Horse liver alcohol dehydrogenase
histidinol oxidation and mechanism of action

Author(s):
Ambar, Abraham

Publication Date:
1981

Permanent Link:
https://doi.org/10.3929/ethz-a-000229537

Rights / License:
In Copyright - Non-Commercial Use Permitted
HORSE LIVER ALCOHOL DEHYDROGENASE: HISTIDINOL OXIDATION AND MECHANISM OF ACTION

A DISSERTATION

submitted to
THE SWISS FEDERAL INSTITUTE OF TECHNOLOGY (ETH)
ZURICH

for the degree of
Doctor of Natural Sciences

presented by
ABRAHAM AMBAR
Dipl. Chem. ETH Zurich

born December 26, 1941
citizen of Israel

accepted on the recommendation of
PD.Dr. H. Dutler, referee
Prof.Dr. H. Zuber, coreferee

Zurich 1981
7. ABSTRACT

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 \(< 100 \, \muM\). 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 \(46 \times 10^{-3} \, \text{s}^{-1}\) was determined. At pH 8.5 \(V, K_m\) and \(K_{eq}\) for His-ol-to-His-al oxidation were found to be \(2.86 \, \text{s}^{-1}\), \(59.12 \, \text{mM}\) and \(1.2 \times 10^{-13} \, \text{M}\), respectively. In experiments for the oxidation of His-ol analogues, Im was found at low concentrations (\(< 5.0 \, \text{mM}\)) to promote Ala-ol- and EtOH-Oxidation; at high concentrations (\(> 120 \, \text{mM}\)) 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.