HORSE LIVER ALCOHOL DEHYDROGENASE: 
HISTIDINOL OXIDATION 
AND MECHANISM OF ACTION

A
DISSERTATION

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His-ol, a metabolite in the His-biosynthesis was found to react with LADH and NAD$^+$, producing His-al and His. The optimal initial His-ol concentration for His-production is <100 µM. At higher concentrations the intermediate product His-al is displaced by His-ol resulting in the suppression of the His-production. In the solution, the intermediate His-al was found to decompose rapidly with a half-life time of ca. 15 min. His-ol-to-His oxidation experiments were carried out in two steps on a stopped-flow machine, producing first His-al at pH 7.0 and upon pH jump to 9.3, His. From these experiments a rate constant for the His-al-to-His oxidation of $46 \times 10^{-3}$ s$^{-1}$ was determined. At pH 8.5 $V$, $K_m$ and $K_{eq}$ for His-ol-to-His-al oxidation were found to be $2.86$ s$^{-1}$, 59.12 mM and $1.2 \times 10^{-13}$ M, respectively. In experiments for the oxidation of His-ol analogues, Im was found at low concentrations (<5.0 mM) to promote Ala-ol- and EtOH-Oxidation; at high concentrations (>120 mM) Im inhibits these reactions, whereas the oxidation of His-ol was inhibited by Im at all concentrations. The $V$ and $K_m$ values for Tyr-ol, Phe-ol, Trp-ol and Pro-ol oxidation as well as for the combined hydrolysis and oxidation of His-ol phosphate and ethanolamine phosphate were obtained from steady state experiments. Cys 174 was derivatized with diazonium-$1H$-tetrazole and the doubly, singly and unmodified LADH-species were
separated by affinity chromatography on Blue-Dextran-Sepharose 6B. The distribution pattern in the eluates showed that strong cooperativity is present between the LADH-subunits during modification reaction. A model for LADH reactions with the active zinc in a flexible square pyramidal coordination with His-ol and His-al as bidentate ligands was developed whereby His-ol is oxidized to His-al and the latter forms a thiohemiacetal with Cys 174. The thiohemiacetal is converted to the corresponding thioester under the consumption of a second NAD$^+$ molecule producing His after hydrolysis. The effects of high EtOH concentrations on His-ol-to-His oxidation were considered. Possible explanations for the known inhibition of the protein-biosynthesis and the formation of addiction producing substances in the organism upon alcohol consumption were given as a consequence of the interference with the His-biosynthesis and accumulation of His-ol and His-al.