MECHANISTIC STUDIES ON SHEEP LIVER ALDEHYDE DEHYDROGENASES

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ABSTRACT

The enzyme aldehyde dehydrogenase has been extensively purified from the cytoplasmic fraction of sheep liver and a study of its kinetic behaviour has been made.

Studies showed that the nucleotide fluorescence of NADH increased on binding to cytoplasmic aldehyde dehydrogenase and the 5.6 fold enhancement of fluorescence has been used to determine the binding site concentration of enzyme solutions. These binding studies showed that the NADH binding sites on the enzyme were all equivalent and possessed a dissociation constant for NADH of 1.2μM. No significant amounts of zinc were detected in the purified enzyme samples.

Steady-state kinetic studies at pH 7.6 showed that the enzyme was capable of utilizing a wide range of aldehydes as substrates and the enzyme also possessed the ability to hydrolyze p-nitrophenyl acetate. The mechanism of action of cytoplasmic aldehyde dehydrogenase using propionaldehyde as a substrate was found to be ordered, with NAD^+ binding prior to the aldehyde and the acid product being released before NADH. Michaelis constants for NAD^+ and propionaldehyde were 2.2μM and 1.4μM respectively while the dissociation constant for NAD^+ was 8μM. At high aldehyde concentration (both for propionaldehyde and acetaldehyde) substrate activation was observed. Steady-state kinetic results were also reported at pH 9.3.

Stopped-flow fluorimetric studies of NADH displacement from aldehyde dehydrogenase using a series of displacing agents (NAD^+, deamino-NAD^+, ADP-ribose and 1,10-phenanthroline) show that this process is biphasic with rate constants of $0.85s^{-1}$ and $0.22s^{-1}$. This has been interpreted as a two step displacement process. The $0.22s^{-1}$ rate constant is similar to the maximum enzyme reaction velocity in the steady-state at high aldehyde concentrations. The association of NADH with the enzyme was also found to be biphasic, one phase being dependent on the NADH concentration while the other was independent.

Stopped-flow experiments where aldehyde dehydrogenase was rapidly mixed with the coenzyme and propionaldehyde showed a burst of NADH formation followed by a slower steady-state turnover. The maximum burst rate constants were $11s^{-1}$ and $23s^{-1}$

for propionaldehyde and acetaldehyde respectively. A mechanism has been postulated for the observed burst and values for various individual rates constants derived.

The general features of the kinetics of sheep liver cytoplasmic aldehyde dehydrogenase have been compared with those of the mitochondrial enzyme from the same source and except for the value of the NAD^+ binding rate constant the two enzymes have been shown to be remarkably similar.

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