STRUCTURAL AND MECHANISTIC STUDIES OF SHEEP LIVER ALDEHYDE DEHYDROGENASE

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Rosemary Lynne MOTION
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ABSTRACT

Studies of NADH displacement in the presence of excess aldehyde dehydrogenase confirmed that a conformational change of the enzyme.NADH binary complex occurs as an essential step in the reaction mechanism.

Modification of sulphydryl groups on the enzyme by the thiol reagent p-(chloromercuri)benzoate (PCMB) produced either activation or inhibition of the enzyme activity, depending upon the relative concentrations of PCMB and aldehyde dehydrogenase, the mixing conditions, and on the concentration of the aldehyde substrate. There was no direct evidence to support the widely held view that a sulphydryl group is catalytically essential.

Studies of the pH dependence of the steady state and presteady state phases of the reaction indicated that there was a change in the rate limiting step as the pH was increased from acyl-enzyme hydrolysis at low pH to release of NADH from the enzyme at high pH. At low pH the release of NADH may occur before acyl-enzyme hydrolysis. Activation by high concentrations of propionaldehyde was shown to occur over the entire pH range (5 to 10).

The reaction could be reversed when acid anhydrides were used to acylate the enzyme.NADH complex but the binding of the substrates for the reverse reaction did not appear to be ordered. Under these conditions other groups on the enzyme were acylated with resultant inhibition or activation of the dehydrogenase activity of the enzyme depending on the relative concentrations of the substrates and reactants, and on the mixing conditions.

The enzyme catalysed the hydrolysis of p-nitrophenylacetate in the presence of NADH but with no significant production of acetaldehyde. It was concluded that ester hydrolysis does not occur at the site of aldehyde oxidation.

Preliminary studies on the reaction of the enzyme with diethylpyrocarbonate indicated that the enzyme may contain a catalytically essential histidine residue.

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