes, and the detection of molecular signals ...”

– Jeremy M. Berg\textsuperscript{11}

Supramolecular chemistry refers to the domain of chemistry beyond that of molecules and focuses on chemical systems consisting of a discrete number of assembled molecular subunits or components. While traditional chemistry focuses on the covalent bond, supramolecular chemistry examines the weak and reversible NCIs between molecules. Therefore, the study of NCIs is crucial to understand supramolecular chemistry. Many biological processes depend on these non-covalent forces for structure and function. For instance, in biological processes, storage and
replication of genetic information depends on hydrogen bonding in the DNA double helix. Four kinds of non-covalent forces are usually distinguished in the field of supramolecular chemistry: electrostatic interactions, hydrogen bonding, van der Waals (VdW) interactions, and hydrophobic effects. A short overview on these NCIs is presented below.

1.2.1 Electrostatic Interactions

Electrostatic interactions are attractive or repulsive forces between charges including dipoles and induced dipoles, which can be subdivided into charge–charge, charge–dipole, and dipole–dipole interactions. A scheme for two point charges interactions is shown in Scheme 1.3. The electrostatic interaction energy is given by Coulomb’s law (Equation 1.1):

\[ E = \frac{q_1 q_2}{4\pi\varepsilon_0 \varepsilon r^2} \]  

(1.1)

Where \( E \) is the interaction energy, \( q_1 \) and \( q_2 \) are the charges on the two atoms, \( \varepsilon_0 \) is the permittivity of vacuum, \( \varepsilon \) is the dielectric constant of the medium, and \( r \) is the distance between the two atoms. As the charge–charge interaction energies exhibit an \( r^{-2} \) distance dependence, the Coulomb forces can be regarded as long-range forces. The dielectric constant of the medium also plays an important role on the strength of the electrostatic interaction, as can be derived from Equation 1.1. In principle, with increasing dielectric constant, the free ion-pair interaction energy decreases significantly. Thus, the strongest electrostatic interaction energy for any free ion pair will be observed in vacuum because it has the smallest dielectric constant (\( \varepsilon = 1 \)) among all media. Salts can be assumed to form a tight contact ion pair in apolar solvents such as dioxane.
or benzene ($\varepsilon = 2–3$)\textsuperscript{12}. On the contrary, loose ion pairs or completely free ions would be predicted if they are solvated in a polar solvent such as acetonitrile ($\varepsilon = 37.5$)\textsuperscript{12} or water ($\varepsilon = 78.4$)\textsuperscript{12}.

\begin{center}
\begin{tikzpicture}
\draw[thick,->] (0,0) -- (1,0) node[above] {$q_1$};
\draw[thick,->] (2,0) -- (1,0) node[above] {$-q_1$};
\draw[thick,->] (1,0) -- (1,1) node[right] {$F$};
\draw[thick,->] (1,0) -- (1,-1) node[right] {$F$};
\draw[thick] (0,0) -- (2,0) node[midway, below] {$r$};
\end{tikzpicture}
\end{center}

**Scheme 1.3** Schematic view of electrostatic interaction between opposite charges.

The interaction between two point charges can be attractive or repulsive, depending on the sign of the charges. Likewise, the force between a charge and a dipole or between two dipoles can become attractive or repulsive, depending on the geometric arrangement. However, the interactions between charges or dipoles and apolar molecules are always attractive because the presence of a charge in proximity to the molecule induces a dipole that is oriented such that the stabilization is maximized.

### 1.2.2 Hydrogen Bonding

Hydrogen bonds formed between two different molecules are called intermolecular hydrogen bonds, whereas those formed within one molecule are called intramolecular hydrogen bonds. It is the intermolecular hydrogen bonds that are responsible for the existence of liquid water under ambient conditions (Figure 1.1) and its high boiling point of 100 °C compared to, e.g. hydrogen sulfide, which builds no hydrogen bonding. Intramolecular hydrogen bonds are partly responsible for the secondary, tertiary, and quaternary structures of proteins and nucleic acids.
Chapter 1

Figure 1.1 Structure model of water molecules in liquid water; the dotted lines represent intermolecular hydrogen bonds.

The hydrogen atom involved in a hydrogen bond is partly shared between two relatively electronegative atoms such as nitrogen (N), oxygen (O), or fluorine (F). It is in closer proximity to one of the electronegative atoms, the so-called hydrogen bond donor (D), than to the other, the hydrogen bond acceptor (A). Thus, a hydrogen bond is commonly written as D–H···A. The hydrogen bond donor atom pulls electron density away from the covalently bound hydrogen atom to create a partial positive charge ($\delta^+$). Subsequently, the hydrogen atom is attracted through electrostatic interaction to the hydrogen bond acceptor, having a partial negative charge ($\delta^-$; Scheme 1.4). If the hydrogen bond acceptor is an anionic compound, the formed hydrogen bond is much stronger than for a neutral acceptor, and is named a ionic hydrogen bond. The hydrogen bond thus is not a truly covalent chemical bond but it has some features of covalent bonds: it is strong and directional, produces interatomic distances shorter than the sum of the VdW radii, and usually involves a limited number of interaction partners, which can be interpreted as a type of valence. As shown in Scheme 1.4, hydrogen bond
strengths in water solution can vary from weak (about 1.9 kcal mol\(^{-1}\)) up to strong (about 38.5 kcal mol\(^{-1}\)). By comparison, the bond energy for a common C–H covalent bond is about 98.0 kcal mol\(^{-1}\), and for a common C–C covalent bond it is about 84.0 kcal mol\(^{-1}\). Thus, even the strongest hydrogen bond cannot compete with covalent bonds.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\delta^-)</td>
<td>(\delta^+)</td>
<td>(\delta^-)</td>
</tr>
<tr>
<td>F—H—F</td>
<td>F—H—F</td>
<td>38.5 kcal mol(^{-1})</td>
</tr>
<tr>
<td>O—H—N</td>
<td>O—H—N</td>
<td>6.9 kcal mol(^{-1})</td>
</tr>
<tr>
<td>O—H—O</td>
<td>O—H—O</td>
<td>5.0 kcal mol(^{-1})</td>
</tr>
<tr>
<td>N—H—N</td>
<td>N—H—N</td>
<td>3.1 kcal mol(^{-1})</td>
</tr>
<tr>
<td>N—H—O</td>
<td>N—H—O</td>
<td>1.9 kcal mol(^{-1})</td>
</tr>
</tbody>
</table>

**Scheme 1.4** Schematic view of hydrogen bonds and their typical bond strengths; the partial charges are shown as \(\delta^+\) and \(\delta^-\).

The total binding energy in complexes supported by multiple hydrogen bonds may sometimes be bigger than that of a single covalent bond. Nevertheless, one must note that the strength of multiple hydrogen bonds would be modulated by the so-called secondary interactions, which can be attractive or repulsive. The presence of relatively strong hydrogen bonds can be characterized by typical shifts of the proton NMR signals and of the IR bands for the D–H···A stretching vibrations. Hydrogen bonds are ubiquitous in supramolecular chemistry. In particular, they are responsible for the overall shape of many proteins, for the recognition of substrates by numerous enzymes, and for the double-helix structure of DNA.
1.2.3 Van Der Waals Interactions

The VdW interactions, named after the Dutch scientist Johannes Diderik van der Waals, are the sum of the attractive and repulsive forces between molecules (or between parts of the same molecule) other than those due to covalent bonds, electrostatic interaction of ions, or hydrogen bonds. They arise from the polarization of an electron cloud by the proximity of an adjacent nucleus, resulting in a weak electrostatic attraction, for instance between two permanent dipoles, between a permanent dipole and a corresponding induced dipole, or between two instantaneously induced dipoles. Even in the absence of a net dipole moment in an unpolarized atom or molecule, a dipole is always induced in this atom or molecule as a result of local fluctuations of the electron density distribution in a second, nearby atom or molecule. Strictly speaking, VdW interactions may be divided into dispersion and exchange-repulsion terms. The dispersion interaction is the attractive component that results from the interactions between fluctuating multipoles in adjacent molecules, while the exchange repulsion balances dispersion at short range defines molecular shape.

The magnitude of VdW interactions decreases very rapidly with distance ($r^{-6}$ dependence) and thus can be regarded as a short-distance attractive force as shown in Scheme 1.5. Although it is very weak, it is additive over the entire association surface and may become the most important attractive force between apolar molecules. For example, VdW interactions are the only reason for the condensation of noble gases under high pressures, and they are most important in formation of inclusion compounds of toluene within the molecular cavity of the calixarene.16 The energetically most favorable distance between atoms or groups is the VdW distance, and thus the atomic
VdW radii are crucial to estimate the VdW interaction energy.\textsuperscript{17} If the distance between atoms becomes smaller than the sum of their VdW radii, a strongly repulsive interaction takes place, with extreme distance dependence ($r^{-12}$ dependence).

\begin{scheme}
\textbf{Scheme 1.5} Energy profile of a van der Waals interaction as two atoms approach each other. The interaction energy is most favorable at the van der Waals contact distance.
\end{scheme}

\subsection{1.2.4 Hydrophobic Effect}

The hydrophobic effect is a key property driving apolar molecules to form intermolecular aggregates in aqueous solution.\textsuperscript{18} The name literally describes the apparent repulsion between water and apolar substances. The hydrophobic effect explains the separation of a mixture of oil and water into two layers or the beadings of water on hydrophobic surfaces such as lotus leaves (Figure 1.2). At the molecular level, the hydrophobic effect is a very important driving force for biological structures and is responsible for protein folding, formation of lipid bilayers, and micelles.\textsuperscript{19}
Figure 1.2 Droplets of water form spherical shapes to minimize contact with the hydrophobic surface of the lotus leaf.

The water molecules in pure water always form as many strong hydrogen bonds as thermodynamically feasible. When an apolar molecule is dissolved in water, the water molecules directly surrounding the apolar molecule cannot form as many such hydrogen bonds as in bulk water, and can also not favorably interact with this apolar surface. Upon association of apolar molecules, the overall apolar surface is reduced. The total number of water molecules directly surrounding these aggregates decreases as compared to the non-aggregate situation, and some of them are released into bulk water. These released water molecules form additional hydrogen bonds with other water molecules, which results in a decrease in enthalpy for the whole system. Furthermore, the water molecules around the apolar surfaces are at a higher level of order than in the bulk solution. The release of many water molecules upon aggregation of apolar surfaces thus also increases the entropy of the system. The larger the apolar surface of the associated molecules, the more favorable is the entropic driving force. Therefore, the
aggregation of apolar molecules in water is favorable both enthalpically and entropically. It is controlled by surface desolvation and is called the classical hydrophobic effect.

1.3 Host–Guest Complexes

1.3.1 Introduction

“Complexes are composed of two or more molecules or ions held together in unique structural relationship by electrostatic forces other than those of full covalent bonds ... A highly structured molecular complex is composed of at least one host and one guest component ... A host–guest relationship involves a complementary stereoelectronic arrangement of binding sites in host and guest ...”

–Donald Cram

Host–guest (H–G) chemistry is one of the fundamental areas of supramolecular chemistry. By definition, host molecules can accommodate other molecules (guests) in their (permanent) intramolecular cavities or extramolecular cavities (where the cavity essentially represents a gap between two or more host molecules). Commonly the host is a large molecule or aggregate such as a synthetic cyclic compound or an enzyme possessing a central hole or pocket. The guest may be a simple anion, cation, or a more sophisticated molecule such as a hormone or a pheromone. Depending on the compatibility of the two binding species, the H–G complex can be considered a non-specific, specific or highly specific complex. In the biological field, hosts often refer to an enzyme, a poly-nucleic acid, or an antibody, which can only bind some specific guest such as an inhibitor, a cofactor, or an antigen. This is called molecular recognition, which implies selectivity of a host for a particular guest molecule, while others are
excluded. According to Fischer’s lock-and-key principle, complementarity between the host and guest is one important factor. A single non-covalent bond is usually not strong, and does not help to specifically bind a guest to a host. Constructing a stable H–G complex demands a host capable of utilizing NCIs in summative, cooperative, or even multiplicative fashion. The more complementary the binding sites of the host and those of the guest are, the higher will be the binding energy. This refers not only to individual non-covalent bonds, but to the whole shape and the whole electrostatic surface of both molecules involved in the binding event.

1.3.2 Host–Guest Complexes in the Condensed Phase

Host molecules can be divided into three major classes according to their preferential binding of specific types of guests, namely simple cations, anions, or sophisticated neutral molecules, respectively. Since the discovery of crown ethers by Pedersen in the 1960s,4,24 many such compounds have been prepared for the complexation of alkali metals, earth alkali metals, and other cationic molecules.5b,5d,5f,25 Usually, crown ether–cation complexes are readily soluble in most other organic solvents, but the solubility is lower in water. This property originates from the structure of these complexes. In particular, the crown ether wraps itself around the cation, which is bound through ion–dipole interactions with the host’s donor oxygen atoms. Upon binding, the apolar methylene groups turn outwards and make the cation soluble in the lipophilic organic phase (Figure 1.3).26 Additionally, the binding geometries of crown ether–cation complexes vary depending on the properties of the cation. For example, the crystal structure of free [18]crown-6 ether is different from that of its cation complex with potassium. The free crown ether displays two anti torsional angles,27 but all torsional
angels are *gauche* in its cationic complex. This suggests that it is the cation and its coordination requirements which organize the structure of the crown ether to which it is bound.

![Crystal structure of [18]crown-6 (left) and its potassium (K+) complex (right).](image)

**Figure 1.3** Crystal structure of [18]crown-6 (left) and its potassium (K+) complex (right).

The size-fit concept has been widely accepted to be one of the most important factors for the stability of crown ether–cation complexes. In particular, the complex formation constant increases with increasing match between the metal’s ionic radius and the cavity radius of the crown ether. For instance, of all the alkali metal ions, [18]crown-6 binds potassium cation strongest because of the size-fit concept, while [15]crown-5 selectively binds sodium cation. However, this concept should not be overestimated. As crown ether chemistry has evolved, aspects such as preorganization, complementarity, and solvation are nowadays accepted to be the most significant factors determining their solution-phase selectivity for cations.²¹

The principle by which crown ethers bind cations has been extended to other cation-binding hosts, such as cryptands⁵,²⁸ and spherands (Figure 1.4).²¹-²²,²⁹ The studies on spherands carried out by Cram and co-workers have demonstrated that complementary
H–G relationships are essential in the formation of specific supramolecular complexes.\textsuperscript{21} Additionally as well as matching of size, shape, and electronic properties of hosts and guests are prerequisites for strong binding.

The first example of successful anion complexation by a synthetic receptor was reported in 1968 by Simmons and Park. Their katapinands have a cryptand-type structure in which two nitrogen centers are linked by three (CH\textsubscript{2})\texttextsuperscript{n} (\textit{n} = 8–10) bridges (Scheme 1.6).\textsuperscript{30} The katapinands are able to include halide ions within their macrobicyclic cavity when \textit{endo}-protonated at the bridgehead nitrogen atoms. This encapsulation behavior was confirmed by X-ray crystal structure determination.\textsuperscript{31} The short N⋯Cl distance of 3.10 Å suggests that the main interaction forces are electrostatic charge–charge interactions and hydrogen bonding. In 1976 Lehn and co-workers described a family of spherical protonated anion receptors that possess a much higher selectivity to halide anions than the non-spherical cryptand-type hosts.\textsuperscript{32} There are quite a few other hosts that have been developed as anion receptor.\textsuperscript{33} In general, anion hosts
obey the same rules that govern the magnitude of binding constants and host selectivity in cation hosts. However, the development of hosts which are capable of binding anions is more challenging in comparison with the development of hosts for cations because of the intrinsic properties of anions. For instance, anions are usually large and differ in shape and geometry, such as halides, SCN\(^-\), CO\(_3^{2-}\), and PO\(_4^{3-}\), requiring anion hosts with considerably larger dimensions and sophisticated binding sites.

![Scheme 1.6 Molecular structure of katapinands and a representation of their complex with hydrogen chloride (HCl).](image)

The design of hosts for neutral guests (usually organic molecules) remains a challenge today. Due to the absence of charges that would provide comparatively high binding energies, the binding of neutral molecules often relies on a combination of hydrogen bonding, VdW interactions, and sometimes the hydrophobic effect. In general, the binding of neutral and apolar organic molecules by the majority of cavitands is relatively weak in apolar solvents, because there is no significant enthalpic gain from H–G interactions except for specific H–G dipole–dipole or hydrogen-bond interactions. In water, polar groups on the host and guest are highly solvated and hence organic guest binding relies mostly on the hydrophobic effect. There are some other
hosts that also play an important role in the binding of neutral guests, such as calixarenes, cucurbiturils and cyclodextrins (CDs) (Figure 1.5).  

![Figure 1.5](image)

**Figure 1.5** Top and side views of crystal structures of a) calix[4]arene, b) cucurbit[6]uril, and c) α-cyclodextrin; hydrogen atoms are omitted for clarity.

CDs are among the most important hosts for neutral guest in aqueous media and are widely applied in food, cosmetics, and especially the pharmaceutical industry owing to their ability to accommodate small molecules of a suitable size as guests (Scheme 1.7) and their particular advantage of being entirely nontoxic over a wide dosage range. Indeed, they are the most studied and cheapest commercially available hosts. The most common of these cyclic glucopyranose oligomers are α-CD, β-CD, and γ-CD (Scheme 1.7). The sugar units are connected through glycosidic α-1,4 bonds to afford a toroidal molecular shape with the wider upper rim lined with secondary hydroxyl groups and the narrower lower rim with primary hydroxyl groups. The internal cavity diameters are in the range of 4.7–5.3 Å for α-, 6.0–6.5 Å for β-, and 7.5–8.3 Å for γ-CD.
Introduction

Scheme 1.7 Molecular structure of cyclodextrins (left) and crystal structure of the complex of α-CD with 4-hydroxybenzoic acid (right).

Since the discovery of CD inclusion compounds by Villiers and Hebd in 1891,39 numerous studies have demonstrated that CDs can host organic compounds to form inclusion complexes in condensed phase.36 Their hydrophilic exterior makes CDs water-soluble, whereas the hydrophobic internal cavity can accommodate the apolar part of a size-matched guest molecule in aqueous solution while leaving any polar part exposed to the bulk solvent. The inclusion complexation of guest molecules by CDs in aqueous solutions results in a substantial rearrangement and removal of the water molecules originally solvated to both the CD and guest molecules, and this process also induces the release of water molecules from the CD cavity into the bulk water. The principal factors involved in binding are believed to be primarily VdW interactions and the hydrophobic effect.36e,36f In principle, the size-fit concept explains the global trend of the thermodynamic properties for complexation processes involving CDs, predicting the highest complex stabilities for the best size-matched H–G pairs.
When the guest molecules cannot be completely accommodated within the CD cavity, steric effects can play an important role. An example is the steric hindrance caused by the ortho-substituent of aromatic guests upon complexation with β-CD. The increase of the chain length of an aliphatic guest will not affect the complex stability when the additional methylene groups cannot be included by the cavity of CDs. The complexes formed between chiral hosts and guests are diastereomers which have unique stabilities and rates of dissociation. For instance, CDs have been used as chiral receptors because of the chirality of their cavities. Chiral recognition by CDs has been applied widely for catalysis and separation processes based on the preferred encapsulation of one specific guest enantiomer inside its cavity. However, most enantiomeric pairs of guests show only small differences in thermodynamic properties upon complexation with CDs. For instance, no obvious differences are found in the thermodynamic quantities for the complexation of α- and β-CD with the enantiomeric pairs of sec-alkanols, norvaline and norleucine, or carbohydrates. Only α-methylbenzylamine gives significantly different thermodynamic quantities for its enantiomeric pair upon complexation with α-CD. Therefore, it seems quite difficult to find significant chiral discriminations in the complexation thermodynamics of enantiomeric guests with CDs, maybe because most of the guest molecules are included only through orientation-independent VdW interactions and hydrophobic effects, and are therefore not rigorously fixed either conformationally or rotationally in the cavity.

1.3.3 Host–Guest Complexes in the Gas Phase

Solvent has a dramatic impact on the properties of H–G complexes in the condensed phase. For instance, the binding constant (log $K_a$) for the complex of [18]crown-6 and
Introduction

potassium ($K^+$) in methanol ($6.10$) is much bigger than in water ($2.06$). The origin of this solvent dependency becomes clear when considering the following complexation equilibrium in solution:

$$\text{Host (solvent)} + \text{Guest (solvent)} \rightleftharpoons [\text{Host–Guest (solvent)}] + \text{free solvent}$$

For ionic H–G complexation processes, the binding event of the host to an ionic guest must compete with solvation. The solvation of the ion becomes stronger with more polar solvent. Therefore, the binding constant of the H–G complex is smaller when a more polar solvent is used. For neutral H–G complexes, the major contribution to the stability is the hydrophobic effect, which is obviously solvent dependent. Therefore, a considerable amount of research has been conducted to study the intrinsic properties of H–G complexes in the gas phase without the influence of solvent. This allows for the solvent effect to be separated out.

With the development of soft ionization methods, mass spectrometry (MS) has become an excellent tool for the characterization of non-covalent H–G complexes. The complexes of crown ethers with alkali ions have been studied extensively in order to understand their intrinsic properties. Most of the early studies attempted to assess the binding selectivity of crown ethers to alkali metal ions directly by applying fast atom bombardment mass spectrometry (FAB–MS), which turned out to be more difficult than expected. The results obtained by electrospray ionization mass spectrometry (ESI–MS) studies are also ambiguous and sometimes contradict the solution-phase studies. For instance, the most prominent signal obtained by ESI–MS was the complex of [18]crown-6 with Li$^+$ ion when spraying a LiCl solution containing [12]crown-4, [15]crown-5, and [18]crown-6. Furthermore, also for the other alkali ions, the
[18]crown-6 complexes were always observed to be the most abundant ones. This is in conflict with the “best-fit” concept in solution because the solvation energy and electrospray response factors should differ for different ions. Sometimes, results for condensed phase monitored by MS differ depending on the particular system and ionization method. Therefore, an increasing number of questions are waiting to be answered by gas-phase reactivity experiments.

Armentrout and co-workers have developed the collision-induced dissociation (CID) threshold determination method in order to quantitatively measure absolute dissociation energies by tandem MS. This has been applied to many crown ether complexes with alkali metal ions (Table 1.1). There are several trends showing up: the binding energy of crown ether–alkali metal complexes under study decreases from Li⁺ to Cs⁺, probably due to the more condensed charge on Li⁺; the binding energy increases from [12]crown-4 to [18]crown-6 for all metal ions, suggesting that the number of oxygen donors in the crown ether is more important than the geometrical fit; geometrical fit must play only a secondary role because the increase in binding energy upon expansion of the cavity from [15]crown-5 to [18]crown-6 is less pronounced for Na⁺ and K⁺ than for Rb⁺ and Cs⁺. Therefore, the intrinsic properties of non-covalent complexes in the gas phase are inherently different to those in solution phase, and the major achievement of the gas-phase studies is a much more profound understanding of the role of solvation in H–G complexation processes.
Table 1.1 Bond dissociation energies (eV) in the gas phase for crown ether–alkali metal complex ions.9

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Li⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Rb⁺</th>
<th>Cs⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>[12]crown-4</td>
<td>3.85</td>
<td>2.61</td>
<td>1.96</td>
<td>0.96</td>
<td>0.88</td>
</tr>
<tr>
<td>[15]crown-5</td>
<td>N/A</td>
<td>3.05</td>
<td>2.12</td>
<td>1.18</td>
<td>1.04</td>
</tr>
<tr>
<td>[18]crown-6</td>
<td>N/A</td>
<td>3.07</td>
<td>2.43</td>
<td>1.98</td>
<td>1.74</td>
</tr>
</tbody>
</table>

[a Data not available.

A number of other H–G complexes have also been studied in the gas phase, including calixarene50 and cucurbituril complexes.51,52 Recently, the focus of the current interests has shifted toward gas-phase studies of CD complexes due to the unclear geometries and NCIs involved in the gas-phase CD complexes. For example, initially (in 1993) the specific mass-to-charge ratios (m/z) of the detected non-covalent species were considered as evidence for the formation of CD inclusion complexes, transferred intact from solution into the gas phase.53 Actually, the signals in mass spectra may serve to quickly determine the stoichiometry of the compounds analyzed, however, these observations alone do not provide any structural information. In 1995, Cunniff and Vouros54 compared amino-acid and peptide complexes of CDs, finding no correlation between the hydrophobic surface area of the amino acid chains and the signal intensities in the mass spectra. They also observed CD adducts in the gas phase even with amino acids and peptides which did not form inclusion complexes in solution. They therefore claimed that these adducts were merely bound by electrostatic interactions and cannot be assigned to specific inclusion complexes. This “false-positive” conclusion was subsequently questioned by Lebrilla and co-workers,55 who reported that the sizes of both the amino acid and the CD cavity did affect chiral selectivity in guest-exchange
reactions in the gas phase, and who therefore concluded that these species would be inclusion complexes rather than non-specific complexes. More recently, Gabelic and co-workers\textsuperscript{56} reported a study on $\alpha$-CD complexes with $\alpha,\omega$-diacids. The relative mass spectral intensities of complexes of guests with different chain lengths between the two carboxylic acid groups did not change much, although the binding constant in solution increased with chain length. This contradiction was considered to be evidence for the formation of non-specific complexes. They also compared the binding constants for the complexes of $\alpha$-CD and its linear analogs maltohexaose with the same guest, finding that the $\alpha$-CD complexes were more stable than the maltohexaose complexes which cannot form inclusion complexes. This was interpreted as evidence for the existence of inclusion complexes. Consequently, both specific and non-specific complexes must have contributed to the ion intensity in the mass spectra. However, it is hard to prove or disprove either result experimentally because of the limitations of the characterization tools available for gas-phase experiments.

Fortunately, Chen and co-workers developed a new method to deconvolute the energy-resolved CID reaction cross sections that is conceived for medium-to-large complexes.\textsuperscript{57} This method, therefore, enables us to quantitatively investigate the intrinsic NCIs in larger H–G systems such as CD complexes. Furthermore, a number of computational studies have served to support the experimental investigations, leading to the general picture that isolated native CDs adopt symmetric conformations with extensive hydrogen bonding networks in their primary and secondary hydroxyl rims.\textsuperscript{58} The coordination of a guest with CD must then compensate for the disruption of such intramolecular hydrogen bonds. Nevertheless, their intrinsic properties are still unclear and attract much attention, such as whether these complexes retain their solution-phase
structures or whether the binding mode changes dramatically due to alteration of the surrounding environment, how strongly bound these complexes are in the gas phase, and which forces predominantly contribute to or affect their kinetic stability.

1.4 Mass Spectrometric Study of Non-covalent Complexes

Mass spectrometry is not a special tool to measure the mass of analytes, but it is a tool by which ions are generated by any suitable method from analytes, then separated by their mass-to charge ratio ($m/z$) and finally detected and quantified by their respective $m/z$ ratio and abundance. Thus, a mass spectrum is the plot of a two-dimensional representation of signal intensity versus $m/z$. All mass spectrometers have a simple basic setup in common: they consist of an ion source, a mass analyzer, and a detector as shown in Scheme 1.8. The mass analyzer and detector are operated under high vacuum while the ion source can either be under vacuum or at atmospheric pressure. The high vacuum in mass spectrometers allows ions to reach the detector without undergoing collisions with other gaseous molecules, which would produce a fragmentation of the ions or lead to loss of charge by collision with the walls of the instrument. The mean free path of an ion under vacuum can be calculated according to the kinetic theory of gases and is inversely proportional to the pressure.59

A critical step in mass spectrometry is the very beginning of each analysis, the ion formation. The ion source is required for the transfer of the sample from the condensed phase into high vacuum. Although a number of ionization methods are commercially available, most of these are more or less incompatible with non-covalent bonds. The relatively weak non-covalent bonding may be disrupted during the ionization process. For instance, for electron or chemical ionization methods the sample must be vaporized
before it can be ionized, and non-covalent complexes usually do not survive this process. FAB has been considered as a soft ionization method in the past, but it has some severe limitations for the analysis of non-covalent complexes. For instance, the available mass range is often limited to light analytes. Peptides, proteins, and larger oligonucleotides are usually heavy and cannot efficiently be ionized by FAB. Furthermore, for the generation of non-covalent complexes, the protic matrices normally used for FAB are often problematic. Currently, matrix-assisted laser desorption/ionization (MALDI) and ESI are widely applied instead of FAB for the detection of non-covalent complexes. MALDI and ESI are often referred to as “soft ionization” techniques because they are capable of efficient ionization of large biomolecules like proteins without unintentional fragmentation.

![Scheme 1.8 General scheme of a mass spectrometer.](image)

**1.5 Conclusions**

The solvent plays a very important role in the molecular recognition for all kinds of H–G complexes in the condensed phase. The complexation of ionic guests needs to compete with solvation. The strength of the main forces, which are hydrogen bonding
and the hydrophobic effect for the complexation of neutral hosts and guests, is solvent dependent. To understand the intrinsic properties of non-covalent interactions, H–G complexes, and the solvent effect, a number of gas-phase experiments have been conducted. Most of the early studies on the crown ether H–G complexes carried out in the gas phase failed to reproduce their properties in solution phase, which indicates that the properties of these crown ether H–G complexes are different in solution versus the gas phase. The gas-phase reactivity experiments conducted later revealed the intrinsic gas-phase properties of crown ether H–G complexes and the solvent effect. However, the intrinsic properties of other H–G complexes such as CD complexes are still unclear because of the limitations of the characterization tools available in the gas phase, although their properties in condensed phase have been studied extensively.

1.6 Scope and Outline of this Thesis

Diverse weak NCIs are fundamental to many biological functions and play a vital role in recognition and reversible association processes. They are also responsible for the higher-level structures of bio-macromolecules such as DNA and proteins. In vivo studies of NCIs are, however, often complicated. Therefore, it is more convenient to study NCIs within a biological mimic, such as CD H–G complexes. ESI–MS has been applied successfully to study non-covalent complexes, to achieve deeper insights into intrinsic NCIs and to NCI-based molecular recognition in the absence of solvent effects. Furthermore, the recently developed energy-resolved CID reaction cross section measurements have proven to be an extremely useful and information-rich approach to obtain absolute bonding dissociation energies. Complementary to gas-phase studies, density functional theory (DFT) calculations can provide more
Chapter 1 geometrical information and, moreover, should well mimic the conditions in the high-vacuum environment of a mass spectrometer. Thus, in this thesis, we explore the field of gas-phase CD chemistry with the aim to elucidate the specificity of CD complexes, the contribution of each non-covalent interaction type to the total complex stability, the absolute bonding dissociation energies. Furthermore, we compare the geometries of and the most important NCIs in these CD complexes in solid state, solution, and the gas phase, as well as the intrinsic properties of the CDs themselves.

Chapter 2 describes the preparation and analysis of non-covalent complexes of α-CD with a series of benzoic acid derivatives (RBA) investigated by ESI–MS and DFT calculations. Collision-induced dissociation experiments reveal exclusive fragmentation into [α-CD]− and neutral RBA and afford the gas-phase kinetic stability trend. DFT calculations further elucidate the experimental observations, suggesting that hydrogen bonding constitutes the primary H–G interaction.

In Chapter 3, a novel deconvolution method for energy-resolved reaction cross sections is applied to determine intrinsic gas-phase dissociation energies for non-covalent CD complexes. The average gas-phase threshold energies for the formation of [CD]− from CD complexes have been measured for the first time for such large H–G complexes. However, the experimental results are not well reproduced by DFT calculations.

In Chapter 4, a series of collision induced dissociation experiments have been carried out in order to further explore the intrinsic properties of CD complexes in the gas phase. The results suggest that the gas-phase stability is affected neither by the size of the guest nor that of the cavity. Any chirality of the guests also does not influence the gas-phase
stability of the corresponding CD complexes. Furthermore, the results indicate that the non-covalent complexes in the gas phase are stabilized primarily by ionic hydrogen bonding, which is governed by the gas-phase basicity of both host and guest moieties. Additionally, the results suggest that all the CD anionic complexes detected by ESI–MS possess properties similar to those of electrostatic adducts in the gas phase.

In Chapter 5, the intrinsic properties of CDs are investigated by mass spectrometry and DFT calculations. The gas-phase basicity of [α-CD]− and [β-CD]− are first studied by the bracketing method. Then the extended kinetic method is performed to determine the exact gas-phase basicity values for both CD anions. DFT calculations provide detailed structural information and theoretical gas-phase basicity values for both [α-CD]− and [β-CD]−, which are consistent with the experimental results.

Chapter 6 reports a structural study of α-CD complexes as solids, in solution, and in the gas phase. The crystal structures of α-CD complexes with 3-methyl benzoic acid and with benzoic acid show 2:1 H–G complexes which have not been reported before for complexes with structurally similar guests. These complexes with the same host and guest moieties show a 1:1 complex model in solution and gas phase, but with different spatial arrangements, revealing a remarkable effect on the NCIs mediated by the surrounding media.

Chapter 7 presents the conclusions and outlook for this thesis.
Chapter 2

Qualitative Study of $\alpha$-Cyclodextrin Complexes in the Gas Phase
The non-covalent interactions in host–guest complexes of α-cyclodextrin (α-CD) with a series of benzoic acid derivatives (RBA) were investigated by electrospray ionization tandem mass spectrometry and density functional theory (DFT) calculations. The 1:1 stoichiometry of the anionic host–guest complexes was unequivocally confirmed by their mass-to-charge ratios (m/z) and isotope patterns. Collision-induced dissociation experiments reveal exclusive fragmentation into [α-CD]− and neutral RBA and afford the gas-phase kinetic stability trend. DFT calculations provide detailed structural assignments and further elucidated the experimental observations, suggesting that the anionic [α-CD·RBA]− inclusion complexes are favored over the non-specific complexes in the gas phase and that hydrogen bonding constitutes the primary host–guest interaction. Additionally, the results provide an estimated gas-phase basicity $\Delta G^0 = 325–327 \text{ kcal mol}^{-1}$ for [α-CD]−.

2.1 Introduction

Compared with the generally accepted conceptions regarding cyclodextrin (CD) complexation in solution, the nature of such host–guest (H–G) complexes in the gas phase remains ambiguous or even controversial.\textsuperscript{65} When the analytes are transferred from solution to the gas phase, the conditions change dramatically. More precisely, H–G interactions, which are competitive with solvation, such as hydrogen bonding and electrostatic interactions, are significantly strengthened in the gas phase.\textsuperscript{66} On the other hand, the hydrophobic effect originates from the gain in entropy of solvent molecules that are released from the solvation shell upon complexation in solution phase, and hence is absent in the gas phase.\textsuperscript{67} Furthermore, in solution the H–G complex may be in equilibrium with the free host and guest, but there is no such equilibrium in the high-vacuum environment of a mass spectrometer: the dissociation of host and guest is irreversible. It would thus be premature to conclude that CDs form inclusion complexes in the gas phase as well. Alternatively, the guest molecule may bind with its polar part to the CD’s exterior through electrostatic interactions or engage in hydrogen bonding with hydroxyl groups on the rim of the CD, for example; such complexes in which the guest is not inside the CD’s cavity are called non-specific.

Here we present an electrospray ionization mass spectrometry (ESI–MS) study of \(\alpha\)-CD complexes with benzoic acid derivatives (RBA) to address the questions whether these complexes retain their solution-state structures or whether the binding mode changes dramatically due to alteration of the surrounding environment, and which forces predominantly contribute to or affect their kinetic stability in the gas phase.
Adducts of CDs with carboxylate-functionalized guests can be detected by ESI-MS. Furthermore, various benzoic acid derivatives are commercially available, which allows us to study the influence of the guest’s substitution. The experimental study is complemented by density functional theory (DFT) calculations, which should well mimic the conditions in the high-vacuum environment of a mass spectrometer. Thus, we characterize the intrinsic properties of these non-covalent complexes, i.e., their preferred gas-phase conformations, binding energies, and dominant interaction forces.

2.2 Results and Discussion

2.2.1 1:1 Host–Guest Complexation

For the ESI–MS investigation of anionic H–G complexes of α-CD with 3,5-diMeBA, 3-MeBA, BA, 3-OHBA, and 3,5-diOHBA, 1:1 mixtures of host and guest were prepared of approximately 10 µM in methanol/water (95:5 v/v). These solutions have a pH around 6 due to dissociation of the RBA, while α-CD with pKa = 12 remains undissociated at this pH. Figure 2.1 shows the ESI–MS spectra obtained for the mixtures of α-CD and RBA. The signals observed at m/z 1121, 1107, 1093, 1109, and 1125 correspond to the expected anionic complexes [α-CD·3,5-diMeBA]−, [α-CD·3-MeBA]−, [α-CD·BA]−, [α-CD·3-OHBA]−, and [α-CD·3,5-diOHBA]−, respectively. However, Kralj and co-workers pointed out recently that such signals may also stem in part from doubly charged dimeric cluster ions [(α-CD·RBA)2]2−. High-resolution mass spectrometric analysis unequivocally excluded the presence of the 2:2 dianionic complexes, as indicated by the unit mass-spaced isotope patterns (see insets in Figure 2.1).
Figure 2.1 Experimental mass spectra and isotope patterns (insets) obtained for 1:1 mixtures of $\alpha$-CD and (a) 3,5-diMeBA, (b) 3-MeBA, (c) BA, (d) 3-OHBA, and (e) 3,5-diOHBA.
2.2.2 Gas-Phase Deprotonation of α-CD

As shown in Figure 2.1, in all five mixtures a common peak at \( m/z \) 971 was observed, which can be assigned to deprotonated α-CD, \([\alpha-CD]^-\). However, this anion could not be detected when spraying a solution of α-CD alone, which is in accordance with its high \( pK_a \) in solution. Thus, the benzoic acid must play a role in the formation of the \([\alpha-CD]^-\) anion, either via proton abstraction from α-CD by benzoate anions in solution or via proton-transfer fragmentation of the anionic H–G complexes \([\alpha-CD-RBA]^-\) in the mass spectrometer. The difference in \( pK_a \) between α-CD and RBA in solution rules out the first possibility, leaving the gas-phase fragmentation as the only plausible pathway. Accordingly, the ion intensity of the deprotonated product \([\alpha-CD]^-\) increased while that of the H–G complex \([\alpha-CD-BA]^-\) decreased when a higher tube lens voltage (from −80 to −100 V) or a higher collision offset was applied, as shown in Figures 2.2 and 2.3.

![Mass spectra obtained for a 1:1 mixture of α-CD and BA at different tube lens voltages.](image)

**Figure 2.2** Mass spectra obtained for a 1:1 mixture of α-CD and BA at different tube lens voltages.
Figure 2.3 CID mass spectra of the \([\alpha\text{-CD·BA}]^-\) complex at different center-of-mass energies \(E_{\text{CM}}\).

2.2.3 Gas-Phase Basicity versus Gas-Phase Stability

One question remains, namely, why does gas-phase fragmentation of the \([\alpha\text{-CD·RBA}]^-\) complexes afford deprotonated \(\alpha\text{-CD}\) rather than the benzoate, as expected on the basis of their \(pK_a\) values? We therefore studied the formation of \([\alpha\text{-CD}]^-\) through collision-induced dissociation (CID) experiments on the \([\alpha\text{-CD·RBA}]^-\) anions with argon as the collision gas. To allow direct comparison, the CID experiments were performed at the same collision energies in the center-of-mass frame (instead of lab frame) for all anionic complexes. As shown in Figure 2.3 for \([\alpha\text{-CD·BA}]^-\), the ion intensity of \([\alpha\text{-CD}]^-\) grows with increasing collision energy while the signal of the non-covalent complex decreases accordingly. The other four \(\alpha\text{-CD}\) anionic complexes showed similar CID behavior; formation of benzoate derivatives ions \([\text{RBA}]^-\) was not observed under any
condition. As can be seen in Figure 2.4, the gas-phase kinetic stability of the anionic H-G complexes increases as follows: $[\alpha\text{-CD}\cdot3,5\text{-diMeBA}]^- < [\alpha\text{-CD}\cdot3\text{-MeBA}]^- < [\alpha\text{-CD}\cdot3\text{-OHBA}]^- < [\alpha\text{-CD}\cdot3,5\text{-diOHBA}]^-$. 

![Figure 2.4](image)

Figure 2.4 CID product spectra of $[\alpha\text{-CD}\cdot\text{RBA}]^-$ anionic complexes at 0.5 mTorr argon and 1.4 eV center-of-mass collision energy, including conversions $R = I_{[\alpha\text{-CD}]^-} / \Sigma I$.

Cai and Cole studied proton-bridged adducts $[A^-\cdots\text{H}^+\cdots B^-]$ formed between anions and polar molecules by ESI-MS. In all cases, CID favored the formation of the anionic species with the lower gas-phase basicity. Furthermore, the most stable adducts formed between species $A^-$ and $B^-$ were of most similar basicity. This relation between gas-phase basicities and adduct stability can be understood by recognizing that the two anionic fragments bind competitively to the bridging proton. If one fragment is more basic, it would bind more strongly to the bridging proton at the expense of the bond to the other fragment, thus reducing the overall kinetic stability of the adduct.

The fact that the only observable CID daughter ion is $[\alpha\text{-CD}]^-$ for all five $[\alpha\text{-CD}\cdot\text{RBA}]^-$ complexes reveals that the gas-phase basicity of $[\alpha\text{-CD}]^-$ is lower than that
of any of the substituted benzoates (RBA\(^{-}\)) used in this study, i.e. the basicity of deprotonated \(\alpha\)-CD versus those of the substituted benzoates is reversed in the gas phase as compared to solution. Thus, the lower the benzoate’s basicity, the closer it is to that of \([\alpha\text{-CD}]^{-}\), and hence the higher the kinetic stability of the \([\alpha\text{-CD-RBA}]^{-}\) complex should be.

The gas-phase basicities \(\Delta G^{0}\) (kcal mol\(^{-1}\)) of four benzoates used in our study are listed in Table 2.1 and follow the order \([3,5\text{-diMeBA}]^{-}\) (333.8\(^71\) > \([3\text{-MeBA}]^{-}\) (333.4\(^71\) > \([\text{BA}]^{-}\) (333.1\(^72\) > \([3\text{-OHBA}]^{-}\) (331.6\(^73\). The gas-phase basicity of \([3,5\text{-diOHBA}]^{-}\) was not available from the literature, but it is necessarily yet smaller than that of \([3\text{-OHBA}]^{-}\) due to the additional electron-withdrawing meta-OH group. This is in agreement with the experimentally determined gas-phase stability order of their adducts with \(\alpha\)-CD (see above).

### Table 2.1 Gas-phase basicities of substituted benzoates (RBA\(^{-}\)).

<table>
<thead>
<tr>
<th>Benzoates (RBA(^{-}))</th>
<th>(\Delta G^{0}) (kcal mol(^{-1}))(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Dimethylbenzoate ([3,5-diMeBA](^{-}))</td>
<td>333.8(^71)</td>
</tr>
<tr>
<td>3-Methylbenzoate ([3-MeBA](^{-}))</td>
<td>333.4(^71)</td>
</tr>
<tr>
<td>Benzoate ([BA](^{-}))</td>
<td>333.1(^72)</td>
</tr>
<tr>
<td>3-Hydroxybenzoate ([3-OHBA](^{-}))</td>
<td>331.6(^73)</td>
</tr>
</tbody>
</table>

\(^a\) Data were taken from the NIST website: http://webbook.nist.gov/chemistry/.

### 2.2.4 Comparison with Solution-State Data

When we compare our gas-phase results with published solution-state data for H–G complexes of \(\alpha\)-CD with benzoic acid derivatives\(^{36e}\), the impact of the environment

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becomes clear. In our MS experiments appreciable fragmentation of \([\alpha\text{-CD-BA}]^–\) requires collision energies that are more than an order of magnitude bigger than its stability in solution of only 1.4 kcal mol\(^{-1}\).\(^{74}\) The aqueous neutral complexes of \(\alpha\text{-CD}\) with substituted benzoic acids are more strongly bound, with Gibbs free energies of association \(–\Delta G_0^\circ = 3.4–4.1\) (BA),\(^{75}\) 3.3–3.7 (3-OHBA),\(^{75b,76}\) and 3.3 kcal mol\(^{-1}\) (3-MeBA).\(^{75a}\) Thus, the solution-state thermodynamic stability trend of these neutral H–G complexes is governed by the steric influence of the meta substitution and differs from the kinetic trend that we observe for their deprotonated counterparts in the gas phase.

It is difficult to obtain a more detailed understanding of the effects of the surrounding medium as mass spectrometric experiments alone do not provide information on the structures of, and hence, the specific interactions in these H–G complexes. However, the high-vacuum environment of a mass spectrometer allows a direct comparison with theoretical studies without the need to model solvent influences. Quantum-chemical calculations, combined with classical characterization techniques in the gas phase such as ESI–MS, thus enable us to derive gas-phase structural details and dissociation energies of the studied complexes, which help to clarify fundamental aspects of the interactions involved and can be useful for the experimentalist’s interpretations. The intrinsic properties in the gas phase can then be compared with those in condensed phase, as influenced by the surrounding solvent, to afford a better understanding of the solvent effects. Therefore, DFT calculations were performed.

### 2.2.5 Calculated Gas-Phase Conformations

One would expect that, in the gas phase, the structure with the largest number of hydrogen bonds is the most stable. Accordingly, a relatively rigid hydrogen-bonded
conformation was found as the global energy minimum of α-CD. The substituted benzoate guests can adopt two general orientations, having the carboxylate pointing either in the same (‘head’) or in the opposite direction (‘tail’) as the taper of the conical α-CD. PM3 searches for either orientation of H–G complexes [α-CD·3-MeBA]−, [α-CD·BA]−, and [α-CD·3-OHBA]− afforded minimum-energy conformations that all had in common that the largest hydrogen-bonding network was formed between α-CD and the benzoate derivative. These geometries were refined by DFT calculations at the M06-L/6-31+G(d,p) level of theory (Figure 2.5). For the head orientation, so-called inclusion conformations are favored having the guest’s aryl group inside the cavity of α-CD, whereas we found non-specific complexes with the aryl group outside α-CD’s cavity as the preferred conformations for the tail orientation. It is worth noting that the conformations for the head orientation (A, B and C in Figure 2.5) found by the DFT calculations are similar to crystal structures for analogous H–G complexes. For instance, in the solid state the guest molecules are hosted by the cavity of α-CD, and the carboxylate or nitro group is usually more or less in the plane of the small rim, just as in our calculations. In contrast, the hydroxyl groups of α-CD point outward to form hydrogen bonds with neighboring solvent or host molecules in the condensed phase, while our gas-phase conformations feature multiple hydrogen bonds of hydroxyl groups on the narrower rim with the guest’s carboxylate group as shown in Figure 2.5. These structural differences reflect the fact that in general, a more delocalized electron density is energetically favorable. Since in the gas phase there is no solvent to interact with the polar groups such as the hydroxyls, the intra- and intermolecular hydrogen bonds, with which the negative charge is efficiently distributed, are believed to constitute the primary interaction in our system.
Figure 2.5 M06-L/6-31+G(d,p) optimized geometries for head (A, B, C, side and top views) and tail (D, E, F) orientations of $[\alpha$-CD·3-MeBA]$^-$, $[\alpha$-CD·BA]$^-$, and $[\alpha$-CD·3-OHBA]$^-$, respectively. Green dashed lines represent hydrogen-bonding interactions.

2.2.6 Host–Guest Bonding Energies

We calculated reaction energies for the dissociation of hydrogen-bonded complexes $[\alpha$-CD·RBA]$^-$ into either deprotonated $[\alpha$-CD]$^-$ anion and neutral RBA (Figure 2.6, pathway 1) or neutral $\alpha$-CD and benzoate $[\text{RBA}]^-$ (Figure 2.6, pathway 2). The first pathway is that observed in our gas-phase experiments, whereas the second pathway is analogous to the reversible H–G complexation in solution according to the pKa values of $\alpha$-CD and $[\text{RBA}]^-$. 

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For either pathway, we calculated larger dissociation energies for the complexes with head orientation of the substituted benzoate than for the corresponding tail conformations (Table 2.2, e.g. $[\alpha\text{-CD} \cdot 3\text{-MeBA}]^-$: 40.59 vs 31.92 kcal mol$^{-1}$ for pathway 1). As illustrated in Figure 2.6, this reflects the fact that the head conformations are more stable than the tail conformations by 8.67, 9.66, and 1.57 kcal mol$^{-1}$ for the complexes with 3-MeBA, BA, and 3-OHBA as the guest, respectively. For $[\alpha\text{-CD} \cdot 3\text{-OHBA}]^-$ the tail conformation is not as much disfavored because it features an additional hydrogen bond between the *meta* hydroxyl group of the guest and a primary hydroxyl on the small rim of the host (Figure 2.5, F), whereas in the head orientation (C) a glycosidic oxygen atom is involved, providing weaker hydrogen bonding. In the electrospray process, the H–G complexes should be able to adjust their structure during the transition to the gas phase; our calculations thus suggest that the detected $[\alpha\text{-CD} \cdot \text{RBA}]^-$ ions would preferentially adopt a head conformation.

![Figure 2.6 Potential energy diagram for the two possible dissociation pathways of $[\alpha\text{-CD} \cdot \text{RBA}]^-$ conformations.](image)

**Figure 2.6** Potential energy diagram for the two possible dissociation pathways of $[\alpha\text{-CD} \cdot \text{RBA}]^-$ conformations.
Table 2.2 Calculated M06-2X//M06-L/6-31+G(d,p) dissociation energies (kcal mol\(^{-1}\)), including BSSE corrections.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Head (\alpha)-CD(^-) + RBA</th>
<th>(\alpha)-CD + [RBA](^-)</th>
<th>Tail (\alpha)-CD(^-) + RBA</th>
<th>(\alpha)-CD + [RBA](^-)</th>
<th>(\Delta\Delta E)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\alpha\text{-CD} \cdot 3\text{-MeBA}]^-)</td>
<td>40.59</td>
<td>48.77</td>
<td>31.92</td>
<td>39.56</td>
<td>8.18</td>
</tr>
<tr>
<td>([\alpha\text{-CD} \cdot \text{BA}]^-)</td>
<td>40.71</td>
<td>47.94</td>
<td>31.05</td>
<td>38.07</td>
<td>7.23</td>
</tr>
<tr>
<td>([\alpha\text{-CD} \cdot 3\text{-OHBA}]^-)</td>
<td>43.95</td>
<td>49.21</td>
<td>42.38</td>
<td>47.28</td>
<td>5.26</td>
</tr>
</tbody>
</table>

^a^ Energy difference between pathways 1 and 2 for the head conformations.

As shown in Table 2.2, the M06-2X//M06-L dissociation energies required for pathway 1 are smaller than for pathway 2 for all studied complexes (\([\alpha\text{-CD} \cdot 3\text{-MeBA}]^-\): \(\Delta\Delta E = 8.18\), \([\alpha\text{-CD} \cdot \text{BA}]^-\): 7.23, \([\alpha\text{-CD} \cdot 3\text{-OHBA}]^-\): 5.26 kcal mol\(^{-1}\)), in agreement with our experimental CID results that afforded only \([\alpha\text{-CD}]^-\) anion and in which no benzoate \([\text{RBA}]^-\) was observable. As the two pathways produce the same species as are involved in the (hypothetical) proton transfer reaction between \([\alpha\text{-CD}]^-\) and \([\text{RBA}]^-\), the difference in dissociation energies \(\Delta\Delta E\) should correspond approximately to the difference in gas-phase basicities of \([\alpha\text{-CD}]^-\) and \([\text{RBA}]^-\), assuming that the Gibbs free energy contributions and any systematic errors largely cancel out. Combining the data in Tables 2.1 and 2.2, we can thus estimate the average gas-phase basicity of \([\alpha\text{-CD}]^-\) to be \(\Delta G^0 = 325–327\) kcal mol\(^{-1}\). No experimental value has been reported yet, and the common methods\(^79\) to determine gas-phase basicities have limitations that particularly apply to measuring the gas-phase basicity of \([\alpha\text{-CD}]^-\). The widely used equilibrium method is based on the determination of the equilibrium constant for the proton exchange reaction in the gas phase and is therefore obviously restricted to volatile
compounds, whereas the kinetic method should be used with care for polyfunctional molecules. However, Kralj et al.\textsuperscript{68} stated that the gas-phase basicity of $[\alpha$-CD$]^{-}$ should be close to that of $p$-nitrophenolate (321 kcal mol$^{-1}$)\textsuperscript{65} as fragmentation of the corresponding H–G complex produces both anions, with which our estimate is in acceptable agreement.

The experimentally observed trend in gas-phase kinetic stabilities $[\alpha$-CD·3-MeBA$]^{-} < [\alpha$-CD·BA$]^{-} < [\alpha$-CD·3-OHBA$]^{-}$ is reproduced by the calculated dissociation energies of 40.59 < 40.71 < 43.95 kcal mol$^{-1}$, respectively (Table 2.2), although we must note that the first two energies are very similar. The results will still be slightly affected by the Gibbs free energy contributions, which we cannot take into account here because the effective temperature of the ions in our experiments is not known. One may expect that the dissociation of $[\alpha$-CD·BA$]^{-}$ is entropically somewhat less favorable than the one of the H–G complexes with substituted benzoates, as the constraints by the host’s cavity on the rotations and vibrations of the substituent are relieved. This effect is probably largest for the complex of 3-OHBA as the hydroxyl substituent undergoes hydrogen bonding with the host. These entropic contributions may thus slightly change the differences, but not the trend, in dissociation energies of the studied H–G complexes. However, such variations are within the accuracy of the computational method used.

### 2.2.7 Hydrogen Bonding Interactions

The observation of proton-transfer dissociation and the DFT calculations clearly indicate that hydrogen bonding plays a decisive role in the gas-phase conformation and kinetic stability of the $[\alpha$-CD·RBA$]^{-}$ complexes. Therefore we investigated these interactions in more detail by natural bond orbital (NBO) analysis\textsuperscript{80} at the
M06-2X/6-31+G(d,p) level of theory. Hydrogen bonding is often described as an electrostatic dipole–dipole interaction. However, it also has some features of covalent bonding: it usually involves a limited number of interaction partners and is directional. Within NBO theory, the second-order perturbation energies $E(2)$ are associated with donor–acceptor delocalization corrections to the idealized Lewis structure, and the interactions between the benzoate’s oxygen lone pairs and the O–H antibonds of $\alpha$-CD reflect the H–G hydrogen bonding strength. In geometries A–C in Figure 2.5, the RBA anion is completely surrounded by $\alpha$-CD and therefore the orientation of the carboxylate group is restricted, which may affect the hydrogen bonding. On the other hand, geometries D–F have the RBA anion outside of the $\alpha$-CD cavity, so that its carboxylate group has more freedom to attain a suitable position to engage in strong hydrogen bonding. Thus, the hydrogen bonding in geometries A–C is expected to be weaker than in D–F. This is corroborated by the calculated hydrogen bond parameters listed in Table 2.3, featuring longer O⋯O distances and more bent O–H⋯O arrangements for geometries A–C than for D–F, and accordingly smaller interaction energies $E(2)$. As a result, the total hydrogen bonding interaction is markedly weaker for the head conformations A–C than for the corresponding tail conformations D–F. One should keep in mind, though, that the interaction energies $E(2)$ are relative to the reference Lewis-like descriptions chosen by the NBO analyses, which are not exactly identical for the different complexes; i.e. their bonds, lone pairs, and antibonds have slightly different electron occupations. Consequently, these results can only serve as a semi-quantitative comparison and cannot be combined with the calculated overall relative energies to accurately estimate the magnitude of the residual H–G interactions that stabilize the head over the tail conformations. Such interactions may include
unconventional hydrogen bonds, such as (C–H⋯O), C–H⋯π interactions, C–H⋯anion interactions, and van der Waals forces. We are currently developing a computational strategy to quantify these bonding contributions. Furthermore, we are performing energy-resolved CID experiments to determine the absolute gas-phase dissociation energies for these complexes, which will serve to validate the computational level.
Qualitative Study of α-Cyclodextrin Complexes in the Gas Phase

Table 2.3 M06-2X//M06-L/6-31+G(d,p) bond lengths (Å) and angles (°) and NBO donor–acceptor interaction energies $E(2)$ (kcal mol$^{-1}$) for intermolecular hydrogen bonds in [α-CD·RBA]$^-$ complexes.

<table>
<thead>
<tr>
<th>Hydrogen bond</th>
<th>$R_{O\cdots O}$</th>
<th>$\angle O-H\cdots O$</th>
<th>$E(2)$</th>
<th>Hydrogen bond</th>
<th>$R_{O\cdots O}$</th>
<th>$\angle O-H\cdots O$</th>
<th>$E(2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A – [α-CD·3-MeBA]$^-$ head conformation</td>
<td></td>
<td></td>
<td></td>
<td>D – [α-CD·3-MeBA]$^-$ tail conformation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O6$^a$H···O</td>
<td>2.721</td>
<td>156.24</td>
<td>20.6</td>
<td>O6$^a$H···O</td>
<td>2.713</td>
<td>174.06</td>
<td>30.1</td>
</tr>
<tr>
<td>O6$^a$H···O</td>
<td>2.891</td>
<td>148.96</td>
<td>8.4</td>
<td>O6$^a$H···O</td>
<td>2.790</td>
<td>167.50</td>
<td>20.2</td>
</tr>
<tr>
<td>O6$^a$H···O'</td>
<td>2.731</td>
<td>157.63</td>
<td>20.3</td>
<td>O6$^a$H···O'</td>
<td>2.714</td>
<td>173.28</td>
<td>29.6</td>
</tr>
<tr>
<td>O6$^a$H···O'</td>
<td>2.904</td>
<td>147.57</td>
<td>7.5</td>
<td>O6$^a$H···O'</td>
<td>2.778</td>
<td>166.44</td>
<td>20.6</td>
</tr>
<tr>
<td>Σ 56.8</td>
<td></td>
<td></td>
<td></td>
<td>Σ 100.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B – [α-CD·BA]$^-$ head conformation</td>
<td></td>
<td></td>
<td></td>
<td>E – [α-CD·BA]$^-$ tail conformation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O6$^a$H···O</td>
<td>2.827</td>
<td>155.76</td>
<td>12.3</td>
<td>O6$^a$H···O</td>
<td>2.732</td>
<td>169.49</td>
<td>24.4</td>
</tr>
<tr>
<td>O6$^a$H···O</td>
<td>2.968</td>
<td>139.67</td>
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<td>2.779</td>
<td>166.60</td>
<td>22.1</td>
</tr>
<tr>
<td>O6$^a$H···O'</td>
<td>2.827</td>
<td>155.79</td>
<td>12.2</td>
<td>O6$^a$H···O'</td>
<td>2.745</td>
<td>169.33</td>
<td>23.3</td>
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<tr>
<td>O6$^a$H···O'</td>
<td>2.969</td>
<td>139.77</td>
<td>3.6</td>
<td>O6$^a$H···O'</td>
<td>2.785</td>
<td>168.81</td>
<td>22.1</td>
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<td>Σ 32.3</td>
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<td></td>
<td>Σ 91.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C – [α-CD·3-OHBA]$^-$ head conformation</td>
<td></td>
<td></td>
<td></td>
<td>F – [α-CD·3-OHBA]$^-$ tail conformation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O6$^a$H···O</td>
<td>2.847</td>
<td>155.35</td>
<td>11.1</td>
<td>O6$^a$H···O</td>
<td>2.683</td>
<td>167.69</td>
<td>13.2</td>
</tr>
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<td>O6$^a$H···O</td>
<td>2.939</td>
<td>143.22</td>
<td>5.3</td>
<td>O6$^a$H···O</td>
<td>2.811</td>
<td>168.16</td>
<td>23.2</td>
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<tr>
<td>O6$^a$H···O'</td>
<td>2.852</td>
<td>154.84</td>
<td>10.5</td>
<td>O6$^a$H···O'</td>
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<td>161.98</td>
<td>15.8</td>
</tr>
<tr>
<td>O6$^a$H···O'</td>
<td>2.938</td>
<td>142.48</td>
<td>5.2</td>
<td>O6$^a$H···O'</td>
<td>2.754</td>
<td>168.81</td>
<td>33.6</td>
</tr>
<tr>
<td>O1$^b$···HO$^m$</td>
<td>3.359</td>
<td>164.32</td>
<td>2.1</td>
<td>O6$^a$H···O'</td>
<td>2.785</td>
<td>167.49</td>
<td>21.1</td>
</tr>
<tr>
<td>Σ 34.2</td>
<td></td>
<td></td>
<td></td>
<td>Σ 106.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O6$^a$–f are the oxygen atoms connected to C6 of the six glucose units a–f in α-CD, with O1$^b$ connecting units b and c; O and O$'$ are the RBA carboxylate oxygen atoms and O$^m$ is the hydroxyl oxygen atom of 3-OHBA.
2.3 Conclusions

The anionic H–G complexes of α-cyclodextrin with substituted benzoates RBA− were studied by ESI–MS and their 1:1 composition was confirmed by the unit-mass spacing of their isotope patterns. The correlation of the gas-phase kinetic stabilities of these complexes, as disclosed by CID experiments, with the basicities of the host and guests involved sheds light on the intrinsic properties of the gas-phase non-covalent complexes. The gas-phase basicity of deprotonated α-CD was found to be lower than that of all investigated benzoate guests as concluded from the dissociation of the [α-CD·RBA]− complexes into [α-CD]− and the neutral RBA. This proton-transfer reaction signifies the existence of hydrogen bonding between host and guest in the gas phase and indicates that the basicity order of deprotonated α-CD vs the benzoates is reversed as compared to solution state. A comparison of the ratios between reactant and product intensities under identical collision conditions with the gas-phase basicities of the used benzoates establishes that the complex is kinetically more stable when the difference in gas-phase basicities of host and guest anions is smaller. The non-covalently bound species of the [α-CD·RBA]− complexes formed in solution are unlikely to retain the same conformation in the gas phase. DFT calculations show a preference for the largest number of hydrogen bonds involved in the [α-CD·RBA]− complexes, in agreement with our experimental results. The calculated dissociation energies suggest that the inclusion complexes are favored over the non-specific complexes in the gas phase, although the hydrogen bonding is weaker in the former conformations. The combination of experimental and computational results provides an estimate for the gas-phase basicity
of $[\alpha\text{-CD}]^{-}$ of 325–327 kcal mol$^{-1}$, which is useful for a better understanding of the properties of gas-phase cyclodextrin complexes.

### 2.4 Experimental Section

#### 2.4.1 Materials

All chemicals were purchased from Aldrich (purity $\geq$ 95%) and were used without further purification. Stock solutions of $\alpha$-CD and benzoic acid derivatives (RBA) were prepared in methanol/water (95:5 v/v) at a concentration of 1.0 mM. For the ESI–MS studies, equal amounts of $\alpha$-CD and RBA stock solution were mixed and diluted to approximately 10 $\mu$M.

#### 2.4.2 Mass Spectrometry

All mass spectrometric experiments were performed on a Finnigan MAT TSQ-7000 triple-stage mass spectrometer equipped with a microspray source. No desolvation or nebulization gas was applied. The experimental parameters were kept as similar as possible to maintain comparable conditions for all samples. The ESI parameters were set at an infusion rate of 1–2 $\mu$L min$^{-1}$, a spray voltage of 1.7–1.9 kV on the needle, the heated capillary was at 150 °C and at $-50$ V, and the tube lens at $-90$ to $-110$ V. The CID spectra were obtained by mass-selecting the desired non-covalent complex anions in the first quadrupole, fragmenting them in the collision octapole, and recording the product anions by scanning the second quadrupole. Argon was used as the collision gas at a pressure of 0.5 mTorr. In order to accurately maintain the collision gas pressure for better reproducibility of the CID experiments, a sensitive Pfeiffer Vacuum IMR 265 hot
cathode/Pirani gauge combination was connected to the CID chamber. The lab-frame collision offsets $V_{\text{coll}}$ were set accordingly for all studied complexes to obtain the same energies in the center-of-mass frame $E_{\text{CM}}$ of 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 eV, as calculated according to equation (1):

$$E_{\text{CM}} = V_{\text{coll}} \times \frac{M_{\text{argon}}}{(M_{\text{argon}} + M_{\text{parent}})} \quad (1)$$

### 2.4.3 Theoretical Calculations

All calculations were carried out using the Gaussian 09 quantum mechanical package. The large size and flexibility of the $\alpha$-CD H–G complexes makes the theoretical investigation of their conformations computationally demanding. We used the semi-empirical PM3 quantum chemical method, which has been widely applied to study CDs due to its relatively low computational cost and has been successfully tested for searching global minimum-energy geometries of CD complexes. The initial geometry of $\alpha$-CD was constructed from the available X-ray structure, in which the hydration water molecules are excluded. The optimized global minimum conformation of $\alpha$-CD was taken for further modeling of the CD complexes with benzoate derivatives. We searched for the global energy minima of $[\alpha$-CD·RBA$]^{-}$ gas-phase complexes analogous to a recently reported method. In GaussView, the $\alpha$-CD molecule was centered with the $C_6$ rotational axis along the Z axis and the wide rim above the XY plane. The [RBA]$^{-}$ anion was placed on the Z axis and two possible orientations were considered: a ‘head’ orientation with the carboxylate group pointing in the negative Z-direction and the opposite, ‘tail’ orientation. The *ipso* carbon atom of [RBA]$^{-}$ was initially located at $+8$ Å on the Z axis and was moved in steps of 0.5 Å until $-8$ Å. Each thus generated starting geometry was optimized without any restriction.
to find the nearest minimum-energy conformation using the semi-empirical method PM3; the computational results are listed in Table 2.4.

Table 2.4 PM3 relative energies (kcal mol$^{-1}$) of conformations, optimized from 33 different starting geometries of [α-CD-RBA]$^-$. 

<table>
<thead>
<tr>
<th>Initial distance (Å)</th>
<th>[α-CD-3-MeBA]$^-$</th>
<th>[α-CD-BA]$^-$</th>
<th>[α-CD-3-OHBA]$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>Tail</td>
<td>Head</td>
<td>Tail</td>
</tr>
<tr>
<td>8.0</td>
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<td>7.5</td>
<td>15.4</td>
<td>4.4</td>
<td>13.5</td>
</tr>
<tr>
<td>7.0</td>
<td>19.7</td>
<td>5.6</td>
<td>16.5</td>
</tr>
<tr>
<td>6.5</td>
<td>12.7</td>
<td>5.8</td>
<td>19.8</td>
</tr>
<tr>
<td>6.0</td>
<td>12.8</td>
<td>6.4</td>
<td>12.1</td>
</tr>
<tr>
<td>5.5</td>
<td>20.7</td>
<td>6.4</td>
<td>22.4</td>
</tr>
<tr>
<td>5.0</td>
<td>20.7</td>
<td>4.6</td>
<td>17.3</td>
</tr>
<tr>
<td>4.5</td>
<td>17.6</td>
<td>8.2</td>
<td>17.4</td>
</tr>
<tr>
<td>4.0</td>
<td>19.6</td>
<td>6.1</td>
<td>23.7</td>
</tr>
<tr>
<td>3.5</td>
<td>19.4</td>
<td>3.0</td>
<td>28.4</td>
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<tr>
<td>3.0</td>
<td>22.9</td>
<td>5.3</td>
<td>28.4</td>
</tr>
<tr>
<td>2.5</td>
<td>19.0</td>
<td>11.4</td>
<td>25.0</td>
</tr>
<tr>
<td>2.0</td>
<td>19.5</td>
<td>6.8</td>
<td>24.4</td>
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<tr>
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<td>19.5</td>
<td>8.1</td>
<td>24.5</td>
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<tr>
<td>1.0</td>
<td>19.5</td>
<td>9.7</td>
<td>24.5</td>
</tr>
<tr>
<td>0.5</td>
<td>19.8</td>
<td>8.8</td>
<td>20.6</td>
</tr>
<tr>
<td>0.0</td>
<td>11.1</td>
<td>9.7</td>
<td>15.8</td>
</tr>
<tr>
<td>-0.5</td>
<td>1.1</td>
<td>8.8</td>
<td>13.9</td>
</tr>
<tr>
<td>-1.0</td>
<td>2.8</td>
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<td>13.0</td>
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<td>0.0</td>
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<td>3.1</td>
<td>9.4</td>
<td>7.7</td>
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<td>0.0</td>
<td>2.4</td>
</tr>
<tr>
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<td>1.7</td>
<td>22.8</td>
<td>1.0</td>
</tr>
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<td>1.6</td>
<td>31.1</td>
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<td>-4.0</td>
<td>22.0</td>
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<td>36.8</td>
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<td>26.4</td>
<td>4.3</td>
<td>33.9</td>
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<td>19.2</td>
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<td>41.9</td>
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<td>20.0</td>
<td>0.0</td>
<td>46.5</td>
</tr>
<tr>
<td>-6.0</td>
<td>20.5</td>
<td>2.6</td>
<td>45.4</td>
</tr>
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<td>-6.5</td>
<td>21.8</td>
<td>0.8</td>
<td>48.5</td>
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<tr>
<td>-7.0</td>
<td>42.9</td>
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<td>47.9</td>
</tr>
<tr>
<td>-7.5</td>
<td>24.4</td>
<td>21.9</td>
<td>20.9</td>
</tr>
<tr>
<td>-8.0</td>
<td>15.8</td>
<td>21.7</td>
<td>47.4</td>
</tr>
</tbody>
</table>
Table 2.5 Calculated energies of species in Hartree.

<table>
<thead>
<tr>
<th>Species</th>
<th>M06-L</th>
<th>M06-2X//M06-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD</td>
<td>$-3664.36214988$</td>
<td>$-3663.44322099$</td>
</tr>
<tr>
<td>[α-CD]$^-$</td>
<td>$-3663.82007571$</td>
<td>$-3662.90321993$</td>
</tr>
<tr>
<td>3-MeBA</td>
<td>$-460.115526056$</td>
<td>$-459.972950488$</td>
</tr>
<tr>
<td>[3-MeBA]$^-$</td>
<td>$-459.558313046$</td>
<td>$-459.419892859$</td>
</tr>
<tr>
<td>BA</td>
<td>$-420.800846719$</td>
<td>$-420.673756421$</td>
</tr>
<tr>
<td>[BA]$^-$</td>
<td>$-420.244837865$</td>
<td>$-420.121812816$</td>
</tr>
<tr>
<td>3-OHBA</td>
<td>$-496.021818825$</td>
<td>$-495.875459347$</td>
</tr>
<tr>
<td>[3-OHBA]$^-$</td>
<td>$-495.468115442$</td>
<td>$-495.326547239$</td>
</tr>
<tr>
<td>[α-CD·3-MeBA]$^-$</td>
<td>head $-4124.01737630$</td>
<td>tail $-4122.95148442$</td>
</tr>
<tr>
<td></td>
<td>tail $-4123.99376640$</td>
<td>$-4122.93306673$</td>
</tr>
<tr>
<td>[α-CD·BA]$^-$</td>
<td>head $-4084.70096925$</td>
<td>$-4083.65178621$</td>
</tr>
<tr>
<td></td>
<td>tail $-4084.67775447$</td>
<td>$-4083.63241999$</td>
</tr>
<tr>
<td>[α-CD·3-OHBA]$^-$</td>
<td>head $-4159.92796935$</td>
<td>tail $-4159.85441543$</td>
</tr>
<tr>
<td></td>
<td>tail $-4159.91732803$</td>
<td>$-4158.86003376$</td>
</tr>
</tbody>
</table>

In order to investigate the involved non-covalent interactions more accurately, the lowest-energy PM3 geometries were refined by DFT calculations with the M06-L density functional. Finally, single-point energies were computed at the M06-2X//M06-L level of theory (Table 2.5). The M06 family of density functionals is designed to accurately model main-group thermochemistry, kinetics, and non-covalent interactions at a reasonable cost. For all the DFT calculations the 6-31+G(d,p) basis set was applied, which is a Pople-type basis set of double-zeta quality, augmented with one set of polarization functions on all elements and one set of diffuse functions on the non-hydrogen atoms. The H–G interaction energies were corrected for basis set superposition errors as calculated with the counterpoise method.
Chapter 3

Quantitative Study of $\alpha$-Cyclodextrin Complexes in the Gas Phase
A novel deconvolution method for energy-resolved reaction cross sections is applied to determine intrinsic gas-phase dissociation energies for non-covalent α-cyclodextrin (α-CD) host–guest complexes. Experimental results were well reproduced by density functional theory (DFT) calculations at the M06-2X//M06-L/6-31+G(d,p) level of theory with counterpoise corrections for the complexes of [α-CD·3-MeBA]−, [α-CD·BA]−, and [α-CD·3-OHBA]−. Additionally, the contribution of intermolecular hydrogen bonds to the stabilization of gaseous complexes has been carefully analyzed and quantified. However, the extension of this combined approach for other CD complexes is limited.

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Chapter 3

3.1 Introduction

The soft electrospray ionization mass spectrometry (ESI–MS) has been applied to the study of cyclodextrin (CD) complexes to achieve deeper insights into intrinsic non-covalent interactions (NCIs) and NCI-based molecular recognition in the absence of solvent effects. The perplexing conclusions derived from qualitative investigations signify the need for a method with which one can reliably evaluate crucial properties of NCIs, e.g., interaction types, strength, and structural information. Among available quantitative methods collision-induced dissociation (CID) threshold determination has been applied to small host–guest (H–G) complexes like crown ether–alkali metal complexes. However, the extraction of absolute dissociation energies from the experimental data by explicit consideration of the internal rovibrational states becomes prohibitive for large systems. Recently, Chen and co-workers developed a new method to deconvolute the energy-resolved CID reaction cross sections, which is conceived for medium-to-large complexes. This method, therefore, enables us to quantitatively investigate the intrinsic NCIs in larger H–G systems such as CD complexes. The ultimate aim of this research is to determine the contributions of individual NCIs to the overall stability of CD complexes in the gas phase. To our knowledge, this quantitative research is unprecedented.

Here we report a combinatorial experimental and theoretical approach to establish the contributions of NCIs to the stability of anionic H–G complexes of α-CD with 3-methylbenzoic acid (3-MeBA), benzoic acid (BA), and 3-hydroxybenzoic acid (3-OHBA) by tandem ESI-MS and density functional theory (DFT) calculations. In order
to establish a reliable connection between experimental data and theoretical results, this combinational approach has been extended to other CD host-guest complexes, named as α-CD with 1-adamantanecarboxylic acid (AA), β-CD with BA, and AA.

3.2 Results and Discussion

3.2.1 CID Threshold Measurements

Preliminary results concerning the ESI-MS investigations and part of the DFT calculations have been presented in Chapter 2 and reported recently. On a customized Finnigan MAT TSQ-700 mass spectrometer, the thermalized anionic parent complexes \( [\alpha-CD \cdot 3-MeBA]^- \) (1), \( [\alpha-CD \cdot BA]^- \) (2), and \( [\alpha-CD \cdot 3-OHBA]^- \) (3) were mass-selected and collided with 20–110 μTorr xenon to monitor the fragmentation into anionic \( [\alpha-CD]^- \) and the neutral benzoic acid derivative, RBA. The energy-resolved reaction cross sections were extrapolated to zero pressure, representing the dissociation cross sections under strict single-collision conditions, which is shown in Figure 3.1 for the dissociation of the anionic complex 1. These curves were fitted with the program L-CID to extract absolute average gas-phase threshold energies \( E_0 \) of 40.8(5), 41.1(5), and 41.8(5) kcal mol\(^{-1}\) for the formation of \( [\alpha-CD]^- \) from complexes 1, 2, and 3, respectively (see the Experimental Section). While the measured dissociation energies differ within the experimental error bounds, each independent experimental result as well as the averaged threshold energies always affords a gas-phase stability trend \( 1 < 2 < 3 \). Both the order and magnitude of the trend are in agreement with our previous qualitative results, in which the extent of fragmentation decreased in the order \( 1 > 2 > 3 \) under identical collision conditions. This illustrates that energy-resolved CID
experiments are suitable to study CD complexes.

Figure 3.1 Energy-resolved reaction cross section for the dissociation of \([\alpha\text{-CD}]^-\) from 1, extrapolated to zero pressure (red circles), and L-CID fitted curve (black line).

3.2.2 The Evaluation of DFT Methods

To complement the experimental data, a detailed theoretical investigation of the complexes was performed by means of DFT calculations with Gaussian 09.\(^{86}\) For all DFT calculations the 6-31+G(d,p) basis set was applied; geometries were optimized with the BLYP\(^{97}\) and M06-L\(^{90,98}\) functionals, followed by counterpoise-corrected\(^ {92a,99}\) single-point energy evaluations with the BLYP, M06-L, and M06-2X\(^ {90-91}\) functionals (see the Experimental Section). The BLYP functional has been widely applied for CDs,\(^ {58b,87c}\) whereas M06-L and especially M06-2X are recommended for the study of nonbonding interactions as well as main-group thermochemistry and kinetics. Furthermore, M06-L is a local functional, allowing the optimization of such large systems as CD complexes at reasonable computational costs.\(^ {89-90,98}\)
Quantitative Study of α-Cyclodextrin Complexes in the Gas Phase

**Table 3.1** Calculated and experimental gas-phase dissociation energies (kcal mol\(^{-1}\)) for formation of \([\alpha\text{-CD}]^-\) from complexes 1–3.

<table>
<thead>
<tr>
<th></th>
<th>BLYP</th>
<th>M06-L</th>
<th>M06-2X//BLYP</th>
<th>M06-2X//M06-L</th>
<th>Exp. (E_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.7</td>
<td>45.5</td>
<td>34.8</td>
<td>40.6</td>
<td>40.8(5)</td>
</tr>
<tr>
<td>2</td>
<td>10.7</td>
<td>45.1</td>
<td>33.2</td>
<td>40.7</td>
<td>41.1(5)</td>
</tr>
<tr>
<td>3</td>
<td>10.8</td>
<td>48.2</td>
<td>36.2</td>
<td>44.0</td>
<td>41.8(5)</td>
</tr>
</tbody>
</table>

![Figure 3.2](image) Structure of complex 1; dotted lines indicate selected O–H···O (green), C–H···O (blue), and C–H···π (yellow) hydrogen bonds.

As shown in Table 3.1, the BLYP functional largely underestimates the experimental dissociation energies for formation of \([\alpha\text{-CD}]^-\) from complexes 1–3 by about 30 kcal mol\(^{-1}\). This result corroborates previous reports that BLYP does not treat weak NCIs well.\(^{100}\) On the other hand, the M06-L optimizations afford dissociation energies that are only slightly (\(ca. 5\) kcal mol\(^{-1}\)) above the experimental values. While the M06-
2X//BLYP single-point calculations still underestimate the experimental dissociation energies by 6–8 kcal mol$^{-1}$, the latter are well reproduced at the M06-2X//M06-L level (40.6, 40.7, and 44.0 kcal mol$^{-1}$, respectively). This suggests that BLYP’s underestimation of NCIs also affects the calculated geometries. Therefore, our computational strategy of using M06-L optimized geometries for subsequent single-point energies with the M06-2X functional appears well suited for an accurate analysis of α-CD complexes. Inspired by these findings, we turned our attention to subdividing the NCIs within these complexes.

### 3.2.3 Decomposition of Non-Covalent Interactions

As shown for complex 1 in Figure 3.2, the calculated geometries indicate that the H–G complexes resemble a neutral α-CD cavity surrounding the anionic benzoate derivative, i.e. only upon collisional activation proton transfer occurs to generate the observed [α-CD]$^-$ anion. Therefore, alternative bonding energies $E_b$ were calculated for the complexes relative to the corresponding neutral host and anionic guest fragments. Furthermore, we calculated the preparation energies $E_P$ required to distort the host and guest fragments to their geometries in the complexes (Table 3.2). The difference between $E_b$ and $E_P$ (complex 1: $E_{int} = -85.5$, 2: $-81.2$, 3: $-84.2$ kcal mol$^{-1}$) corresponds to the total attractive interactions between host and guest. These total interaction energies were further decomposed into contributions from various NCIs.
Quantitative Study of α-Cyclodextrin Complexes in the Gas Phase

Table 3.2 M06-2X//M06-L alternative bonding energies $E_b$, preparation energies $E_P$, total O–H⋯O hydrogen bond energies $\sum E_{OH⋯O}$, and remaining interaction energies $E_R$ (kcal mol$^{-1}$) in complexes 1–3.

<table>
<thead>
<tr>
<th></th>
<th>$E_b$</th>
<th>$E_P$</th>
<th>$E_{int}$</th>
<th>$\sum E_{OH⋯O}$</th>
<th>$E_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−48.8</td>
<td>36.7</td>
<td>−85.5</td>
<td>−43.1</td>
<td>−42.4</td>
</tr>
<tr>
<td>2</td>
<td>−48.2</td>
<td>33.0</td>
<td>−81.2</td>
<td>−38.6</td>
<td>−42.6</td>
</tr>
<tr>
<td>3</td>
<td>−49.5</td>
<td>34.7</td>
<td>−84.2</td>
<td>−41.6</td>
<td>−42.6</td>
</tr>
</tbody>
</table>

The six hydroxyl groups on the narrower rim of the α-CD host form three groups of hydrogen bonds, namely two strong and one weaker interaction with the guest’s carboxylate group (Figure 3.2). The contribution of each hydrogen bond pair to the H–G bonding energy was estimated by calculating the effect of replacing the OH groups by H atoms according to a hypothetical host-exchange reaction. Specifically, according to Figure 3.3, hydroxyls of interest were replaced by hydrogen atoms, maintaining the directions of the original C–O bonds and assuming a C–H bond length of 1.070 Å in the optimized [α-CD·RBA] geometries. Without re-optimization, counterpoise-corrected [host·RBA]$^-$ bond energies were calculated. Thus, geometric and energetic contributions from the changed interactions within the modified host are suppressed or largely cancel out. According to the hypothetical host-exchange reaction in Figure 3.3, the contribution of each hydrogen bond pair to the H–G bonding energy was estimated by calculating the effect of replacing the OH groups by H atoms. Table 3.3 lists structural parameters for these hydrogen bonds relative to the carboxylate. This afforded strengths of 23.0, 16.7, and 3.4 kcal mol$^{-1}$ for the three groups of hydrogen bonds in complex 1. These individual contributions are nearly additive. For example, when all...
six OH groups are replaced by H atoms, the total host–3-MeBA bonding interactions decrease by 44.7 kcal mol$^{-1}$ as compared to 1, which is in good agreement with the sum of the above numbers (43.1 kcal mol$^{-1}$). Thus, our subdivision analysis seems reliable.

Likewise, we calculated the hydrogen bond strengths in complexes 2 and 3, including that for the interaction of the OH group of 3-OHBA with a glycosidic oxygen atom in α-CD (see Table 3.3).

![Figure 3.3 General approach for O–H⋯O hydrogen bond strength analysis. R = Me, H, OH.](image)

After subtraction of the hydrogen bond strengths from the total attractive energies $E_{\text{int}}$, interaction energies $E_{R}$ remain of 42.4, 42.6, and 42.6 kcal mol$^{-1}$ for complexes 1 and 2 and 3, respectively (Table 3.2). These stem from e.g. C–H⋯O and C–H⋯π (Figure 3.2) as well as electrostatic (ion–dipole) and van der Waals interactions. While individual contributions are not easily extracted, it is notable that the values for $E_{R}$ are very similar for complexes 1–3, suggesting similar structural features for the remaining NCIs. The calculated geometries of the three complexes are overlaid in Figure 3.6 (see
the Experimental Section), confirming that the spatial arrangements are closely comparable. As listed in Table 3.4, the C–H···O contacts feature C···O distances within the typical range of 3.1–3.5 Å for so-called “weak” hydrogen bonds.\textsuperscript{101} The interaction energies of similar C–H···O hydrogen bonds with aromatic C–H donors in the 1,4-benzoquinone dimer are significant.\textsuperscript{102} Furthermore, the C–H···π contacts are within range for attractive interactions. At 4.5 Å separation, the CH\textsubscript{4}···C\textsubscript{6}H\textsubscript{6} interaction is \textit{ca} 1 kcal mol\textsuperscript{−1} stabilizing,\textsuperscript{103} which may be further enhanced in complexes 1–3 by the substitution on both the hydrogen bond donor and acceptor. Thus, it is believed that C-H···O and C-H···π interactions (Figure 3.2) provide significant stabilization.

\textbf{Table 3.3} M06-2X//M06-L distances (Å) and angles (°),\textsuperscript{a} and hydrogen bond strengths (kcal mol\textsuperscript{−1}) in complexes 1–3.

<table>
<thead>
<tr>
<th>Type</th>
<th>RO····O</th>
<th>∠O–H···O</th>
<th>E_{OH···O}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Primary</td>
<td>2.727</td>
<td>156.94</td>
<td>−23.0</td>
</tr>
<tr>
<td>Secondary</td>
<td>2.898</td>
<td>148.27</td>
<td>−16.7</td>
</tr>
<tr>
<td>Tertiary</td>
<td>4.340</td>
<td>125.85</td>
<td>−3.4</td>
</tr>
<tr>
<td>2 Primary</td>
<td>2.827</td>
<td>155.78</td>
<td>−20.9</td>
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<tr>
<td>Secondary</td>
<td>2.969</td>
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<td>−13.4</td>
</tr>
<tr>
<td>Tertiary</td>
<td>3.386</td>
<td>105.19</td>
<td>−4.3</td>
</tr>
<tr>
<td>3 Primary</td>
<td>2.850</td>
<td>155.09</td>
<td>−20.8</td>
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<tr>
<td>Secondary</td>
<td>2.939</td>
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<td>−14.3</td>
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<tr>
<td>Tertiary</td>
<td>3.447</td>
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<td>−4.4</td>
</tr>
<tr>
<td>A\textsubscript{t}OH···O\textsuperscript{CD}</td>
<td>3.359</td>
<td>164.32</td>
<td>−2.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values for hydrogen bond pairs are averaged.
3.2.4 Comparison with Solid-State Study

Over 20 crystal structures of α-CD complexes with similar features as those calculated for complexes 1–3 were retrieved from the Cambridge structural database and listed in Table 3.4. In the crystal structures, the guest molecules are formally neutral and most of the polar hydroxyl groups of the host point outward, instead of inward as in our calculated geometries, in order to engage in intermolecular hydrogen bonding with neighboring H–G units. Nonetheless, the aryl moieties adopt very similar locations and orientations within the cavity of α-CD as found in our calculations (Figure 3.4), i.e. the guest attains the same alignment in both the condensed and the gas phase. Since the crystal structures contain no solvent molecules in the α-CD cavity, comparable C–H···O and C–H···π contacts also exist in the condensed phase. Thus, at least in our case, these interactions appear to be partly responsible for the alignment of the guest inside the cavity of α-CD.

![Calculated structure of complex 1 (blue) overlaid with crystal structures of H–G complexes of α-CD with 4-iodophenol (gray), 4-hydroxybenzoic acid (red), and 4-nitrophenol (green). Hydrogen atoms are omitted for clarity.](image_url)

**Figure 3.4** Calculated structure of complex 1 (blue) overlaid with crystal structures of H–G complexes of α-CD with 4-iodophenol (gray), 4-hydroxybenzoic acid (red), and 4-nitrophenol (green). Hydrogen atoms are omitted for clarity.
Table 3.4 Bond lengths (Å) for C–H⋯O ($R_{C-O}$) and C–H⋯π hydrogen bonds ($R_{C-\pi}$: distance between the carbon atom and the center of benzene ring) for calculated geometries and crystal structures of α-CD complexes.

<table>
<thead>
<tr>
<th>Guest</th>
<th>$R_{C-O}$</th>
<th>$R_{C-\pi}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MeBA</td>
<td>3.366</td>
<td>4.421</td>
<td>$^a$</td>
</tr>
<tr>
<td>BA</td>
<td>3.396</td>
<td>4.574</td>
<td>$^a$</td>
</tr>
<tr>
<td>3-OHBA</td>
<td>3.358</td>
<td>4.583</td>
<td>$^a$</td>
</tr>
<tr>
<td>4-chlorophenol</td>
<td>3.364</td>
<td>4.319</td>
<td>$^{104a}$</td>
</tr>
<tr>
<td>4-bromophenol</td>
<td>3.356</td>
<td>4.331</td>
<td>$^{104b}$</td>
</tr>
<tr>
<td>4-iodophenol</td>
<td>3.318</td>
<td>4.324</td>
<td>$^{104c}$</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>3.350</td>
<td>4.281</td>
<td>$^{104c}$</td>
</tr>
<tr>
<td>4-nitrophenol</td>
<td>3.359</td>
<td>4.327</td>
<td>$^{78}$</td>
</tr>
<tr>
<td>3-nitroaniline</td>
<td>3.422</td>
<td>4.545</td>
<td>$^{104d}$</td>
</tr>
</tbody>
</table>

$^a$ This work, M06-L optimizations

3.2.5 Extension to Other CD Complexes

In a similar fashion, the absolute bond dissociation energies of the thermalized anionic parent complexes [α-CD·AA]$^-$ (4), [β-CD·BA]$^-$ (5), and [β-CD·AA]$^-$ (6) were measured to be 40.2(5), 39.8(5), and 38.9(4) kcal mol$^{-1}$, respectively. All experimental values are within a rather narrow range of 38.9–41.8 kcal mol$^{-1}$, although one would expect a relatively large difference in bonding, especially between complexes 2 and 4. Specifically, the guest BA can perfectly penetrate the cavity of α-CD in solution, while the guest AA is too large to be included. This is reflected by the solution-phase enthalpy change of 9.4$^{75,105}$ vs 3.7$^{106}$ kcal mol$^{-1}$ for the association of α-CD with BA and AA,
respectively. Conversely, the similar gas-phase bonding energies would indicate similar geometrical orientations for all these studied CD complexes.

**Figure 3.5** M06-L/6-31+G(d,p) optimized geometries for head (A, B, C, side and top views) and tail (D, E, F) orientations of \([\alpha\text{-CD-}\text{AA}]^-, [\beta\text{-CD-BA}]^-, \text{and } [\beta\text{-CD- AA}]^-\), respectively. Green dashed lines represent hydrogen-bonding interactions.

Geometry optimizations were performed by means of DFT calculations with the M06-L functional, which has been used previously. The method reported in Chapter 2 for locating the global energy-minima of CD complexes was applied here to complexes 4, 5, and 6. The minimum-energy geometries found for both the “head” and “tail” orientation are presented in Figure 3.5. The inclusion geometry of 4 (Figure 3.5, A) is
calculated to be energetically more stable than the non-specific geometry D, which is against expectation. Certainly, the cavity of α-CD has less repulsive interactions with BA than with AA. However, the attractive interactions between the cavity and the guest AA still provide enough energy to maintain the inclusion geometry in vacuum. In the case of the β-CD complexes, the minimum-energy geometries for both the head and tail orientations are inclusion conformations, which differ with respect to the bonding sites. Geometries B and C involve hydrogen bonds between the carboxylate guest and the hydroxyls on the narrow rim of β-CD, versus those on the wide rim for conformations E and F. Energetically, geometry B is less stable than E whereas geometry C is more stable than F, although the energy differences are very small.

Table 3.5 Calculated and experimental gas-phase dissociation energies (kcal mol\(^{-1}\)) for formation of [CD]\(^-\) from complexes 4–6.

<table>
<thead>
<tr>
<th>Method</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>M06-2X//M06-L</td>
<td>33.0</td>
<td>36.3</td>
<td>44.5</td>
</tr>
<tr>
<td>Exp. (E_0)</td>
<td>40.2(5)</td>
<td>39.8(5)</td>
<td>38.9(4)</td>
</tr>
</tbody>
</table>

For the most stable geometries, the dissociation energies were reevaluated by single-point calculations with the M06-2X functional, including corrections for the basis set superposition error (BSSE), as listed in Table 3.5. The agreement between the experimental and theoretical results is poor for complexes 4–6, although they matched well for 1–3. This indicates that the theoretical calculations at this level cannot reproduce the experimental results to sufficient extent for such large CD complexes, at
least from the energetic point of view. Therefore, an unambiguous conclusion about the geometry of these CD complexes under study cannot be drawn from our results.

### 3.3 Conclusions

In summary, the anionic H–G complexes of α-CD with three benzoates were studied by CID threshold measurements to provide the first absolute gas-phase dissociation energies for large non-covalent H–G complexes. Our experimental results are well reproduced by DFT calculations at the M06-2X//M06-L level of theory with counterpoise corrections for the complexes of α-CD and 3-MeBA, BA, and 3-OHBA, respectively. The intermolecular O–H···O hydrogen bond interactions have been carefully assigned and quantified. However, the extension of this combinational approach to other CD complexes is not successful. This suggests that the aforementioned good agreement between experimental and theoretical results might be a coincidence. Thus, the geometry of CD complexes in the gas phase remains ambiguous. A systematic study to reveal the mysteries of CD complexes will be discussed in the next Chapter.

### 3.4 Experimental Section

#### 3.4.1 Materials

All chemicals were purchased from Aldrich (purity \( \geq 95\% \)) and were used without further purification. Stock solutions of CDs and guests were prepared in methanol/water (95:5 v/v) at a concentration of 1.0 mM. For the ESI–MS studies, equal amounts of CDs and guest-stock solutions were mixed and diluted to approximately 10 \( \mu \)M.
3.4.2 Theoretical Calculations

All calculations were carried out using the Gaussian 09 quantum mechanical package.\textsuperscript{86} For all the DFT calculations the 6-31+G(d,p) basis set was applied, which is a Pople-type basis set of double-zeta quality, augmented with one set of polarization functions on all elements and one set of diffuse functions on the non-hydrogen atoms. The H–G interaction energies were corrected for BSSEs as calculated with the counterpoise method (see Table 3.5 and 3.6).\textsuperscript{92}

**Table 3.5** Absolute energies (in Hartree) and BSSEs corrections for complexes $[\alpha$-CD·RBA]$^-$.  

<table>
<thead>
<tr>
<th>Species</th>
<th>$[\alpha$-CD·3-MeBA]$^-$</th>
<th>$[\alpha$-CD·BA]$^-$</th>
<th>$[\alpha$-CD·3-OHBA]$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLYP/6-31+G(d,p)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[\alpha$-CD]$^-$</td>
<td>$-3663.18531201$</td>
<td>$-3663.18531201$</td>
<td>$-3663.18531201$</td>
</tr>
<tr>
<td>RBA</td>
<td>$-460.014613308$</td>
<td>$-420.720582508$</td>
<td>$-495.941484822$</td>
</tr>
<tr>
<td>$\alpha$-CD</td>
<td>$-3663.71994112$</td>
<td>$-3663.71994112$</td>
<td>$-3663.71994112$</td>
</tr>
<tr>
<td>$[\text{RBA}]^-$</td>
<td>$-459.466215744$</td>
<td>$-420.173495220$</td>
<td>$-495.397040720$</td>
</tr>
<tr>
<td>$[\alpha$-CD·RBA]$^-$</td>
<td>$-4123.22502066$</td>
<td>$-4083.93146826$</td>
<td>$-4159.15394097$</td>
</tr>
<tr>
<td>$\alpha$-CD$^#$</td>
<td>$-3663.66570261$</td>
<td>$-3663.67042715$</td>
<td>$-3663.67006507$</td>
</tr>
<tr>
<td>$[\text{RBA}]^#$</td>
<td>$-459.464793865$</td>
<td>$-420.17023044$</td>
<td>$-495.393311103$</td>
</tr>
<tr>
<td>BSSE</td>
<td>$0.009318652034$</td>
<td>$0.008656210341$</td>
<td>$0.009867986480$</td>
</tr>
<tr>
<td>M06-L/6-31+G(d,p)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[\alpha$-CD]$^-$</td>
<td>$-3663.82007571$</td>
<td>$-3663.82007571$</td>
<td>$-3663.82007571$</td>
</tr>
<tr>
<td>RBA</td>
<td>$-460.115526056$</td>
<td>$-420.800846719$</td>
<td>$-496.021818825$</td>
</tr>
<tr>
<td>$\alpha$-CD</td>
<td>$-3664.36214988$</td>
<td>$-3664.36214988$</td>
<td>$-3664.36214988$</td>
</tr>
<tr>
<td>$[\text{RBA}]^-$</td>
<td>$-459.558313046$</td>
<td>$-420.244837865$</td>
<td>$-495.468115442$</td>
</tr>
<tr>
<td>$[\alpha$-CD·RBA]$^-$</td>
<td>$-4124.01719450$</td>
<td>$-4084.70035040$</td>
<td>$-4159.92752294$</td>
</tr>
<tr>
<td>$\alpha$-CD$^#$</td>
<td>$-3664.31109470$</td>
<td>$-3664.31669106$</td>
<td>$-3664.315252$</td>
</tr>
</tbody>
</table>

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Table 3.6 M06-2X//M06-L/6-31+G(d,p) absolute energies (in Hartree) and BSSE corrections, and hydrogen bond strengths (in kcal mol$^{-1}$) according to the host-exchange reaction.

<table>
<thead>
<tr>
<th>Species$^a$</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
<th>All OH groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>[α-CD-3-MeBA]$^-$</td>
<td>-4122.95148443</td>
<td>-4122.95148443</td>
<td>-4122.95148443</td>
<td>-4122.95148443</td>
</tr>
<tr>
<td>BSSE</td>
<td>0.01062506952</td>
<td>0.01062506952</td>
<td>0.01062506952</td>
<td>0.01062506952</td>
</tr>
<tr>
<td>Host$^a$</td>
<td>-3512.99436525</td>
<td>-3513.01025390</td>
<td>-3512.99436526</td>
<td>-3212.23648113</td>
</tr>
<tr>
<td>α-CD$^a$</td>
<td>-3663.38647011</td>
<td>-3663.38647011</td>
<td>-3663.38647011</td>
<td>-3663.38647011</td>
</tr>
</tbody>
</table>

$^a$ Entries marked with $^a$ use the geometries of host or guest in the corresponding H–G complex.
Quantitative Study of α-Cyclodextrin Complexes in the Gas Phase

<table>
<thead>
<tr>
<th>Complex</th>
<th>Host−</th>
<th>BSSE</th>
<th>H–G bonding diff.</th>
<th>BSSE</th>
<th>Host#</th>
<th>α-CD#</th>
<th>BSSE</th>
<th>H–G bonding diff.</th>
<th>BSSE</th>
<th>α-CD#</th>
<th>BSSE</th>
<th>H–G bonding diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Host·3-MeBA]−</td>
<td>−3972.52129989</td>
<td>−3972.54706069</td>
<td>−3972.54850882</td>
<td>−3671.72688061</td>
<td>0.00913123507</td>
<td>0.00902253961</td>
<td>0.01042333431</td>
<td>0.00731580590</td>
<td>0.00902253961</td>
<td>0.01042333431</td>
<td>0.00731580590</td>
<td>−23.0</td>
</tr>
<tr>
<td>[α-CD·BA]−</td>
<td>−4083.65178621</td>
<td>−4083.65178621</td>
<td>−4083.65178621</td>
<td>−4083.65178621</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>0.00993653840</td>
<td>−23.0</td>
</tr>
<tr>
<td>[Host·3-OHBA]−</td>
<td>−4158.86003652</td>
<td>−4158.86003652</td>
<td>−4158.86003652</td>
<td>−4158.86003652</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>0.01131288502</td>
<td>−20.9</td>
</tr>
</tbody>
</table>

Entries marked with ‡ use geometries derived from the corresponding [α-CD·RBA]− complex.

Figure 3.6 Overlaid structures of complexes 1 (green), 2 (blue), and 3 (red). Hydrogen atoms are omitted for clarity.
3.4.3 Threshold–CID Measurements

The determination of absolute gas-phase reaction barriers requires the reactant ions to be prepared with well-defined, narrow distributions in their kinetic and internal energies prior to collision. On the customized Finnigan MAT TSQ-700 mass spectrometer, this is accomplished by thermalizing the electrosprayed ions with 10 mTorr argon in a gas cell mounted on a radiofrequency 24-pole ion guide. Retardation measurements, in which the parent ion intensity is recorded as a function of the \( \text{dc} \) bias potential applied to the collision octapole in the absence of collision gas, afford well-defined near-Gaussian distributions of the ions’ kinetic (and presumably internal) energies (Figure 3.7 for 1) as required for subsequent deconvolution of the cross-section curves. For the CID threshold measurements, the parent ion was mass-selected in the first quadrupole and allowed to react with xenon in the octapole collision cell. Intensities of the reactant and product ions were recorded as a function of the collision offset voltage (Figure 3.8 for 1). These intensities were converted to reactive collision cross sections according to Ervin et al., extrapolated to zero collision-gas pressure to impose single-collision conditions, and fitted with L-CID. Three independent data sets were acquired on different days; the Figure is derived from data set 3. For each data set, at least 15 acceptable fits were selected to determine the confidence intervals of the fitting parameters, and the final values of the parameters were obtained as averages over the data sets. For an accurate treatment of the kinetic shift, one needs to distinguish between so-called “loose” and “tight” transition states. A loose transition-state (TS) model is appropriate for reactions in which the dissociation into products is rate limiting (i.e. a reverse activation barrier is absent); otherwise, a tight TS model should be used. Both loose and tight transition-state models were considered (Tables 3.7–3.9); DFT
calculations indicate that the rate-limiting transition state is loose for the fragmentation of \([\alpha\text{-CD}\cdot\text{RBA}]^-\) into \([\alpha\text{-CD}]^-\) and RBA. Using these arguments, we obtained benchmark values for the threshold dissociation energy \(E_0\) of 40.8(5), 41.1(5), and 41.8(5) kcal mol\(^{-1}\) for the complexes 1, 2, and 3, respectively.

**Figure 3.7** Distribution of uncorrected ion kinetic energies in the laboratory frame for \([\alpha\text{-CD}\cdot3\text{-MeBA}]^-\); the Gaussian fit (red line) has a full width at half-maximum of 1.15 eV.

**Figure 3.8** Ion-intensity curves for CID of \([\alpha\text{-CD}\cdot3\text{-MeBA}]^-\) with xenon as collision gas at different pressures (\(\mu\text{Torr}\)).
**Figure 3.9** Threshold reactive collision cross-section curves for the dissociation of \([\alpha-\text{CD}]^-\) from \([\alpha-\text{CD}\cdot3-\text{MeBA}]^-\) at various collision gas pressures in \(\mu\text{Torr}\), extrapolation to zero pressure, and fitted curve from L-CID assuming no reverse barrier.

**Table 3.7** Fitted parameters and standard deviations (in parentheses) for three independent data sets of the one-channel reactive cross-section curves for dissociation of \([\alpha-\text{CD}]^-\) from \([\alpha-\text{CD}\cdot3-\text{MeBA}]^-\), assuming either a tight or a loose transition-state model.

<table>
<thead>
<tr>
<th>Data set</th>
<th>(V_{cen}) (V)</th>
<th>(fwhm) (V)</th>
<th>(E_0) (eV)</th>
<th>(\nu_{eff}) (cm(^{-1})) (\alpha) (cm(^{-1}))</th>
<th>(E_0) (eV)</th>
<th>(\nu_{eff}) (cm(^{-1})) (\alpha) (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(-2.27)</td>
<td>1.28</td>
<td>1.715(27)</td>
<td>689(23) (5766(241))</td>
<td>1.144(17)</td>
<td>832(40) (272(120))</td>
</tr>
<tr>
<td>2</td>
<td>(-2.75)</td>
<td>1.20</td>
<td>1.792(17)</td>
<td>878(46) (5661(190))</td>
<td>1.216(18)</td>
<td>946(43) (257(82))</td>
</tr>
<tr>
<td>3</td>
<td>(-2.58)</td>
<td>0.97</td>
<td>1.800(23)</td>
<td>933(35) (5739(241))</td>
<td>1.205(23)</td>
<td>959(39) (258(138))</td>
</tr>
<tr>
<td>Average (1—3)</td>
<td></td>
<td></td>
<td>1.77(2)</td>
<td>8.3(4)(\times10^2) (5.7(2)(\times10^3)</td>
<td>1.19(2)</td>
<td>9.1(4)(\times10^2) (2.6(12)(\times10^2)</td>
</tr>
</tbody>
</table>
Table 3.8 Fitted parameters and standard deviations (in parentheses) for three independent data sets of the one-channel reactive cross-section curves for dissociation of $[\alpha\text{-CD}]^-$ from $[\alpha\text{-CD-BA}]^-$, assuming either a tight or a loose transition-state model.

<table>
<thead>
<tr>
<th>Data set</th>
<th>$V_{\text{cen}}$ (V)</th>
<th>fwhm (V)</th>
<th>$E_0$ (eV)</th>
<th>$\nu_{\text{eff}}$ (cm$^{-1}$)</th>
<th>$\alpha'$ (cm$^{-1}$)</th>
<th>$E_0$ (eV)</th>
<th>$\nu_{\text{eff}}$ (cm$^{-1}$)</th>
<th>$\alpha'$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2.58</td>
<td>1.15</td>
<td>1.725(30)</td>
<td>672(36)</td>
<td>5908(259)</td>
<td>1.121(20)</td>
<td>786(53)</td>
<td>230(110)</td>
</tr>
<tr>
<td>2</td>
<td>-2.75</td>
<td>1.63</td>
<td>1.806(14)</td>
<td>791(66)</td>
<td>5918(225)</td>
<td>1.213(19)</td>
<td>903(37)</td>
<td>255(100)</td>
</tr>
<tr>
<td>3</td>
<td>-2.69</td>
<td>1.08</td>
<td>1.816(18)</td>
<td>905(34)</td>
<td>5927(166)</td>
<td>1.245(14)</td>
<td>962(30)</td>
<td>288(87)</td>
</tr>
<tr>
<td>Average</td>
<td>(1—3)</td>
<td></td>
<td>1.78(2)</td>
<td>7.9(5)$\times10^2$</td>
<td>5.9(2)$\times10^3$</td>
<td>1.193(18)</td>
<td>8.8(4)$\times10^2$</td>
<td>2.6(10)$\times10^2$</td>
</tr>
</tbody>
</table>

Table 3.9 Fitted parameters and standard deviations (in parentheses) for three independent data sets of the one-channel reactive cross-section curves for dissociation of $[\alpha\text{-CD}]^-$ from $[\alpha\text{-CD-3-OHBA}]^-$, assuming either a tight or a loose transition-state model.

<table>
<thead>
<tr>
<th>Data set</th>
<th>$V_{\text{cen}}$ (V)</th>
<th>fwhm (V)</th>
<th>$E_0$ (eV)</th>
<th>$\nu_{\text{eff}}$ (cm$^{-1}$)</th>
<th>$\alpha'$ (cm$^{-1}$)</th>
<th>$E_0$ (eV)</th>
<th>$\nu_{\text{eff}}$ (cm$^{-1}$)</th>
<th>$\alpha'$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2.41</td>
<td>1.64</td>
<td>1.768(26)</td>
<td>656(44)</td>
<td>6155(234)</td>
<td>1.159(12)</td>
<td>800(49)</td>
<td>244(103)</td>
</tr>
<tr>
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<td>909(34)</td>
<td>5570(148)</td>
<td>1.283(21)</td>
<td>968(30)</td>
<td>291(108)</td>
</tr>
<tr>
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<td>-2.57</td>
<td>0.96</td>
<td>1.824(20)</td>
<td>814(40)</td>
<td>5922(170)</td>
<td>1.235(13)</td>
<td>922(37)</td>
<td>284(125)</td>
</tr>
<tr>
<td>Average</td>
<td>(1—3)</td>
<td></td>
<td>1.81(2)</td>
<td>7.9(4)$\times10^2$</td>
<td>5.9(19)$\times10^3$</td>
<td>1.226(16)</td>
<td>9.0(4)$\times10^2$</td>
<td>2.7(11)$\times10^2$</td>
</tr>
</tbody>
</table>
Chapter 4

Intrinsic Properties of Cyclodextrin Complexes in the Gas Phase
Series of collision induced dissociation experiments are carried out by ESI–MS/MS in order to find evidence for the existence of inclusion complexes in the gas phase. However, the results suggest that the gas-phase stability is neither affected by the size of either the guest or the host’s cavity, nor by the chirality of the guest. Furthermore, the results indicate that it is the ionic hydrogen bonding, which governed by the gas-phase basicity of both host and guest moieties, primarily stabilizes the non-covalent complexes in the gas phase. Additionally, the behavior of all the CD anionic complexes detected by ESI–MS is similar to that of electrostatic adducts such as [CD-Cl]⁻ or [CD-Br]⁻. Therefore, all these results strongly point to the formation of non-specific complexes in the gas phase. We further conclude that the solution-phase CD inclusion complexes likely dissociate during the ionization process, followed by the formation of non-specific complexes in the gas phase.
4.1 Introduction

Electrospray mass spectrometry (ESI–MS) has found wide application as a rapid and powerful analytical tool to study host–guest (H–G) complexes.\(^\text{65a,65c-e,65g,109}\) However, even as the specific mass-to-charge ratios \((m/z)\) of the detected species may serve to quickly determine their stoichiometries, these observations alone do not provide any structural information. Therefore, what still remains unclear is the extent of structural changes as a result of solvent removal during the electrospray ionization process, and the interactions that govern the stability of the desolvated species in the gas phase. In 1995, Cunniff and Vouros\(^\text{54}\) observed CD complexes with amino acids and peptides in the gas phase, even for guests which did not form inclusion complexes in solution. They therefore claimed that these adducts were merely bound by electrostatic interactions and cannot be assigned to specific inclusion complexes. Nevertheless, some recent studies on chiral selectivity in guest-exchange reactions have suggested that the CD–amino acid H–G complexes produced in solution maintain their inclusion structure even in the gas phase.\(^\text{55}\) As the tools available for gas-phase structural characterization are very limited, it remains difficult to resolve whether such non-covalent systems are specific complexes or not in the gas phase.

When analytes are transferred from solution to the gas phase, the conditions change dramatically. More precisely, all the solvent molecules, such as water, are removed in the gas phase. This would affect the binding affinity of non-covalent complexes. For example, the main impetus for the formation of CD inclusion complexes in solution is the hydrophobic effect,\(^\text{36a,36f}\) which originates from the gain in entropy of solvent
molecules that are released from the solvation shell upon complexation. Obviously, the
hydrophobic effect is absent in the gas phase.\textsuperscript{67} On the other hand, non-covalent
interactions that solvation is in competition with, such as hydrogen bonding and
electrostatic interactions, are significantly strengthened in the gas phase.\textsuperscript{66a,110} One
specific aspect of hydrogen bonds should be pointed out here, namely that both the bond
distance and bond angle affect the hydrogen bonding strength. Thus, the binding
partners preferably arrange themselves to form the strongest hydrogen bonding possible,
and then the non-specific complex would be favored thermodynamically since the guest
molecule has more freedom than in an inclusion complex.

Another major difference between solution and gas phase is the fact that the
equilibrium and thermodynamic properties have to be taken into consideration in
solution phase. In the gas phase, no equilibrium can occur between free host and guest
versus the stable H–G complex, because all like-charged ions in a mass spectrometer
repel each other and are separated in a high vacuum environment unless the conditions
necessary for collisions are deliberately and carefully set up. Moreover, in a mass
spectrometer any ions that kinetically survive the ionization process will be detected,
which is not restricted to the most stable conformer. Thus, any conclusions based on
gas-phase studies of H–G complexes should be taken with great caution considering the
abovementioned differences between solution and gas phase, and the specific conditions
used in such experiments.

Herein we present an ESI–MS study of $\alpha$- and $\beta$-CD complexes with a variety of
guests (Scheme 4.1) as a continuation of our research on the intrinsic properties of CD
complexes in the gas phase.\textsuperscript{96,111} The stabilities of gaseous complexes having different
sizes of both guest and cavity of the host are compared to test the concept of the size-
matching principle in the gas phase. The concept of chiral selectivity is also systematically investigated for CD–amino acid complexes. Additionally, series of experiments are performed to address the questions of which forces mainly contribute to or affect their stability, and whether these complexes retain their solution-state structures in the gas phase.

![Molecular structures of guests employed in this study.](image)

**Scheme 4.1** Molecular structures of guests employed in this study.

### 4.2 Results and Discussion

#### 4.2.1 The Size-Matching Principle in the Gas Phase

The size-matching principle is regarded as one of the most important prerequisites for the existence of inclusion complexes in the condensed state.\(^{36c,36e,36f}\) We previously reported a study of \(\alpha\)-CD complexes with several benzoate derivatives in the gas phase,\(^{96,111}\) but the difference in size between those guests was too small to test the size-
matching principle. Thus, several guests with makeable different sizes, including pentanoic acid (PA), benzoic acid (BA), and 1-admantanecarboxylic acid (AA), were chosen to address the question whether their CD complexes obey the size-matching principle in the gas phase.

Figure 4.1 Mass spectra and isotope patterns (insets) obtained for mixtures of β-CD and (a) PA, (b) BA, and (c) AA.

For the ESI–MS investigation of anionic H–G complexes, 1:5 mixtures of host and guest were prepared at approximately $10^{-5}$ mol L$^{-1}$ in methanol/water (95:5 v/v). Figure 4.1 shows the full ESI–MS spectra obtained for the mixtures of β-CD with PA, BA, and AA. The signals observed at mass-to-charge ratios $m/z$ 1236, 1256, and 1314
correspond to the expected 1:1 monoanionic complexes $[\beta$-CD·PA]$^-$, $[\beta$-CD·BA]$^-$, and $[\beta$-CD·AA]$^-$ as confirmed by their isotope patterns (insets in Figure 4.1). The complexes of 2:1 stoichiometry were also observable under milder ESI conditions, such as at lower tube lens voltages, analogous to reports of Kralj and co-workers.\textsuperscript{68} Conversely, harsher conditions, such as a higher tube lens voltage of –95 V, eliminate all the 2:1 complexes. As we liked to focus in this study on the 1:1 H–G complexes, a tube lens voltage of –95 V was applied in all experiments unless noted otherwise.

![Figure 4.2](image)

**Figure 4.2** Product mass spectra for collision-induced dissociation of $[\beta$-CD·RBA]$^-$ anionic complexes with 0.5 mTorr argon at 1.2 eV center-of-mass collision energy, including daughter-ion conversions $R = I_{[\text{daughter}]^{-}} / \Sigma I$.

In a mass spectrometer almost all processes occur under high-vacuum conditions, and one should be aware of the difference to chemical reactions in condensed phase.\textsuperscript{112} The mass-spectrometric intensities cannot be simply taken as quantitative indicators for solution concentrations of different analytes, because several factors, such as the desolvation efficiency, influence the ESI response.\textsuperscript{113} Thus, the study of the intrinsic...
properties of the analytes requires the use of gas-phase techniques, for example collision-induced dissociation (CID) experiments. To allow direct comparison, CID experiments for all anionic complexes were performed at the same collision energy in the center-of-mass frame. As shown in Figure 4.2, the deprotonated β-CD rather than the carboxylate anion was observed, as would have been expected based on their gas-phase basicities (GBs) since CID favors the formation of the anionic product with the lower GB. The apparent gas-phase stability (GS) of CD complexes can be defined by the fraction of daughter ions \((R)\) as we did before, affording a ranking of these anionic H–G complexes by increasing stability as follows: \([\beta\text{-CD} \cdot \text{PA}]^- (R = 0.44) < [\beta\text{-CD} \cdot \text{AA}]^- (R = 0.33) < [\beta\text{-CD} \cdot \text{BA}]^- (R = 0.30).

### Table 4.1 Average enthalpies of α- and β-CD aqueous complexes with FA, PA, BA, and AA, respectively, and gas-phase basicities (GB) of the guest anions.

<table>
<thead>
<tr>
<th></th>
<th>(-\Delta H) (kcal mol(^{-1}))</th>
<th>GB (kcal mol(^{-1})) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.7(^{105a})</td>
<td>338.5(^{72,115})</td>
</tr>
<tr>
<td>PA</td>
<td>7.1(^{116})</td>
<td>339.2(^{72,117})</td>
</tr>
<tr>
<td>BA</td>
<td>9.4(^{75,105})</td>
<td>333.0(^{72,115b,115c})</td>
</tr>
<tr>
<td>AA</td>
<td>3.7(^{106})</td>
<td>336.3(^{72,120})</td>
</tr>
<tr>
<td>β-CD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>N/A(^b)</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>3.6(^{116})</td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>4.4(^{75b,105b,116,118})</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>5.3(^{106b-d,119})</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Data were taken from the NIST website; \(^b\) Experimental data was not available.

The size-matching principle does not afford an explanation for the observed stability trend of β-CD complexes in the gas phase. It is obvious that the guest size follows the order PA < BA < AA, and all three guests are capable of penetrating into the cavity of β-CD in solution. From the thermodynamic point of view, the best-fitting guest to the
The cavity of $\beta$-CD is the spherical AA, followed by the planar BA, and least the linear PA. However, in our experiments we observe no correlation between the size of the guest and the GS of these complexes, even though the size-matching principle appears to work very well in solution. For example, as shown in Table 4.1, the average enthalpies of the aqueous $\beta$-CD inclusion complexes are $3.6$ (PA), $4.4$ (BA), $75b,105b,116,118$ and $5.3$ kcal mol$^{-1}$ (AA)$^{18b-d,19}$. To confirm that our experimental results are reliable under a large scale of collision energy, series of CID control experiments were carried out under the same conditions except for the collision offset, which was varied from $1.0$, $1.2$, $1.4$, to $1.6$ eV. As shown in Figure 4.3, the daughter-ion fraction grows with increasing collision offset, and the GS order of these three $\beta$-CD complexes is maintained throughout the experimental conditions.

**Figure 4.3** Daughter-ion fractions $R$ for carboxylate complexes of $\alpha$- and $\beta$-CD, derived from CID experiments at $0.5$ mTorr argon and several center-of-mass collision energies.

The size-matching principle cannot be applied to elucidate the stability trend of $\alpha$-CD complexes in the gas phase. In a similar fashion, daughter-ion fractions were derived from CID experiments for $\alpha$-CD anionic complexes at different collision energies. As
Intrinsic Properties of Cyclodextrin Complexes in the Gas Phase

can be seen in Figure 4.3, the GS trend of these anionic H–G complexes increases also in the order \([\alpha\text{-CD·PA}]^- < [\alpha\text{-CD·AA}]^- < [\alpha\text{-CD·BA}]^-\), as was the case for the \(\beta\text{-CD analogues. These gas-phase experimental findings are contradictory with solution-phase studies on \(\alpha\text{-CD inclusion complexes. For instance, the affinity or enthalpy (kcal mol}^{-1})\) of these aqueous complexes increases as follows: \([\alpha\text{-CD·AA}] (3.7^{106}) < [\alpha\text{-CD·PA}] (7.1^{116}) < [\alpha\text{-CD·BA}] (9.4^{75,105})\) (see also Table 4.1). Specifically, the stability order between \([\alpha\text{-CD·AA}]^-\) and \([\alpha\text{-CD·BA}]^-\) agrees with solution-phase studies, which shows that \(\alpha\text{-CD has a lower affinity for AA than for BA because the guest AA cannot be included in the relatively small cavity of } \alpha\text{-CD. The linear guest PA is capable of penetrating into the cavity of } \alpha\text{-CD, however, the GS of the complex } [\alpha\text{-CD·PA}]^-\) is smaller than that of \([\alpha\text{-CD·AA}]^-\).

Additionally, no large differences on the GS of these CD complexes are observed due to the change in cavity size (Figure 4.3), which is also in conflict with the results obtained in solution (Table 4.1). For instance, in solution, the complexation enthalpies of \(\alpha\text{-CD with PA and BA are larger than for the corresponding } \beta\text{-CD complexes, while the big guest AA fits better in the big cavity of } \beta\text{-CD than into the small cavity of } \alpha\text{-CD. On the other hand, in the gas phase, changing either the size of the guest or the cavity of the host hardly influences the GS of these CD complexes. Therefore, our results suggest that the GS trend of these CD complexes do not obey the size-matching principle, and imply that the van der Waals interactions between the hydrophobic part of the guest and the cavity of the host are not the dominant contribution to the GS of such complexes.}

4.2.2 Main Contributions to the Gas-Phase Stability

The negative results with regard to the importance of the size-matching principle in the
gas phase would be explained by the existence of non-specific complexes. Such non-
specific complexes will be formed between the carboxylate group and the exterior
hydroxyl groups of the CD through hydrogen bonding, which rules out any effect due to
the size of both cavity and guest. The main factor affecting the apparent GS of these
complexes would then be the GBs of host and guest. This hypothesis would also explain
the observed trends in GS of both α- and β-CD anionic complexes with different guests.
As we reported previously, the most stable complexes are formed between CD and
guest anions of most similar GB,96,111 and the GBs of deprotonated CDs are lower than
327 kcal mol\(^{-1}\).96 Our current experiments show a GS order of [CD·PA]\(^-\) < [CD·AA]\(^-\) <
[CD·BA]\(^-\) for both α- and β-CD anionic complexes, which can be rationalized
straightforwardly by the GB values (kcal mol\(^{-1}\)) of the respective guest anions,
following the order PA (339.2\(^{72,117}\)) > AA (336.3\(^{72,120}\)) > BA (333.0\(^{72,115b,115c}\)).

Under the same conditions, CID efficiency, which governs the appearance of CID
mass spectra, is determined not only by the intrinsic interaction between host and guest
moieties, but also by the collision cross section (CCS) or the size of the H–G complex.
The larger the complexes, the more collisions will occur during the CID process, and
hence the probability increases that sufficient activation energy will be supplied to
induce a chemical reaction. Actually, we did not observe any obvious effect of the size
of the guest on the collision cross section of the complexes, e.g. for AA vs BA or PA. It
might be that the differences are not large enough to change the GS order of these
complexes. Thus, the smallest carboxylic acid, formic acid (FA), was tested for the
guest’s size influence in the gas phase.
Intrinsic Properties of Cyclodextrin Complexes in the Gas Phase

Figure 4.4 Daughter-ion fractions $R$ for $[\alpha$-CD-carboxylate]$^-$ complexes derived from CID experiments at 0.5 mTorr argon and several center-of-mass collision energies.

As shown in Table 4.1, the GB of the FA anion is slightly lower than that of the PA anion, but bigger than that of both the AA and the BA anion. Thus, the GS of the $[\text{CD} \cdot \text{FA}]^-$ complex should be similar to that with PA, and smaller than those of the corresponding AA and BA complexes. However, this is not the fact according to our experimental results as shown in Figure 4.4, which clearly indicates that the stability of these complexes increases as follows: $[\alpha$-CD-PA]$^- < [\alpha$-CD-FA]$^- \approx [\alpha$-CD-AA]$^- < [\alpha$-CD-BA]$^-$. The CCS of the complexes under study may play an important role in this case. From FA to PA, the size of the guest changes dramatically from one to five carbon moieties. This explains why a larger daughter-ion fraction $R$ is observed in the CID experiments for the complex $[\alpha$-CD-PA]$^-$ than for $[\alpha$-CD-FA]$^-$ although the GBs of the FA and PA anions are similar (Figure 4.4). The CCS, and hence the collision efficiency decreases going from complex $[\alpha$-CD-AA]$^-$ to $[\alpha$-CD-FA]$^-$. On the other hand, the intrinsic interactions within the complex $[\alpha$-CD-AA]$^-$ are stronger than in $[\alpha$-CD-FA]$^-$ as governed by the GB values, which would compensate the effect of CCS on the CID
efficiency. As a result, the apparent GS of the complexes \([\alpha\text{-CD} \cdot \text{FA}]^-\) and \([\alpha\text{-CD} \cdot \text{AA}]^-\) are similar. The smaller GS of complex \([\alpha\text{-CD} \cdot \text{FA}]^-\) than \([\alpha\text{-CD} \cdot \text{BA}]^-\) can be illustrated by the large GB difference between FA and BA anions.

Similar results were obtained for the \(\beta\text{-CD}\) analogue (see the Experimental Section). This indicates that the intrinsic GS of these H–G complexes is governed by the hydrogen bonding between host and guest moieties, whose strength primarily depends on the GB values of both host and guest moieties. Size changes have no effect on the intrinsic GS of the complexes but merely influence the apparent stability quantified by \(R\) from the CID experiments. Furthermore, the similar properties of the CD complexes with AA, BA, PA and FA indicate that all of these are non-specific complexes in the gas phase.

4.2.3 Relationship between Gas-Phase Basicity and Stability

In order to estimate the relationship between GB and GS of these complexes, one needs to minimize the influence caused by the differences in CCS. Thus, an array of \(para\)-substituted benzoic acids (4-RBA, where \(R = \text{OMe}, \text{Me}, \text{F}, \text{H}, \text{Cl}, \text{CF}_3\)) was chosen as guests, which have almost identical CCS. Additionally, the substituent in the \(para\) position of BA is unlikely to have any steric effects on the binding in these complexes. The results shown in Figures 4.5 and 4.6 were obtained after optimizing the instrument settings for signal intensity in order to minimize experimental errors. For instance, a relatively high tube lens voltage causes significant dissociation of the CD complexes as we reported before,\(^9^6\) but it also improves their ionization efficiency. Thus, the tube lens voltage was set at \(-80\) and \(-90\) V for the study of \(\alpha\)- and \(\beta\)-CD complexes, respectively,
in order to obtain maximum parent ion intensity. The daughter ion intensity was increased by applying a higher collision gas pressure of 0.85 mTorr.

**Table 4.2** Gas-phase basicities and Hammett constants $\sigma$ of *para*-substituted benzoates.

<table>
<thead>
<tr>
<th>R</th>
<th>GB (kcal mol$^{-1}$)$^a$</th>
<th>$\sigma^{121}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMe</td>
<td>333.8$^{122}$</td>
<td>−0.27</td>
</tr>
<tr>
<td>Me</td>
<td>334.0$^{73}$</td>
<td>−0.17</td>
</tr>
<tr>
<td>F</td>
<td>330.0$^{73}$</td>
<td>0.06</td>
</tr>
<tr>
<td>Cl</td>
<td>328.5$^{73}$</td>
<td>0.23</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>325.2$^{123}$</td>
<td>0.54</td>
</tr>
</tbody>
</table>

$^a$ Data were taken from the NIST Web site: http://webbook.nist.gov/chemistry/.

**Figure 4.5** Hammett correlations for the proton-transfer reaction of complexes [α-CD·4-RBA]$^-$, using the daughter-ion fractions $R_R$ derived from CID experiments at 0.85 mTorr argon pressure and several center-of-mass collision energies.
As shown in Figure 4.5, a linear free-energy relationship with the Hammett $\sigma$ constant is found for the dissociation of $\alpha$-CD complexes. Apparently, the dissociation process of these CD complexes involves a proton transfer reaction. Standard gas-phase experiments, however, cannot probe the reaction thermodynamics rigorously due to the uncertainty in temperature and entropy under the given experimental conditions. Nevertheless, a widely accepted approximation introduced by Cooks assumes a linear free-energy relationship for the gas-phase ions.$^{124}$ Chen and co-workers have also observed a linear free-energy relationship in a gas-phase study of gold benzylidene complexes.$^{125}$ It is no great surprise that we find a linear correlation of the daughter-ion fractions with the Hammett $\sigma$ constants (Table 4.2), given the putative electrostatic nature of the binding in these CD complexes; similar results were obtained for the $\beta$-CD complexes as detailed in the experimental section. The measurements for all of the CD complexes with 4-RBA were repeated at collision energies in the center-of-mass frame of 1.0, 1.2, 1.4, and 1.6 eV to confirm that the observed trends are not simply coincidental. This finding also indicates that the proton transfer reaction for the dissociation of these CD complexes involves similar rate-determining step.$^{126}$

The electron-withdrawing ability of para substituents is expressed by their Hammett $\sigma$ constants, which are based on the solution-phase pKa values of the corresponding benzoic acids. Hence, these should in principle be directly correlated to the GBs of the respective conjugate bases RBA. For instance, a more strongly electron-withdrawing group would pull electron density away from the carboxylate group, leading to a smaller GB of the RBA anion, and vice versa. It was previously reported that a linear relationship exists between the GBs of 4-RBA and the Hammett $\sigma$ constants.$^{73,127}$ On the other hand, a stronger GS of the CD complexes is predicted with increasing similarity in
GB for host and guest anion species.\textsuperscript{70,96} In our case, the lower the GB of the guest, the higher the stability of the respective complex is. Thus, we correlated the GS of the H–G complexes to the GBs of the guests (Table 4.2) as plotted in Figure 4.6. Essentially linear relationships were found with regression coefficients $R^2$ larger than 0.98. One cannot guarantee that such a linear correlation will exist beyond the range of substituents studied, i.e. for other guests RBA with GBs below 325 or above 334 kcal mol$^{-1}$. Nevertheless, our previous conclusion that the intrinsic GS of the CD complexes observed in the gas phase is determined by the GBs of the binding moieties is confirmed, which also implies that these CD complexes are electrostatic adducts.

![Figure 4.6](image)

**Figure 4.6** Daughter-ion fractions ($R$) for [$\alpha$-CD·4-RBA]$^-$ complexes plotted against the guest’s gas-phase basicity.

### 4.2.4 Chiral Recognition in the Gas Phase

In an ongoing search for indications of the conformation of CD complexes in the gas phase, we tested the chiral recognition of CDs to amino acids by negative-mode ESI–MS experiments. All samples were prepared and analyzed as described before for the
[CD-RBA]$^-$ complexes, and the species of interest were observable with high signal intensity. A tube lens voltage of $-95$ V was used to suppress the 2:1 complexes. Figure 4.7 shows the full ESI–MS spectra obtained for the mixtures of α-CD with L-Alanine (L-Ala), L-Isoleucine (L-Ile), and L-Phenylalanine (L-Phe). The signals observed at mass-to-charge ratios $m/z$ 1060, 1102, and 1136 correspond to the expected anionic complexes $[\alpha\text{-CD} \cdot \text{L-Ala}]^-$, $[\alpha\text{-CD} \cdot \text{L-Ile}]^-$, and $[\alpha\text{-CD} \cdot \text{L-Phe}]^-$, respectively. The 1:1 stoichiometry of these complexes is confirmed by their isotope patterns (see insets in Figure 4.7). Subsequently, CID experiments were carried out under the same conditions as in other CID experiments by careful mass selection of the complexes of interest.

![Full mass spectra and isotope patterns (insets) obtained for mixtures of α-CD and L-Ala, L-Ile, and L-Phe.](image)

**Figure 4.7** Full mass spectra and isotope patterns (insets) obtained for mixtures of α-CD and (a) L-Ala, (b) L-Ile, and (c) L-Phe.

As shown in Figure 4.8, the apparent GS of these α-CD complexes with several L-amino acids increases as follows: $[\alpha\text{-CD} \cdot \text{L-Ile}]^- \leq [\alpha\text{-CD} \cdot \text{L-Ala}]^- < [\alpha\text{-CD} \cdot \text{L-Phe}]^-$. 91
Again, the apparent GS trend of these α-CD–amino acid complexes cannot simply be explained with the size-matching principle, although the most stable α-CD complex is that with the biggest guest, L-Phe. The comparable GS of complexes [α-CD·L-Ala]− and [α-CD·L-Ile]− is not consistent with the size-matching principle. Furthermore, Ala is unlikely to be encapsulated by, or would only form a very weak H–G complex with α-CD due to its highly hydrophilic character. In contrast, Ile does form relatively strong H–G inclusion complexes with α-CD in solution. In order to test the chiral recognition principle in the gas phase, CID experiments have also been carried out for the α-CD complexes with the corresponding D-amino acids (Figure 4.8). However, no distinct difference was observable for the GS of the complexes of L- and D-amino acid guests with α-CD. This implies that chiral recognition is insignificant in gas-phase anionic α-CD-amino acid complexes.

![Figure 4.8](image-url)

**Figure 4.8** Daughter-ion fractions $R$ for [α-CD·amino acid]− anionic complexes derived from CID experiments with 0.5 mTorr argon at several center-of-mass collision energies.
The GS trend of these anionic \( \alpha \)-CD–amino acid complexes can be rationalized with the same concept as we used for the carboxylate H–G complexes. Comparing the alkyl substitution in the amino acids Ala, Ile, and Phe, the benzyl group in the latter is more capable of accommodating electron density. Consequently, the negative charge on the carboxylate group would be reduced more significantly for deprotonated Phe than for Ala and Ile. Thus the GB of Phe anion would be smaller than for Ala and Ile, which is in reasonable agreement with the higher apparent GS of \([\alpha\text{-CD-Phe}]^-\) than of the analogous Ala and Ile complexes. The similar GS of complexes \([\alpha\text{-CD-Ala}]^-\) and \([\alpha\text{-CD-Ile}]^-\) can be interpreted by the compensation of the effect caused by the difference in GB and CCS. Therefore, the GS of these CD–amino acid complexes is determined primarily by the GBs of both host and guest, even though the guests are remarkably different in shape, size, and chirality. These experimental results are at odds with the solution-phase behavior of the inclusion complexes, and suggest that the complexes observed in the gas phase are non-specific.

4.2.5 Complex Formation from Solution to the Gas Phase

Lebrilla and co-workers have concluded that the complexes they observed in the gas phase were intact inclusion complexes that had been formed in solution.\(^{55}\) After failing to find indications for size matching and chiral recognition for these CD complexes in the gas phase, we started considering the transfer of the CD complexes from solution into the gas phase. A series of control experiments were carried out in order to address whether these are inclusion complexes formed in solution and transferred to the gas phase intact, or whether they are merely electrostatic adducts formed during the ionization process. In short, the host \( \alpha \)-CD was kept at a fixed concentration of \(5 \times 10^{-6} \text{ M}\).
for all samples, while 1, 3, 10, or 30 equivalents of the guest BA or AA were used. All of these samples were subjected to an ESI–MS study in order to find a relationship between the complex’s concentration in solution and its signal intensity in the gas phase.

Table 4.3  ESI–MS intensities of complex ion at different host:guest ratios.

<table>
<thead>
<tr>
<th>Equiv. guest</th>
<th>Signal intensity ($\times 10^4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[\alpha$-CD·BA]$^-$</td>
</tr>
<tr>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td>10</td>
<td>24.6</td>
</tr>
<tr>
<td>30</td>
<td>18.6</td>
</tr>
</tbody>
</table>

A similar behaviour was observed for in the case of $\alpha$-CD and AA mixtures, in which no inclusion complex of $\alpha$-CD and AA forms. As shown in Table 4.3, the ion intensities of complexes $[\alpha$-CD·BA]$^-$ and $[\alpha$-CD·AA]$^-$ increase up to sixfold and threefold, respectively, when the host/guest concentration ratio is increased from 1:1 to 1:10. Only when 30 equivalents of guest were used did the signal intensity decrease moderately, which is a common phenomenon for too concentrated analyte solutions that will generally suppress the ionization process. According to the ion evaporation model, the benzoate anions desolvate by direct evaporation from the surface of highly charged micro-droplets. During this evaporation process, some of the isolated guest anions may engage with neutral hosts to form stable electrostatic adducts with multiple hydrogen bonds in the gas phase. At a higher concentration of the guest in solution, the probability for this recombination is higher and therefore a larger amount of non-
specific complexes would be detected by mass spectrometry, which agrees well with the results of our experiments. Thus, we believe that in solution the hydrophobic effect stabilizes inclusion complexes, such as CD H–G complexes, which however dissociate during the ionization process. Therefore, our results suggest that the complexes detected by ESI–MS are non-specific, and their intensity is determined by the amount of guest and host, not by the H–G complexes present in solution.

4.2.6 Non-Specific Complexes in the Gas Phase

If the gas-phase CD H–G complexes are non-specific, then adducts of any ions that are able to form strong electrostatic interactions or hydrogen bonding with CD should be observable in the gas phase, such as the CD–Na⁺ electrostatic adduct reported by Cunniff and Vouros. Likewise, anions such as fluoride, chloride, or bromide have been reported to be capable of attaching to glucose, and can also form hydrogen bonded adducts with CDs in the gas phase. In fact, the non-specific complexes \([\alpha-CD\cdotCl]^- (m/z 1007.5), [\alpha-CD\cdotBr]^- (m/z 1052), [\beta-CD\cdotCl]^- (m/z 1169.5), \) and \([\beta-CD\cdotBr]^- (m/z 1214)\) are readily detectable from mixtures of \(\alpha\)- or \(\beta\)-CD with NaCl or KBr, and their unique isotope patterns are shown in Figure 4.9. It should be noted that these anions would be solvated and surrounded by polar solvent molecules such as water, instead of binding to the CD in solution. Additionally, the complexes cannot adopt an inclusion conformation in either solution or gas phase. Therefore, it is reasonable to propose that the observed complexes are the result of the relatively strong hydrogen bonding between the negatively charged halide and the hydroxyl groups in the CD.
Intrinsic Properties of Cyclodextrin Complexes in the Gas Phase

Figure 4.9 Isotope patterns (insets) of [CD-halide]− complexes and product mass spectra for CID with 0.5 mTorr argon at 1.6 eV center-of-mass collision energy, including daughter-ion fractions $R$.

The CID mass spectra shown in Figure 4.9 indicate that the apparent stability for these non-specific complexes is also determined by the GBs of host and guest moieties. Namely, given that the GBs of chloride and bromide are 328.1$^{115h,131}$ and 318.3 kcal mol$^{-1}$,132 respectively, the complexes of CDs with chloride should be generally less stable than the bromide complexes. Indeed, the GS of these complexes follow the trend: $[\alpha$-CD-Cl]$^- \approx [\beta$-CD-Cl]$^- < [\alpha$-CD-Br]$^- \approx [\beta$-CD-Br]$^-$. Furthermore, the complex $[\alpha$-
CD·Cl$^-$ is more stable than $[\alpha$-CD·Phe]$^-$ although the GB values of these two guests are similar, which can be explained by the fact that the CCS of complex $[\alpha$-CD·Cl]$^-$ is much smaller than that of $[\alpha$-CD·Phe]$^-$. Therefore, the same principles which have been used to rationalize the experimental results for the CD complexes with carboxylate derivatives and amino acids are also valid for the CD–halide complexes, which indicates that all of these gas-phase CD complexes are merely electrostatic adducts.

To the best of our knowledge, all reported gas-phase CD complexes have in common that the guest possesses at least one polar group, which is able to form either a negatively charged carboxylate group or a positively charged ammonium group. One could argue that the charge is a prerequisite for the detection of such complexes by mass spectrometry. In the preliminary screening, we synthesized several charged CDs, such as mono-6-(1-butyl-3-imidazolium)-6-deoxy-β-cyclodextrin chloride and mono-6-(p-picolinyl)-6-deoxy-β-cyclodextrin chloride, and tried to find stable CD complexes with neutral apolar guests. Not surprisingly, no 1:1 complexes of charged CDs and guest could be observed in the gas phase. Apparently, H–G complexes only stabilized by van der Waals interactions do not survive the ionization process. Notably, no water adducts with host, guest, or H–G complexes were observed by ESI–MS, either. Probably more than ten water molecules form relatively strong hydrogen bonds to host and guest molecule in solution, but all of them are removed during the ionization process. Thus, also merely neutral hydrogen bonding is too weak to withstand the ionization conditions, i.e. the description of ESI as a “soft” ionization technique should not be overvalued. All in all, only H–G complexes stabilized by strong hydrogen bonding or electrostatic interactions have been observed in the gas phase. Furthermore, Qinyuan and co-workers studied the non-covalent carbonic anhydrase-inhibitor
complexes in the gas phase, and reported that the GS of these complexes was primarily
determined by polar surface interactions.\textsuperscript{134} The study of protein–ligand complexes in
the gas phase also highlights the intrinsic stability of electrostatic interactions.\textsuperscript{66b}
Additionally, we found that the GS of CD complexes is determined by ionic hydrogen
bonding between host and guest moieties, and both size matching and chiral recognition
are insignificant in the gas phase. Therefore, in light of our experimental results it seems
reasonable to presume that any deprotonated anions, including substituted carboxylates,
may afford electrostatic complexes with CDs in the gas phase, analogous to chloride or
bromide.

As far as we know, only two strong indications have been reported for the existence
of inclusion complexes in the gas phase. One is the observation by Lebrilla and co-
workers\textsuperscript{55} that the chiral selectivity in guest-exchange reactions is affected by the size of
both the guest and the cavity of the CD host. Probably, kinetically generated non-
specific complexes are converted to the more stable inclusion complexes upon
thermalization by collisions with surrounding guest molecules before the
characterization and detection. The fact that the gas-phase stabilities of α-CD complexes
are higher than those of the corresponding maltohexaose complexes has been taken as
another indication for the existence of inclusion complexes in the gas phase.\textsuperscript{56} However,
our calculations have shown that multiple hydrogen bonds are formed between the guest
and hydroxyl groups on the rim of α-CD even in the non-specific complexes.\textsuperscript{96,111}
Maltohexaose is a linear analogue of α-CD and incapable of forming inclusion
complexes in solution. It cannot form as many hydrogen bonds with the guest due to its
more linear geometry. This implies that the stabilities of the maltohexaose complexes
ought to be lower than those of the corresponding α-CD complexes anyways. Thus, the
observed stabilities are not unambiguous evidence for the existence of inclusion complexes in the gas phase. It should be pointed out here that we do not claim that all reported gas-phase CD complexes are non-specific. Rather, we would like to encourage a systematic investigation by gas-phase studies on each class of non-covalent complexes detectable by mass spectrometer.

4.3 Conclusions

The results presented here show that size matching and chiral recognition are not significant in the gas phase for the studied CD anionic complexes, even though these aspects are the most important for predicting the stability of inclusion complexes in solution. Only the similarity in GB of host and guest appears to affect the intrinsic stability of these gas-phase CD complexes, which is consistent with ionic hydrogen bonding being the principal H–G interaction. The size of the guest merely affects the CCS, which has an influence on the apparent GS as quantified by the fraction of daughter ions produced under predefined CID conditions. Additionally, the results suggest that all the anionic CD complexes detected by ESI–MS behave similarly, including gas-phase adducts [CD·Cl]− and [CD·Br]−. Therefore, the results point against the existence of inclusion complexes in the gas phase, and strongly suggest the formation of non-specific complexes. We are inclined to believe that the inclusion complexes in solution dissociate during the ionization process, upon which the non-specific complexes are formed, which are stabilized primarily by ionic hydrogen bonding. Our results shed light on the intrinsic stabilization by ionic hydrogen bonding and electrostatic interactions in the gas phase, and the significant differences in solution
and the gas phase for non-covalent complexes with respect to the properties of geometry and intrinsic interaction.

### 4.4 Experimental Section

#### 4.4.1 Materials

All chemicals were purchased from Aldrich (purity $\geq 95\%$) and were used without further purification. Stock solutions of $\alpha$- and $\beta$-CD and all the studied guests were prepared in methanol/water (95:5 v/v) at a concentration of 1.0 mM. For the ESI–MS studies, CDs and RBA stock solutions were mixed at a 1:5 ratio and diluted to approximately 10 $\mu$M.

#### 4.4.2 Mass Spectrometry

All mass spectrometric experiments were performed on a Finnigan MAT TSQ-7000 triple-stage mass spectrometer equipped with a microspray source. No desolvation or nebulization gas was applied. The experimental parameters were kept as similar as possible to maintain comparable conditions for all samples. The ESI parameters were set at an infusion rate of 1–2 $\mu$L min$^{-1}$, a spray voltage of 1.6–1.7 kV on the needle, the heated capillary at 150 °C and at $-50$ V, and the tube lens at $-95$ V. The collision-induced dissociation (CID) spectra were obtained by mass-selecting the desired non-covalent complex anions in the first quadrupole, fragmenting them in the collision octapole, and recording the product anions by scanning the second quadrupole. Argon was used as the collision gas at a pressure of 0.85 mTorr. In order to accurately maintain the collision gas pressure and therefore get better reproducibility of the CID
experiments, a sensitive Pfeiffer Vacuum IMR 265 hot cathode/Pirani gauge combination was connected to the CID chamber. The lab-frame collision offsets $V_{\text{coll}}$ were set accordingly for all complexes studied to obtain the same energies in the center-of-mass frame, $E_{\text{CM}}$.

**Figure 4.10** Mass spectra and isotope patterns (insets) obtained for mixtures of $\alpha$-CD and a) PA, b) BA, and c) AA.
Figure 4.11 CID product mass spectra for \([\alpha\text{-CD·RBA}]^−\) anionic complexes at 0.5 mTorr argon and 1.2 eV center-of-mass collision energy.

Figure 4.12 Daughter-ion fractions \(R\) for \([\beta\text{-CD·amino acid}]^−\) anionic complexes derived from CID experiments with 0.5 mTorr argon at several center-of-mass collision energies.
Figure 4.13 Hammett correlations for the proton-transfer reaction of complexes $[\beta$-CD·4-RBA]$^-$, using the daughter-ion fractions $R_R$ derived from CID experiments at 0.85 mTorr argon pressure and several center-of-mass collision energies.

Figure 4.14 Daughter-ion fractions $R$ for $[\beta$-CD·4-RBA]$^-$ complexes plotted against the guest’s gas-phase basicity.
Chapter 5

Intrinsic Properties of Cyclodextrins in the Gas Phase
Using the bracketing method, the gas-phase basicities (GBs) of [α-CD]− and [β-CD]− are found to be in between those of [4-CF3BA]− (325.3 kcal mol−1) and [2,6-diOHBA]− (307.4 kcal mol−1). This is in agreement with GB values that are determined by the extended kinetic method to be 321.7 ± 10.4 and 320.1 ± 6.1 kcal mol−1 for [α-CD]− and [β-CD]−, respectively. Complementary DFT calculations afforded the alcohol-alcohol (A-A) conformation as the global energy minimum for both α- and β-CD. The hydroxyl groups on the narrow rim are indicated to be most prone to deprotonation. Additionally, the calculated GB values of 325.7 kcal mol−1 for [α-CD]− and 321.0 kcal mol−1 for [β-CD]− reproduce the experimental results. These results provide an important set of benchmarks, relevant to the gas-phase chemistry of CDs.

Manuscript in preparation
5.1 Introduction

We recently reported that the gas-phase stabilities (GSs) of $\alpha$-CD complexes correlate with the gas-phase basicities (GBs) of the guest anions used.\(^{96,135}\) Similarly, the GS of different CD complexes with the same guest is expected to depend on the GB of the CD anions themselves. To the best of our knowledge, studies on the thermodynamic properties, such as GB, of isolated CD anions are rare. We previously reported an estimated GB of 325–327 kcal mol\(^{-1}\) for $[\alpha$-CD]$^-$, as obtained from the combination of experimental and theoretical results.\(^{96}\) Furthermore, although there are a few reports on the low-energy conformations of neutral CDs,\(^{77a,87}\) a systematic structural investigation of both neutral and anionic CDs is highly desirable to explore the conformations of their host–guest (H–G) complexes. This suggests the need for a study on the intrinsic properties of CDs themselves to provide a deep understanding of the intrinsic properties of these complexes.

GBs are commonly determined experimentally by equilibrium measurements, by reaction bracketing, or by the kinetic method.\(^{79b,136}\) Equilibrium measurements are obviously restricted to volatile compounds and therefore cannot be applied to CDs. For hydrogen-bonded acid–base adducts, the bracketing method can afford GBs within a relatively narrow range depending on the availability of appropriate reference bases.\(^{137}\) The kinetic method, developed by Cooks and co-workers,\(^{117,138}\) is widely used for the determination of GBs in the gas phase due to the operational simplicity and its applicability with most commercially available tandem mass spectrometers.
Herein we present an electrospray ionization tandem mass spectrometric (ESI–MS/MS) study of CD complexes with substituted benzoates [RBA]− to address the intrinsic properties of CDs. Specifically, both the bracketing and the kinetic method were applied to determine the GBs of α- and β-CD anions. The benzoates [RBA]− were chosen as reference bases because various benzoic acid derivatives are commercially available for which GBs have been reported, and because complexes of CDs with carboxylate functionalized guests are detectable by ESI–MS. The experimental study is complemented by density functional theory (DFT) calculations, which allows for a direct comparison as they mimic the conditions in the high-vacuum environment of a mass spectrometer. Furthermore, the calculations provide structural indications, which cannot be addressed by ESI–MS/MS.

5.2 Results and Discussion

5.2.1 Stoichiometry of CD Complexes

For the ESI–MS/MS investigation of anionic H–G complexes of α- and β-CD with benzoic acid derivatives RBA, 1:5 mixtures of host and guest were prepared of approximately 10 μM in methanol/water (95:5 v/v). The full ESI–MS spectra obtained for the mixtures of α- and β-CD with 2-OHBA are exemplary for all H–G systems studied herein (Figure 5.1). The signals observed at mass-to-charge ratios m/z 1109.3 and 1271.4 correspond to the anionic complexes [α-CD·2-OHBA]− and [βCD·2-OHBA]−, respectively. However, as Kralj and co-workers reported recently, such signals may stem not only from the monomer but can also have a contribution from doubly charged dimeric cluster ions, [(CD·RBA),]2−. The high-resolution capabilities of
the used ESI-Qq-TOF-MS instrument enabled us to unambiguously determine the stoichiometry of the gas-phase species. α-CD formed exclusively the 1:1 monomeric complex \([\alpha\text{-CD·2-OHBA}]^-\) as indicated by the unit mass-spaced isotope pattern of the species at \(m/z\) 1109.3 (see inset in Figure 5.1a). Conversely, for the analogous β-CD complex the signal at \(m/z\) 1271.4 is in part due to dimeric cluster ions \([(\beta\text{-CD·2-OHBA})_2]^2^-\) according to the half-unit mass-spaced isotope pattern (see inset Figure 5.1b).

![Figure 5.1](image)

**Figure 5.1** Full mass spectra and isotope patterns (insets) obtained from mixtures of 2-OHBA with a) α-CD and b) β-CD.

The presence of such dimeric species would preclude the determination of the GB of \([\beta\text{-CD}]^-\). By increasing the in-source collision energy from 40 to 95 V, the β-CD dimeric complex vanished completely while the intensity for the 1:1 H–G ion remained high (Figure 5.2). Comparing the contribution of dimeric ions \([(\beta\text{-CD·2-OHBA})_2]^2^-\) to the signal of 1271.4 in parent-mode (Figure 5.1b) and daughter-mode (Figure 5.2a), about 20% of the dimeric ions \([(\beta\text{-CD·2-OHBA})_2]^2^-\) were fragmented relative to
monomeric ions by applying an in-source collision energy of 40 V. This suggests that the dimeric ions [(β-CD·2-OHBA)_2]^2− are less stable than the 1:1 H–G ions [β-CD·2-OHBA]^−. It is reasonable to assume that the interactions between β-CD molecules should be weaker than the interactions within the H–G complexes, because electrostatic interactions and multiple O–H···O hydrogen bonding all contribute to the stability of the latter complexes.96 One possible explanation for the absence of dimeric complex [(α-CD·2-OHBA)_2]^2− under the same conditions is that the β-CD dimeric complex is more stable than the α-CD dimeric complex. Actually, dimeric α-CD complexes have been detected under milder conditions.68 The same in-source collision energy of 95 V was applied for all other studied CD complexes to ensure comparability of the results.

**Figure 5.2** Experimental daughter-mode mass spectra and isotope patterns (insets) obtained by selecting the ions of interest at m/z 1271.4 without collision gas, but with in-source collision energy at a) 40 V and b) 95 V.
5.2.2 Bracketing Method for Gas-Phase Basicity Determination

The fragmentation of hydrogen-bonded adducts \([A\cdots H\cdots B_i]^-\) in a mass spectrometer leads to the formation of the anionic species with the lower GB (Scheme 5.1); when the GBs of \([A]^-\) and \([B_i]^-\) are comparable, both fragments are observed. Thus, the unknown GB of anion \([A]^-\) can be bracketed by CID experiments with adducts of a suitable series of reference bases \([B_i]^-\) with known GB. The use of a series of reference bases with small differences in GB allows the GB value for the analyte to be bracketed within a narrow range.

\[
\begin{align*}
[A\cdots H\cdots B_i]^- & \xrightarrow{k_a} [A]^- + HB_i & \text{GB}([A]^-) << \text{GB}([B_i]^-) \quad (a) \\
& \xrightarrow{k_b} AH + [B_i]^- & \text{GB}([A]^-) >> \text{GB}([B_i]^-) \quad (b)
\end{align*}
\]

**Scheme 5.1** Possible dissociation pathways of hydrogen-bonded adduct \([A\cdots H\cdots B_i]^-\).

Figure 5.3 shows the CID product spectra for the anionic complexes of \(\alpha\)-CD with 4-CF\(_3\)BA, 2-OHBA, and 2,6-diOHBA, whose GBs are listed in Table 5.1. The signals observed at \(m/z\) 1161.3, 1109.3, and 1125.3 correspond to the respective 1:1 complexes exclusively, as is confirmed by the isotope patterns (insets in Figure 5.3). With decreasing GB of the benzoate derivative, the observed CID reactivity of the complex changes from exclusive loss of \([\alpha\text{-CD}]^-\) (path (a) in Scheme 5.1) to loss of \([RBA]^-\) (path (b)). Thus, the GB of \([\alpha\text{-CD}]^-\) is bracketed between those of \([2,6\text{-diOHBA}]^-\) (307.4 kcal mol\(^{-1}\))\(^{139}\) and \([4\text{-CF}_3\text{BA}]^-\) (325.3 kcal mol\(^{-1}\))\(^{73}\) and is very close to that of \([2\text{-OHBA}]^-\) (317.8 kcal mol\(^{-1}\)).\(^{73}\) Similarly, the GB of \([\beta\text{-CD}]^-\) was determined to be in the same range as for \([\alpha\text{-CD}]^-\) (see the Experimental Section). Apparently, deprotonated
CDs have lower GBs than deprotonated $\alpha$-D-glucose, whose GB has been determined by the bracketing method to be in the range of 328.2–336.3 kcal mol$^{-1}$.\textsuperscript{70}

**Figure 5.3** CID mass spectra and isotope patterns (insets) obtained for complexes of $\alpha$-CD and a) 4-CF$_3$BA, b) 2-OHBA, and c) 2,6-diOHBA at a collision energy $E_{\text{CM}} = 0.7$ eV.


Table 5.1 Gas-phase basicities (GBs) and proton affinities (PAs) (kcal mol\(^{-1}\)) of substituted benzoates.

<table>
<thead>
<tr>
<th>Benzoate</th>
<th>GB(^a)</th>
<th>PA(^a)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Trifluoromethylbenzoate, ([4-\text{CF}_3\text{BA}]^\text{−})</td>
<td>325.3</td>
<td>332.3</td>
<td>140</td>
</tr>
<tr>
<td>4-Cyanobenzoate, ([4-\text{CNBA}]^\text{−})</td>
<td>320.8</td>
<td>327.8</td>
<td>73</td>
</tr>
<tr>
<td>4-Nitrobenzoate, ([4-\text{NO}_2\text{BA}]^\text{−})</td>
<td>320.0</td>
<td>327.0</td>
<td>73</td>
</tr>
<tr>
<td>2-Hydroxybenzoate, ([2-\text{OHBA}]^\text{−})</td>
<td>317.8</td>
<td>325.5</td>
<td>73</td>
</tr>
<tr>
<td>2,6-Dihydroxybenzoate, ([2,6-\text{diOHBA}]^\text{−})</td>
<td>307.4</td>
<td>314.3</td>
<td>139</td>
</tr>
</tbody>
</table>

\(^a\) Data was taken from the NIST website.

5.2.3 Extended Kinetic Method for Gas-Phase Basicity Determination

For hydrogen-bonded acid-base adducts \([A\cdots\text{H}\cdots\text{B}_i]^\text{−}\) both dissociation pathways in Scheme 5.1 occur competitively when the analyte \([A]^\text{−}\) and the reference base \([B_i]^\text{−}\) have similar GBs. In this case, the GB of \([A]^\text{−}\) can be determined with the extended kinetic method, which is based upon the thermodynamic formulation of transition state theory, involving approximations and assumptions that have been discussed extensively\(^{141}\) and result in equations (5.1) and (5.2):

\[
\ln\left(\frac{k_1}{k_2}\right) = \ln\left(\frac{I_{[B_i]^\text{−}}}{I_{[A]^\text{−}}}\right) \approx \frac{[\text{PA}([A]^\text{−}) - \text{PA}([B_i]^\text{−})]}{RT_{\text{eff}}} - \Delta\Delta S^\ddagger / R \tag{5.1}
\]

\[
\text{GB}_{\text{app}}([A]^\text{−}) = \text{PA}([A]^\text{−}) - T_{\text{eff}} \Delta\Delta S^\ddagger \tag{5.2}
\]

where \(I_{[A]^\text{−}}\) and \(I_{[B_i]^\text{−}}\) are the observed product intensities, and \(\text{PA}([A]^\text{−})\) and \(\text{PA}([B_i]^\text{−})\) are the corresponding proton affinities. The effective temperature \(T_{\text{eff}}\) reflects the rovibrational excitation of the hydrogen-bonded adduct undergoing dissociation, and \(\Delta\Delta S^\ddagger\) is the difference in entropies of activation for the two dissociation pathways. The
value of the apparent gas-phase basicity $G_{\text{app}}$ as defined by equation (5.2) is determined for a given $T_{\text{eff}}$ value, and differs from the true $G_B$ of the analyte. The extended kinetic method requires multiple measurements under different experimental conditions using several reference bases that are structurally similar, in which case the entropy term $\Delta \Delta S^\ddagger$ can be assumed to be constant and $PA([A^-])$ and $T_{\text{eff}}$ can be determined by regression analysis.

Figure 5.4 CID mass spectra and isotope patterns (insets) obtained for complexes of $\alpha$-CD with a) 4-CNBA, b) 4-NO$_2$BA, and c) 2-OHBA at a collision energy $E_{\text{CM}} = 0.9$ eV.

Experiments were performed using $\alpha$- and $\beta$-CD complexes with a series of reference bases RBA. Figure 5.4 shows the CID experiments for three $\alpha$-CD anionic complexes with structurally similar RBA for which both dissociation pathways (a) and
(b) occurred competitively. The signals observed at m/z 1118.3, 1138.3, and 1109.3 correspond to the expected anionic complexes \([\alpha\text{-CD}\cdot 4\text{-CNBA}]^-\), \([\alpha\text{-CD}\cdot 4\text{-NO}_2\text{BA}]^-\), and \([\alpha\text{-CD}\cdot 2\text{-OHBA}]^-\), respectively. Their 1:1 stoichiometries were confirmed by their isotope patterns (see insets in Figure 5.4). Furthermore, similar results were obtained for \(\beta\text{-CD}\) anionic complexes with these three RBA molecules (see the Experimental Section).

![Graph](image)

**Figure 5.5** Extended kinetic method for determination of the PA and entropy change \((\Delta\Delta S^\ddagger)\) for \([\alpha\text{-CD}]^-\). Plot (a) was obtained for PA of three RBAs \([4\text{-CNBA}]^-\), \([4\text{-NO}_2\text{BA}]^-\), and \([2\text{-OHBA}]^-\) versus \(\ln([A^-]/[B_i^-])\) at different collision energy; Plot (b) was obtained for \(\frac{G_{\text{app}}}{RT_{\text{eff}}}\) versus \(\frac{1}{RT_{\text{eff}}}\) at different \(T_{\text{eff}}\).

CID product mass spectra of \(\alpha\)- and \(\beta\)-CD complexes with these reference bases were acquired at five different collision energies \(E_{\text{CM}} = 0.7, 0.8, 0.9, 1.0,\) and 1.1 eV.
The data analysis of the extended kinetic method was done by considering two plots: firstly, $\ln([A]/[B])$ was plotted against $PA(B)$, from which the slopes $1/RT_{eff}$ and the intercept $GB_{app}$ were deduced (Figure 5.5a); secondly, plotting $GB_{app}/RT_{eff}$ versus $1/RT_{eff}$ afforded the enthalpic and entropic quantities $PA$ and $\Delta\Delta S^\ddagger$ of the analyte. The $PA$ of the analyte was obtained from the slope of the second plot, whereas $\Delta\Delta S^\ddagger$ of the analyte was estimated from the y-intercept (Figure 5.5b).138,141d,141g,142

The current data analysis method gives good results, for instance, the values for $PA(\alpha-CD)$ of $317.1 \pm 9.0$ kcal mol$^{-1}$ and for $\Delta\Delta S^\ddagger(\alpha-CD)$ of $-15.3 \pm 17.4$ cal mol$^{-1}$ K$^{-1}$ were obtained with a correlation coefficient $R^2 = 0.998$ (Figure 5.5). Likewise, the values for $PA(\beta-CD)$ of $318.1 \pm 5.2$ kcal mol$^{-1}$ and for $\Delta\Delta S^\ddagger(\beta-CD)$ of $-6.6 \pm 10.5$ cal mol$^{-1}$ K$^{-1}$ were obtained (see the Experimental Section). It has been shown by Bouchoux that the GBs measured by the extended kinetic method are close to the “true” GBs as obtained with the equilibrium method.143 Thus, the GB values for $\alpha$- and $\beta$-CD of $321.7 \pm 10.4$ and $320.1 \pm 6.1$ kcal mol$^{-1}$, respectively, as determined by the extended kinetic method are used throughout the discussion below.

The GB values of $\alpha$- and $\beta$-CD from the extended kinetic method are in between those of $[4-CF_3BA]$ (325.3 kcal mol$^{-1}$) and $[2,6-diOHBA]$ (307.4 kcal mol$^{-1}$), and thus agree with the previous experimental results by the bracketing method. Furthermore, there should be no large difference in the thermodynamic properties of the deprotonated CDs themselves, since the chemical composition of the sugar units and the spatial arrangement of both CDs are identical. So, very similar GBs for both CD anions would be expected. Additionally, while any changes in experimental conditions would affect the ion intensity of both parent and daughter ions, that would hardly change the
ratio between the two daughter-ion intensities for the competitive proton transfer reactions, since this ratio should in principle only be dependent on the GBs of the two daughter ions. Thus, the errors for the extended kinetic method are largely suppressed and the obtained results should be more reliable than those from the bracketing method.

5.2.4 Calculated Gas-Phase Conformations

One would expect that, in the gas phase, the CD conformation that maximizes the number of hydrogen bonds is the most stable one. On the basis of the possible hydrogen bond orientations, several representative free α- and β-CD geometries were optimized at the M06-L/6-31+G(d,p) level of theory. This method was chosen because of its relatively low computational cost and its good performance in searching for global minimum-energy structures of CD complexes. Accordingly, as a consequence of the asymmetry of the glucose units, six relatively rigid hydrogen-bonded conformations were found as local energy minima for α- and β-CD each. The structures adopt one of two spatial arrangements on the narrower rim of CD cavity, namely either the alcohol-alcohol (A-A) or the alcohol-ether (A-E) type of hydrogen bonding (Figure 5.6). As the orientation of the hydrogen bonds can be clockwise (cw) or counterclockwise (cc) on both the narrow and the wide rim of the CD, four distinct hydrogen bond orientations are found for the conformations of A-A type. Likewise, two different hydrogen bonding orientations are possible for the A-E type conformations. Conformations A-A-cwcc and A-E-cwcw as from the top view of the narrow rim are shown in Figure 5.6, where cw (cc) denotes the (counter) clockwise orientation of the hydrogen bonds on the narrow rim (first entry) and the wide rim (second entry) of the CD. This definition has been used throughout the text.
Intrinsic Properties of Cyclodextrins in the Gas Phase

Figure 5.6 Top view of representative minimum-energy α-CD.

These optimized conformations were subjected to single-point energy calculations with the M06-2X functional, as Zhao and Truhlar reported recently that the M06 family of density functionals shows good performance for non-covalent interactions and M06-2X is the best for such interactions. As shown in Table 5.2, the A-A-type conformations are in general more stable than A-E-type conformations. For instance, the conformer A-A-cwcc of α-CD is more stable by 20.2 kcal mol\(^{-1}\) than the conformer A-E-cwcc for the same hydrogen bond orientation. Thus, hydrogen bonding interactions for A-A-type conformations (O6–H···O6) should be stronger than those of A-E-type (O6-H···OE'). This is supported by the corresponding hydrogen bond distances which are indicative for the hydrogen bond strengths. All the intramolecular hydrogen bond distances and relative energies for both α- and β-CD conformers are listed in Table 5.2. The hydrogen bond distances for O6–H···O6 are all smaller than for O6–H···OE'. The differences in energies and hydrogen bond distances for the A-A-type conformers are small. However, for these conformers which have the same chemical composition and chemical bond connectivity, but only differ in hydrogen bond orientation, the calculated
relative energies should be quite accurate. Therefore, the global minimum-energy conformers are found to be A-A-cwcw for α-CD and A-A-cccw for β-CD.

**Table 5.2** M06-2X/M06-L/6-31+G(d,p) calculated relative energies $\Delta E$ (kcal mol$^{-1}$) and bond lengths (Å) for the six orientations of hydrogen bonds in α- and β-CD.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta E$</th>
<th>$R_{O6'\cdots O6}$</th>
<th>$R_{O6'\cdots O}$</th>
<th>$R_{O2\cdots O3}$</th>
<th>$R_{O2'\cdots O3'}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>α-CD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-A-cccc</td>
<td>2.7</td>
<td>2.791</td>
<td>2.888</td>
<td>3.106</td>
<td></td>
</tr>
<tr>
<td>A-A-cccw</td>
<td>1.5</td>
<td>2.787</td>
<td>2.857</td>
<td>3.102</td>
<td></td>
</tr>
<tr>
<td>A-A-cwcw</td>
<td>0.0</td>
<td>2.811</td>
<td>2.855</td>
<td>3.141</td>
<td></td>
</tr>
<tr>
<td>A-A-cwcc</td>
<td>1.7</td>
<td>2.813</td>
<td>2.884</td>
<td>3.145</td>
<td></td>
</tr>
<tr>
<td>A-E-cwcw</td>
<td>18.8</td>
<td>2.944</td>
<td>2.864</td>
<td>3.152</td>
<td></td>
</tr>
<tr>
<td>A-E-cwcc</td>
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<td>2.953</td>
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<td><strong>β-CD</strong></td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>2.945</td>
<td>2.882</td>
<td>3.058</td>
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5.2.5 Calculated Gas-Phase Basicities of Deprotonated CDs

In order to calculate the GB of deprotonated CDs, it is obviously necessary to identify the most stable conformations of both their neutral and deprotonated forms. While the global minimum-energy conformations have been found for the neutral CDs, the deprotonation site is ambiguous as there are three different types of hydroxyl groups on the rims of CD, considering C$_6$ symmetry for $\alpha$-CD and C$_7$ symmetry for $\beta$-CD. Thus, geometry optimizations and vibrational analyses were performed at the M06-L/6-31+G(d,p) level of theory for all three possible anionic CD conformers of both $\alpha$-CD and $\beta$-CD, followed by single-point energy evaluations at the M06-2X level of theory in order to determine the global energy-minima of the deprotonated CDs. The GBs of the CD anions were calculated as described in the computational section.

Figure 5.7 Top views of the relevant anionic $\alpha$-CD and $\beta$-CD conformations. A and D, B and E, and C and F have the deprotonation site at the O2, O3, or O6 position, respectively.
As shown in Figure 5.7, the conformers that are deprotonated at O2, O3, or O6 are readily distinguished for both α- and β-CD anions. It is worth noting that multiple proton shuttling occurred during the geometry optimizations of conformers C and F. This suggests that the hydrogen bonding pattern in these conformers is much more dynamic than that in the neutral CDs. No such proton shuttling occurred during the optimizations of the other conformers. For both anionic α- and β-CD, the conformer deprotonated at O6 was found to be the most stable one (Figure 5.7, C and F; Table 5.3). This is consistent with the fact that nucleophilic substitution of CDs in solution usually takes place at the O6 site.133a,146

Table 5.3 M06-2X//M06-L/6-31+G(d,p) calculated relative energies ΔE and GB values (kcal mol⁻¹) for α- and β-CD anion, deprotonated at either the O2, O3, or O6 site.

<table>
<thead>
<tr>
<th>α-CD</th>
<th>ΔE</th>
<th>GB</th>
<th>β-CD</th>
<th>ΔE</th>
<th>GB</th>
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<td>A</td>
<td>12.3</td>
<td>D</td>
<td>D</td>
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<tr>
<td>B</td>
<td>8.7</td>
<td>E</td>
<td>E</td>
<td>22.6</td>
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</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>325.7</td>
<td>F</td>
<td>0.0</td>
<td>321.0</td>
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</table>

Taking the most stable conformations of both the neutral and deprotonated CDs, theoretical GB values of 325.7 and 321.0 kcal mol⁻¹ were obtained for anionic α- and β-CD⁻, respectively (Table 5.3). These calculated GB values match well with our experimental results. Moreover, the GBs of deprotonated CDs should be smaller than of the corresponding deprotonated single sugar unit, because the negative charge can delocalize much more extensively through their hydrogen bonding networks. Indeed,
the GB of α-D-glycopyranose has been determined experimentally to be in the range of 328.2–336.3 kcal mol\(^{-1}\).\(^{70}\)

5.3 Conclusions

The anionic H–G complexes \([\text{CD} \cdot \text{RBA}]^-\) have been generated in 1:1 stoichiometry under high in-source collision energy, and their existence is unequivocally confirmed by their mass-to-charge (\(m/z\)) ratios and isotope patterns. The GB of \([\alpha\text{-CD}]^-\) and \([\beta\text{-CD}]^-\) were found to be in between that of \([4\text{-CF}_3\text{BA}]^-\) and \([2,6\text{-diOHBA}]^-\) by the reaction bracketing method. Subsequent extended kinetic experiments were performed to determine the GB values of \([\alpha\text{-CD}]^-\) and \([\beta\text{-CD}]^-\) at 321.7 ± 10.4 and 320.1 ± 6.1 kcal mol\(^{-1}\), respectively, which are consistent with the results from the bracketing method. The theoretical GB values calculated at the M06-2X//M06-L/6-31+G(d,p) level of theory for both \([\alpha\text{-CD}]^-\) and \([\beta\text{-CD}]^-\) are in agreement with experiment. Additionally, the calculations suggest that the alcohol-alcohol type of conformation is the global minimum-energy conformer for both \(\alpha\)- and \(\beta\)-CD, and that deprotonation should occur from a hydroxyl group on the narrow rim of the cavity. The combined experimental and theoretical investigations of both the thermodynamic and geometric properties shed light on the intrinsic properties of CD in the gas phase.

5.4 Experimental Section

5.4.1 Materials

All chemicals were purchased from Aldrich (purity ≥ 95%) and were used without further purification. Stock solutions of \(\alpha\text{-CD}, \beta\text{-CD}\) and benzoic acid derivatives (RBA)
were prepared in distilled methanol/water (95:5 v/v) at $1.0 \times 10^{-3}$ M concentration. For the ESI–MS/MS studies, CD and RBA stock solutions were mixed in 1:5 ratio and diluted to approximately $1.0 \times 10^{-5}$ M in CD concentration.

### 5.4.2 Mass Spectrometry

All mass spectrometric experiments were performed on a ESI-Qq-TOF-MS tandem mass spectrometer (Bruker Daltonik, Zürich, Switzerland). The instrument was controlled via the Compass ver. 1.3 (Bruker Daltonik) software package. Optimal ESI settings were a spray rate of 1–2 $\mu$L min$^{-1}$, a capillary voltage of 4.5 kV, a nebulizer gas pressure of 0.4 bar, and a drying gas (N$_2$) flow of 4.0 L min$^{-1}$; the transfer capillary was heated to 200 °C. Experimental parameters were kept as constant as possible to maintain comparable conditions. The CID spectra were obtained by mass-selecting the desired non-covalent anions and fragmenting them in the collision cell. Argon was used as the collision gas at 50% flow rate for all CID experiments. The lab-frame collision offsets were set accordingly for all studied complexes to obtain the same energies in the center-of-mass frame ($E_{CM}$).
Intrinsic Properties of Cyclodextrins in the Gas Phase

Figure 5.8 CID mass spectra and isotope patterns (insets) obtained for complexes of β-CD and a) 4-CF₃BA, b) 2-OHBA, and c) 2,6-diOHBA at collision energy $E_{CM} = 0.7$ eV.

Figure 5.9 CID mass spectra and isotope patterns (insets) obtained for complexes of β-CD with a) 4-CNBA, b) 4-NO₂BA, and c) 2-OHBA at a collision energy $E_{CM} = 0.9$ eV.

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Figure 5.10 Extended kinetic method for determination of the PA and entropy change ($\Delta \Delta S^\ddagger$) for [β-CD]⁺; Plot (a) was obtained for PA of three RBAs [4-CNBA]⁻, [4-NO₂BA]⁻, and [2-OHBA]⁻ versus ln([A]⁻/[Bi]⁻) at different collision energy; Plot (b) was obtained for GB\textsubscript{app} / RT\textsubscript{eff} versus 1/RT\textsubscript{eff} at different T\textsubscript{eff}.

5.4.3 Theoretical Calculations

All calculations were carried out using the Gaussian 09 quantum mechanical package. Geometries were optimized using the pure functional M06–L in combination with the 6-31+G(d,p) basis set, which is a Pople-type basis set of double-zeta quality with one set of polarization functions on all elements and one set of diffuse functions on the
non-hydrogen atoms.\textsuperscript{92} The initial geometries of $\alpha$-\textsuperscript{88} and $\beta$-CD\textsuperscript{147} were constructed from the available X-ray structures, excluding hydration water molecules and imposing $C_6$ and $C_7$ symmetry, respectively. All geometries were optimized and confirmed to be energy minima by subsequent frequency calculations, which also afforded thermochemical corrections. In order to investigate the thermochemical properties more accurately, single-point energies were calculated with the M06-2X density functional.\textsuperscript{90-91} The GB of a molecule is defined as the free energy change for deprotonation at the most acidic site, i.e. $\text{GB} = G([\text{CD}]^-) + G(H^+) - G(\text{CD})$. Theoretical GB values were calculated from the M06-2X single-point energies in combination with the M06-L Gibbs free-energy corrections at 298 K. Assuming ideal gas behavior,\textsuperscript{148} the free energy of $H^+$ was calculated according to the following equation:

$$G(H^+) = 2.5 \, RT - TS(H^+) = 6.26 \text{ kcal mol}^{-1}$$

**Table 5.4** M06–2X//M06–L/6-31+G(d,p) energies of species in Hartree at.

<table>
<thead>
<tr>
<th>Conformation</th>
<th>$G_{corr}$ \textsuperscript{a}</th>
<th>$E_{M06-2X}$</th>
</tr>
</thead>
<tbody>
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</tr>
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**β-CD**

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**β-CD−**

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\(^a\) M06-L/6-31+G(d,p) thermal correction to the free energy.
Chapter 6

On the Structure of α-Cyclodextrin Complexes in the Solid State, Solution, and the Gas Phase
Crystal structures of α-cyclodextrin complexes with 3-methyl benzoic acid and benzoic acid were obtained, revealing unusual 2:1 host–guest complexes. In solution and in the gas phase, 1:1 complexes are observed with different spatial arrangements, revealing that the structures of host–guest complexes and the relevant inherent non-covalent interactions are strongly affected by the surrounding medium.

Manuscript in preparation
6.1 Introduction

The main impetus for the existence of cyclodextrin (CD) inclusion complexes in solution and in the solid state are the hydrophobic effect and van der Waals interactions.\textsuperscript{36e,36f,40,149} Conversely, in the gas phase, host and guest do not have to compete with solvent molecules, so that interactions of e.g. electrostatic nature are significantly strengthened.\textsuperscript{66a,110} Actually, according to our previous study, the main contribution to the stability of CD complexes in the gas phase comes from electrostatic interactions, especially hydrogen bonding.\textsuperscript{96,135} As these non-covalent interactions (NCIs) in CD complexes differ for different physical states, the structures of these complexes should also be different. Herein, we present a study on $\alpha$-cyclodextrin ($\alpha$-CD) complexes with benzoic acid derivatives (RBA) in the solid state, in solution, and in the gas phase. To get a detailed understanding of the complexation processes involving $\alpha$-CD, three different guests, 3-methybenzoic acid (3-MeBA), benzoic acid (BA), and 3-hydroxybenzoic acid (3-OHBA) are chosen for this study. The structures of and NCIs in these $\alpha$-CD complexes are then compared for the same physical state as well as across different phases.

A search for crystal structures of $\alpha$-CD host–guest (H–G) complexes in the Cambridge Structural Database revealed that with BA analogues mainly H–G species of 1:1 stoichiometry are formed (Figure 6.1 (a)).\textsuperscript{78,104,150} A 2:1 H–G configuration (Figure 6.1 (b)) is rarely observed for small guests. One structure was found in which $\alpha$-CD formed a 2:1 complex with the guest 4,4’-biphenyldicarboxylic acid, whose two carboxylic functionalities and rigid biphenyl moiety determine its preference for a 2:1 rather than a 1:1 stoichiometry.\textsuperscript{151} These studies, nevertheless, prove that the type of
H-G complex that α-CD can attain is highly dependent on the nature of the guest. In our study, the 1:1 H–G configuration is expected to be favored for the α-CD complexes with 3-MeBA, BA, and 3-OHBA due to the small size of these three guest molecules. To date, no crystal structures have been reported for these three H–G complexes. Thus, we set out to obtain such crystals in order to further elucidate the structures of these complexes in the solid state.

Figure 6.1 Proposed complexation modes of α-CD with benzoic acid derivatives (RBA):
(a) 1:1 H–G and (b) 2:1 H–G stoichiometry.

6.2 Results and Discussion

6.2.1 Structures of α-CD Complexes in the Solid State

Single crystals of α-CD complexes with BA and 3-MeBA were grown from equimolar aqueous solutions of α-CD and the corresponding guest by slow evaporation of the solvent at room temperature, and were subjected to X-ray crystallography (see Experimental Section). Two different kinds of crystals were obtained from the 1:1 solution of α-CD and BA. One was identified to consist of only the guest molecule, and
the other was elucidated to be of the 2:1 H–G complex \([(\alpha-CD)_{2}\cdot BA]\) (1, Figure 6.2), which was against our expectations. For the other guest, the crystals were also characterized to be of the 2:1 H–G complex \([(\alpha-CD)_{2}\cdot 3\text{-MeBA}]\) (2). At first glance, it was hard to distinguish between the crystals of these two \(\alpha\text{-CD}\) complexes because the unit cell dimensions, space group, and molecular structures are very similar. Therefore, the experiments were repeated to confirm the already found structure of 2. Unfortunately, the preparation of crystals of \(\alpha\text{-CD}\) with 3-OHBA was not successful. However, the crystal structures of 1 and 2 were very surprising as they differ from the commonly observed 1:1 stoichiometry in all previously reported crystal structures of \(\alpha\text{-CD}\) with guests similar to BA, such as 4-hydroxybenzoic acid and 3-nitroaniline. How could such 2:1 H–G complexes of \(\alpha\text{-CD}\) with BA and 3-MeBA form in the solid state?

**Figure 6.2** Side and top views of the crystal structure of \([(\alpha-CD)_{2}\cdot BA]\); the green dashed lines indicate hydrogen bonding between the two \(\alpha\text{-CD}\) molecules. Water molecules were omitted for clarity.

As the solvent slowly evaporates, crystallization starts once the concentration of a species reaches or exceeds its saturation point. According to the close packing theory of
Kitagorodsky\textsuperscript{152} crystallization is often simply a manifestation of the maximization of favorable van der Waals interactions, the molecules will prefer to undergo a shape simplification and progress towards dimers, trimers, higher oligomers, and ultimately crystals. The formation of 2:1 H–G inclusion structures in the solid state for BA and 3-MeBA meets the requirement for the maximization of favorable van der Waals interactions, and avoids the unfavorable water–arene contacts. These reported crystal structures of α-CD and BA analogues, such as 3-nitroaniline, which contain at least one more extra hydrophilic group than BA, are 1:1 stoichiometry. The extra hydrophilic group would maintain the favorable hydrogen bonding with surrounding water molecules, and thus no 2:1 H–G crystals were reported. Therefore, the close packing theory accounts for the formation of our reported 2:1 H–G structures in the solid state.

As the 2:1 H–G crystals grow, the excess guest will become more concentrated and finally exceed its saturation point as well. Subsequently, the guest will precipitate or crystallize out besides the 2:1 complex, as is the case in the crystal preparation of I, where separate crystals of BA were also obtained. For the complex of α-CD with 3-OHBA, the extra hydrophilic hydroxyl group might disfavor the formation of a 2:1 complex. All of the α-CD complexes with similar arene derivatives bearing two hydrophilic substituents, e.g., 4-hydroxybenzoic acid\textsuperscript{150} 3-nitroaniline\textsuperscript{104d} are complexes of 1:1 stoichiometry in the solid state. Nakai and co-workers reported that the crystals of α-CD with BA, 3-OHBA, and 4-OHBA were 2:1 complexes from powder X-ray diffractometry\textsuperscript{153} However, at least the crystal of α-CD with 4-OHBA is clearly 1:1 stoichiometry in the solid state.\textsuperscript{150} Therefore, the results from Nakai’s report is suspicious, and we assume that the complex of α-CD with 3-OHBA would preferably form a 1:1 structure in the solid state.
6.2.2 Evaluation of Binding Energies by DFT Calculations

It is difficult to determine experimentally the energetics of the NCIs that are responsible for the binding in H–G complexes. Fortunately, we could not locate any water molecules in the cavity of α-CD when carefully inspecting the crystal structures of 1 and 2. This allows the direct analysis of the binding energies by theoretical studies without the need to partition out explicit solvent interactions. Since the guest 3-MeBA was distorted in the crystal structure, no accurate experimental coordinates were available for complex 2. Thus, only complex 1 was studied by density functional theory (DFT) calculation with Gaussian 09.86 The coordinates were taken from the crystal structure of 1 without geometry optimization. The M06-2X functional90-91, which has been recommended for the study of non-bonding interactions within CD complexes in our previous studies,96,135 was used for single-point energy evaluations. For all the DFT calculations the 6-31+G(d,p) basis set was applied, and the H–G interaction energies were corrected for basis-set superposition errors (BSSEs) as calculated with the counterpoise method.92 The two α-CD molecules have different binding strengths with BA: the more strongly bound one (on the right-hand side in Figure 6.2A) supposedly represents the 1:1 H–G binding in solution, while the more weakly bound molecule would dissociate rapidly in solution. As shown in Figure 6.3, the calculated binding energy for the more strongly bound α-CD unit is 11.4 kcal mol$^{-1}$, which is comparable with the reported complexation enthalpy in water solution of 9.7 kcal mol$^{-1}$ on average.105a,105b,154 The other unit is bound by only 2.2 kcal mol$^{-1}$, while the hydrogen bonding between both α-CD molecules would play a minor role for the stability of the 2:1 H–G complex in solution as there will be competitive interactions with the solvent. Therefore, the DFT calculations are in agreement with our suggestion that in solution,
the second α-CD would dissociate readily and therefore the 2:1 H–G complexes are not detected.

![Figure 6.3 M06-2X/6-31+G(d,p) potential energy diagram (in kcal mol\(^{-1}\), including BSSE corrections) for the bonding of each α-CD in the 2:1 complex \([(α-CD)_2·BA]\).](image)

6.2.3 Comparison between Solution and Solid State

The formation of α-CD inclusion complexes with RBA in solution is by now well documented\(^{36c,36e,36f}\). Furthermore, the 1:1 H–G inclusion complexes of α-CD with RBA have also been confirmed by NMR spectroscopic studies. The fact that two hydrogen atoms located closely in space can exhibit a Nuclear Overhauser Effect (NOE), resulting in a cross-peak between the relevant proton signals in the NOESY or ROESY spectra, can be used to obtain structural indications.\(^{36f}\) A 2D NOESY NMR experiment was performed for the α-CD complex with BA in D\(_2\)O solution, for which the spectrum is shown in the experimental section. The cross-peaks between the protons (H\(_3\) and H\(_5\)) in the cavity of α-CD, and those on the aryl ring (ortho and meta position) clearly indicate the formation of an inclusion complex in solution.\(^{155}\) By analogy, the guests 3-MeBA
and 3-OHBA are also expected to form inclusion complexes with α-CD in solution. Additionally, the aromatic protons show a dramatic downfield shift upon complexation with CDs, and thus Job plots can be used to determine the stoichiometry of the H–G complexes.\textsuperscript{156} As shown in Figure 6.4, for all three studied complexes the maximum of the plot is close to a mole ratio of the guest molecule ($R_G$) of 0.5, corresponding to a 1:1 stoichiometry of the H–G complexes in D$_2$O at room temperature.

![Job plots for determination of the stoichiometry of α-CD complexes with 3-MeBA, BA, and 3-OHBA in D$_2$O, respectively.](image)

**Figure 6.4** Job plots for determination of the stoichiometry of α-CD complexes with 3-MeBA, BA, and 3-OHBA in D$_2$O, respectively.

In the solid state, as there are no water molecules in the cavity of complex 1, the structural parameters defining the relative orientations of α-CD and BA should represent the optimal geometry as governed by van der Waals interactions only. Thus, the binding between host and guest in solution should be tighter than in the solid state. Additionally, from a structural point of view, in order to maintain the 2:1 stoichiometry of complex 1, the inclusion of guest BA should be much looser in the solid state than in the 1:1 complex in solution. As an example, the overlaid crystal structures of 1 and [α-CD·4-
OHBA\[150 are shown in Figure 6.5. The extent of penetration of the guest clearly reveals that the inclusion of BA by α-CD is much looser than for 4-OHBA, even though the latter guest is more hydrophilic, which should in principle result in a more loose inclusion of the guest 4-OHBA than of BA by α-CD in solution. There are some water molecules surrounding the guest 4-OHBA in the crystal lattice of [α-CD·4-OHBA]. The deeper inclusion for 4-OHBA in crystals of [α-CD·4-OHBA] compared to BA in crystals 1 is probably due to the presence of hydrophobic interactions. Anyway, the structure geometries of and the NCIs in these H–G complexes are different in the solid state and in solution, at least in the case of α-CD complexes with BA and 3-MeBA. Therefore, crystal structures do not always reflect the solution-phase structures accurately, especially with respect to the involved NCIs.

Figure 6.5 Side and top views of overlaid crystal structures of 1 (red) and complex [α-CD·4-OHBA] (blue). Water molecules and hydrogen atoms are omitted for clarity.

6.2.4 Comparison between Solution and Gas Phase

In our previous study, we determined the gas-phase binding energies for the α-CD
complexes with 3-MeBA, BA, and 3-OHBA to be 40.8, 41.1, and 41.8 kcal mol$^{-1}$, respectively (Table 3.1).$^{135}$ The binding energies in the gas phase are much larger than the complexation enthalpies in solution of 11.7,$^{157}$ 9.7,$^{105a,105b,154}$ and 11.4$^{154b,157}$ kcal mol$^{-1}$ on average, respectively. Whereas electrostatic interactions, i.e. ionic hydrogen bonding, primarily determine the stability of the α-CD complexes in the gas phase, these interactions are significantly weakened or even absent in solution.$^{66a,110}$ On the other hand, it is the relatively weak NCIs, such as van der Waals interactions and hydrophobic effects, which stabilize the α-CD complexes in solution. Thus, not only the magnitude of the interaction energy changes dramatically, but also the dominating NCIs are different in the gas phase versus in solution.

6.2.5 Comparison between Solid State and Gas Phase

Both the structures and main NCIs involved in H–G CD complexes are different between solid state and the gas phase. In Figure 6.6 the calculated inclusion geometry$^{96}$ of gas-phase complex [α-CD•BA]$^-$ is overlaid with the crystal structure of 1. In the solid state, the hydroxyl and carboxylate groups would interact with water molecules surrounding the capsule of complex 1, which leaves the van der Waals interactions between the cavity and BA intact to maintain the inclusion structure. In the gas phase, if the inclusion structure is formed, the guest BA is calculated to penetrate deeper than it is the case in the crystal structure. Thus, the van der Waals interactions between the cavity and BA would be less attractive in the gas phase than in the solid state. Additionally, there is more spatial freedom in the non-specific structures than in the inclusion structure, which is critical for the formation of strong hydrogen bonding. As our previous study in Chapter 4 suggests that the complexes observed in the gas phase
are non-specific complexes, apparently the increased van der Waals interactions in host–guest inclusion complexes do not compensate the weaker hydrogen bonding in this configuration. Therefore, the structure geometries of, and dominating NCIs in these H–G complexes differ in the solid state and gas phase, too.

**Figure 6.6** Side and top views of the crystal structure of 1 (red) overlaid by the calculated inclusion geometry of [α-CD·BA]− (blue). Water molecules and hydrogen atoms are omitted for clarity.

### 6.3 Conclusions

In summary, we performed a structural comparison of α-CD complexes with three substituted benzoic acids RBA in the solid state, in solution, and in the gas phase. We obtained crystal structures of 1 and 2 with an unusual 2:1 H–G configuration, which have not been reported before for α-CD complexes with structurally similar guests. However, solution-phase and gas-phase studies on these complexes indicate a 1:1 H–G complexation. Our experimental results are supported by DFT calculations at the M06-2X level of theory with counterpoise corrections. The structural comparison and
analysis of the interactions involved in these complexes affords a deeper understanding of the role of NCIs in solids, in solution, and in the gas phase.

### 6.4 Experimental Section

#### 6.4.1 Materials

All chemicals were purchased from Aldrich and were used without further purification (purity ≥ 95%).

#### 6.4.2 X-ray Crystallography Experiments

Crystal data for [(α-CD)$_2$·BA]: 2(C$_{36}$H$_{60}$O$_{30}$), C$_7$H$_6$O$_2$, 15(H$_2$O), $M =$ 2338.03, $Z$ = 4, orthorhombic, space group $P 2_1 2_1 2_1$, $a = 13.9319(4)$ Å, $b = 24.3940(7)$ Å, $c = 30.8882(9)$ Å, $V = 10497.5(5)$ Å$^3$, $T = 100(2)$ K, $\rho_{\text{calcd}} = 1.478$ g cm$^{-3}$, $\mu = 1.166$ mm$^{-1}$; $R(F) = 0.0688$ for 36198 reflections with $F^2 > 2\sigma$, $wR(F^2) = 0.2005$ for all 16388 independent reflections with 1431 refined parameters, difference map between −0.592 and +0.782 eÅ$^{-3}$.

Crystal data for [(α-CD)$_2$·3-MeBA]: 2(C$_{36}$H$_{60}$O$_{30}$), C$_8$H$_8$O$_2$, 16(H$_2$O), $M =$ 2370.07, $Z$ = 4, orthorhombic, space group $P 2_1 2_1 2_1$, $a = 14.0947(18)$ Å, $b = 24.368(3)$ Å, $c = 31.015(4)$ Å, $V = 10652.5(2)$ Å$^3$, $T = 100(2)$ K, $\rho_{\text{calcd}} = 1.478$ g cm$^{-3}$, $\mu = 1.162$ mm$^{-1}$; $R(F) = 0.0605$ for 38141 reflections with $F^2 > 2\sigma$, $wR(F^2) = 0.1626$ for all 17025 independent reflections with 1568 refined parameters, difference map between −0.618 and +0.793 eÅ$^{-3}$.

Several disordered hydroxyl groups, water molecules from both crystals, and the 3-MeBA molecule from the later crystal were refined over two positions with some
geometrical restraints. Further crystallographic details may be found in the CIF files deposited in the CCDC 8888101 ([(α-CD)$_2$·BA]) and CCDC 888100 ([(α-CD)$_2$·3-MeBA]) for this thesis.

**Figure 6.7** Side view of the major conformation in the crystal structure of [(α-CD)$_2$·3-MeBA]; water molecules are omitted for clarity.

**Figure 6.8** Top view of the major conformation in the crystal structure of [(α-CD)$_2$·3-MeBA]; water molecules are omitted for clarity.
6.4.3 DFT Calculations

All calculations were carried out using the Gaussian 09 quantum mechanical package. Basis set superposition errors (BSSEs) were computed with the counterpoise approach.

Table 6.1 M06-2X/6-31+G(d,p) absolute energies and BSSEs (in Hartree) for complex 1 ([α-CD·BA·α-CD’] in Figure 6.3).

<table>
<thead>
<tr>
<th>species</th>
<th>α-CD</th>
<th>BA</th>
<th>α-CD’</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>–3662.899547590</td>
<td>–420.610667316</td>
<td>–3663.201595660</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>species</th>
<th>[α-CD·BA]</th>
<th>[α-CD’·BA]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>–4083.513796590</td>
<td>–4083.832365880</td>
</tr>
<tr>
<td>BSSE</td>
<td>0.00245636385</td>
<td>0.00669600686</td>
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6.4.4 NMR Experiments

For the stoichiometry determination of α-CD complexes using Job plots, stock solutions of α-CD and RBA were prepared in D2O at identical concentrations of 0.01 M. Predetermined quantities of α-CD and RBA solution were mixed in 5 mm NMR tubes, and $^1$H NMR data were acquired at room temperature on a Bruker 300 MHz spectrometer. Chemical shift ($\delta$) values were determined for the ortho-H atoms of RBA; these are listed along with the corresponding guest ratio $R_G$ in Table 6.2. The 2D NOESY NMR spectrum (Figure 6.9) of a 1:1 mixture of α-CD and BA in D2O solution was recorded on a Bruker 400 MHz spectrometer.
On the Structure of $\alpha$-Cyclodextrin Complexes in the Solid State, Solution and the Gas Phase

Figure 6.9 $^1$H NOESY spectrum of $\alpha$-CD complex with BA in D$_2$O solution at room temperature.

Table 6.2 The chemical shift ($\delta$) at specific guest ratio $R_G$ required for the Job plot in order to determine the stoichiometry of the complexes of $\alpha$-CD with RBA.

<table>
<thead>
<tr>
<th>$\alpha$-CD-BA</th>
<th>$\alpha$-CD-3-MeBA</th>
<th>$\alpha$-CD-3-OHBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_G$</td>
<td>$\delta$</td>
<td>$\Delta\delta\cdot R_G$</td>
</tr>
<tr>
<td>1.0</td>
<td>8.065</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.9</td>
<td>8.09</td>
<td>0.0225</td>
</tr>
<tr>
<td>0.7</td>
<td>8.179</td>
<td>0.0798</td>
</tr>
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<td>0.6</td>
<td>8.231</td>
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</tr>
<tr>
<td>0.5</td>
<td>8.274</td>
<td>0.1045</td>
</tr>
<tr>
<td>0.4</td>
<td>8.313</td>
<td>0.0992</td>
</tr>
<tr>
<td>0.3</td>
<td>8.331</td>
<td>0.0798</td>
</tr>
<tr>
<td>0.1</td>
<td>8.323</td>
<td>0.0258</td>
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<tr>
<td>0.0</td>
<td>0.0000</td>
<td>0.0000</td>
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</table>

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Conclusions and Outlook

The anionic host–guest cyclodextrin (CD) complexes were studied by electrospray mass spectrometry (ESI–MS) and their 1:1 composition was confirmed by the unit-mass spacing of their isotope patterns. The correlation between the gas-phase stabilities of these complexes, as disclosed by CID experiments, and the gas-phase basicities (GB) of the host and guests involved sheds light on the intrinsic properties of these non-covalent complexes in the gas phase. The CID threshold measurements provide the first measured absolute gas-phase dissociation energies for such large non-covalent CD complexes. Our results were further examined by means of DFT calculations, which suggest that the inclusion geometries are more stable than the non-specific geometries. However, for some of these large systems, the calculated energies were not in good agreement with experimental results. Thus, the coordination geometries of the CD complexes detected in the gas phase remain unclear.

Subsequently, a systematic study was performed on the specificity of CD complexes in the gas phase. The results indicate that only the GB values affect the intrinsic gas-phase stabilities of these CD complexes. The size of the guest merely affects the collision cross section, which has an influence on the apparent gas-phase stability as
Conclusions and Outlook

quantified by the daughter-ion fractions in CID experiments. Additionally, the behavior of all the CD anionic complexes studied by ESI–MS was similar to that of the gas-phase electrostatic adducts [CD·Cl]− and [CD·Br]−. This highlights the intrinsic stabilization by electrostatic interactions in the gas phase and the differences between non-covalent complexes in solution and in the gas phase. Therefore, our results argue against the existence of inclusion complexes in the gas phase, and strongly point to the formation of non-specific complexes during the desolvation process.

It is still not well understood whether some fraction of specific complexes also contributed to the total peak intensity observed by ESI–MS. Several questions cannot yet be fully answered: What is the minimal interaction strength a complex should have in order to survive the ionization process? What exactly is the relationship between the interaction strength and the complex stability? How do the types of non-covalent interactions change during the transfer from solution to gas phase? From the current state of knowledge, it becomes clear that it is not the affinity between host and guest moieties in solution which determines the complex stability observed in the gas phase, but the non-covalent interactions existing in the gas phase. With the development of ion mobility mass spectrometry (IM–MS), the aforementioned questions might become addressable. IM is an established technique for studying the shape and conformation of small molecules and proteins in the gas phase. When IM is coupled to MS, the overall topology of non-covalent complexes can be determined simultaneously with their molecular mass and, consequently, the composition of the subunits. If the difference in collision cross section between the specific and non-specific complexes is large enough to be resolved by IM–MS, the contribution of both conformations can be determined. We believe that the application of combined CID threshold measurements, IM-MS
studies, and DFT calculations is of great advantage for the study of non-covalent complexes in the gas phase. Therefore, we recommend following this approach to gain even deeper insight into the intrinsic properties of gas-phase host–guest complexes.
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References


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(139) The GB of [2,6-diOHBA]− was calculated according to the strategy of gas-phase basicity (GB) calculations for [CDs]−, but at the B3LYP/6-311++G(d,p) level of theory.


