Sheep Liver Cytosolic Aldehyde Dehydrogenase; A fresh perspective

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Abstract

The pre-steady-state mechanism of aldehyde dehydrogenase has been further investigated using synthesised deuterated 4-*trans*-(N,N-dimethylamino) cinnamaldehyde as a substrate.

Reporter groups of the active site of ALDH have indicated the presence of a divalent or trivalent metal electrophile, shown in chapter 3 as being either $Fe^{(II)}$ or $Fe^{(III)}$.

Studies of the spectral properties of NADH bound to aldehyde dehydrogenase have revealed the presence of at least two spectrally different enzyme-NADH species. The consequences of this information are important in interpretation of the kinetic data and understanding apparently contradictory experimental results from different research workers.

The steady-state kinetics of ALDH have been further investigated.

A sensitive substrate for use in enzyme immunoassays has been designed and synthesised. The preliminary kinetic behaviour observed using this substrate has been studied with three enzymes.

Aldehyde dehydrogenase has been used as a model system for studying the effects of electromagnetic radiation on biological systems.

Acknowledgements

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Introduction

Sheep liver cytosolic aldehyde dehydrogenase is an enzyme which is involved in the metabolism of aldehdyes. It has been suggested that aldehyde dehydrogenase has a multifunction purpose, its broad substrate specificity allowing it to oxidise a wide variety of aldehydes to the corresponding carboxylic acids. Hence the physiological role of aldehyde dehydrogenase appears to be that of a general detoxifier (Weiner, 1989).

Aldehyde dehydrogenase was first purified by Kathy Kitson (nee Crow, thesis), and she has continued her involvement in the improvement enzyme quality thoughout. Kathy Kitson has been predominantly researching the physiological factors that control the rate alcohol metabolism, of which aldehyde dehydrogenase is one (Crow and Hardman, 1989).

The steady state and pre-steady state kinetics of sheep liver aldehyde dehydrogenase was first studied by MacGibbon (thesis). The significant outcomes of this work was that the product NADH was shown to be released from ALDH in a biphasic manner. The biphasic release of NADH was found to be the rate limiting process for many aldehyde substrates. MacGibbon also showed that ALDH could catalyse the hydrolysis of unstable esters. Later a group at Hull University, under the leadership of Mark Dickinson also started research on this enzyme. It was found that NADH could reduce the ALDH-acyl species (the reverse reaction) formed by reaction of the enzyme active site nucleophile with an anhydride. This group contributed much pre-steady state and steady information (Hart and Dickinson, 1982)

The rate of acyl enzyme hydrolysis (for many substrates) using proton release was predominantly studied by Bennett (thesis). Bennett also studied the effect of magnesium ions on the enzyme, magnesium ions being

available in abundance in vivo (Soman et al, 1970). Motion (thesis) studied the kinetic effects of pH, buffers and furthered the studies on the reverse reaction. Dunn and Buckley (1982,1985), studied the formation of a coloured quassi-stable ALDH-acyl species formed as an intermediate in the oxidation of 4-trans-(N,N-dimethylamino) cinnamaldehyde. Jeremy Hill examined the enzyme structural equilibrium between the tetrameric state and the dissociated state (Buckley et al., 1991).

Trevor Kitson made the first stable ALDH-acyl species (Kitson T., 1989) which was found to have been incorporated with the residue cysteine 302 (Kitson et al.,1991). In conjunction with Kerry Loomes, Kitson studied the effects of a variety of sulfhydral modifiers on the kinetics of aldehyde dehydrogenase (Kitson T., (1989,b)).

A major focus of the research work presented in this thesis is to further increase the kinetic understanding of aldehyde dehydrogenase.