Doctoral Thesis

Synthesis of heparin oligosaccharides and the creation of heparin microarrays

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Publication Date:
2007

Permanent Link:
https://doi.org/10.3929/ethz-a-005412748

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Synthesis of Heparin Oligosaccharides
and the Creation of Heparin Microarrays

A dissertation submitted to the
EIDGENÖSSISCHE TECHNISCHE HOCHSCHULE ZÜRICH

for the degree of

DOCTOR OF NATURAL SCIENCES

presented by

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Zürich 2007
Abstract

Heparin is a highly sulfated, linear polymer that is composed of disaccharide units consisting of an uronic acid 1,4-linked to a D-glucosamine unit. A prototypical heparin disaccharide contains three sulfate groups. These sulfate groups render heparin one of the most acidic macromolecules in nature. Heparin participates in a plethora of biological processes by interaction with many proteins such as fibroblast growth factors, chemokines, and selectins.

The chemical complexity and heterogeneity of heparin can explain the fact that, despite its widespread medical use as an anticoagulant drug, the structure-function relationship of defined heparin sequences is still poorly understood.
In this thesis the chemical synthesis of a library containing heparin oligosaccharides ranging from di- to hexamers of different sequences and sulfation patterns is presented. An amine-terminated linker was placed at the reducing end of the synthetic structures to allow for immobilization onto N-hydroxysuccinimide activated glass slides and creation of heparin microarrays. Key features of this modular synthesis, such as the influence of the amine linker on glycosidation efficiency, the use of 2-azidoglucose as glycosylating agents for oligosaccharide assembly, and the compatibility of the protecting group strategy with the sulfation-deprotection steps, are discussed.

Carbohydrate microarrays, carrying tens or hundreds of different sugars that are bound covalently in small spots on solid surfaces, are becoming a standard tool for glycobiologists to screen large numbers of sugars and to elucidate the role of carbohydrates in biological systems. Moreover, carbohydrate microarrays possess a plethora of potential applications in glycomics including the rapid determination of the binding profile of carbohydrate-binding proteins, the detection of specific antibodies for the diagnosis of diseases, the characterization of carbohydrate-cell recognition events and the high-throughput screening of inhibitors to prevent carbohydrate-protein interactions.
Heparin arrays are expected to fundamentally assist in the establishment of structure-activity relationships for heparin sequences. Therefore, with the synthetic oligosaccharides at hand, heparin microarrays were constructed using a robotic printer and employed to characterize the carbohydrate binding affinities of heparin-binding proteins. Different FGF's and chemokines that are implicated in angiogenesis, cell growth and differentiation were studied. These heparin chips aided in the discovery of novel, sulfated sequences that bind to these proteins, and in the determination of the structural requirements needed for recognition by using picomoles of protein on a single slide. The results presented in this work highlight the potential of combining oligosaccharide synthesis and carbohydrate microarray technology to establish a structure-activity relationship in biological processes.

The limitation of preparing hundreds or thousands of heparin oligosaccharides needs to be overcome to enable wider applications of heparin chips. The best way to prepare heparin oligosaccharides in a fast way and in significant quantities is the use of automated solid phase synthesis as is now common for DNA and peptide synthesis.
Solid phase synthesis displays immense advantages compared to solution phase synthesis since the reaction can be driven to completion by removal of excess reagents by simply washing the resin. The number of chromatographic steps required is minimized by purification of the reaction products at the end of synthesis.

Despite the impressive advances in the automated assembly of oligosaccharides during the last decade, the solid-phase synthesis of HLGAGs is still a difficult goal to achieve. In this context, one chapter of this thesis reports the efforts to synthesize heparin oligosaccharides by automation in order to expand the complexity and utility of heparin arrays.

The combination of automated oligosaccharide synthesis and carbohydrate microarray technology may become essential to better understand the role of heparin in biological processes.
Zusammenfassung


Obwohl dieses Polysaccharid seit mehreren Jahrzehnten als Gerinnungshemmer eingesetzt wird, ist die Struktur-Funktionsbeziehung von genau definierten Heparinsequenzen wegen seiner chemischen Komplexität und Heterogenität bis heute noch nicht geklärt.


Um das Potential von Heparin Chips richtig ausschöpfen zu können, müssen hunderte und tausende von verschiedenen Heparin Oligosacchariden synthetisiert werden, was nach wie vor ein Problem darstellt. Mittels der Kombination von Festphasensynthese und Automatisierung, wie sie heutzutage bereits erfolgreich bei der Synthese von DNA-Strängen und Peptiden angewendet wird, sollte dieses Hindernis auch umgangen werden können.
Im Vergleich zur Synthese in Lösung können bei der Festphasen Synthese die mit Überschuss eingesetzten Reagenzien durch Waschen des Trägers einfach und schnell vom Produkt getrennt werden. Daher ist eine Aufreinigung des Zielmoleküls nur nach vollendeter Synthese erforderlich.

Die Kombination aus automatischer Zucker-Synthese und Kohlenhydrat-Microarrays könnte in der nahen Zukunft an Bedeutung gewinnen, da daraus wichtige Informationen über die Rolle von Heparin in biologischen Prozessen erhalten werden können, was sich wiederum in der Entwicklung neuer Medikamente widerspiegeln wird.