Kinetic And Mechanistic Studies on Aldehyde Dehydrogenases From Sheep Liver

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Abstract

The mechanisms of sheep liver aldehyde dehydrogenases have been further investigated by both steady-state and pre-steady-state kinetic methods.

By utilizing the acid/base indicator phenol red, a burst in the production of protons has been detected for both the cytoplasmic and mitochondrial isoenzymes. The rates and amplitudes of the two proton bursts were almost identical to those for the NADH bursts which both isoenzymes exhibit. After a consideration of the kinetic data, the electronic structure of various aldehydes and computer simulation studies, the proton burst process was postulated as arising from a conformational change on aldehyde binding to the enzyme-NAD^+ binary complex. The proton release arises from the perturbation of the pKa of a protonated functional group from about 8.5 to below 5.0

The effects of the Mg^{2+} ion on the cytoplasmic isoenzyme were also studied. The presence of millimolar concentrations of this ion resulted in marked inhibition of the enzyme activity, and a lowering of the dissociation constants for both the E.NAD^+ and E.NADH binary-enzyme complexes. Steady-state and pre-steady-state studies showed that the major effect of $MgCl_2$ on the enzyme mechanism was to slow the steady-state rate-limiting step, which was NADH dissociation at high propionaldehyde concentrations and an unidentified step, possibly involving deacylation, at low propionaldehyde concentrations.

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INTRODUCTION

The ingestion of excess ethanol by mammals results in symptoms such as flushing, sweating, respiratory difficulties and, in humans, a severe headache, these symptoms being collectively known as a hangover. These effects are believed to result mainly from a build up in the bloodstream of acetaldehyde, the primary product of ethanol metabolism. Acetaldehyde introduced into the bloodstream can reproduce the effects mentioned above, and is thought to exert its effect by a variety of means which include, the production of alcohols from biogenic amines by shifting their catabolism to a reductive pathway (Truitt and Walsh, 1971) and direct condensation with biogenic amines (Cohen and Collins, 1970). A further possible effect of acetaldehyde in mammals is the formation of tetrahydropapaveroline (Davis and Walsh, 1970), an alkaloid similar to morphine which is a possible link with the addiction to alcohol seen with alcoholics.

During normal metabolism, the level of acetaldehyde in the blood is kept to a relatively low level by three aldehyde oxidising systems, aldehyde oxidase, xanthine oxidase and aldehyde dehydrogenase. The first two enzymes have much lower affinities for aldehydes (Rajogopalan and Handler, 1964; Mackler *et al.*, 1954) than aldehyde dehydrogenase (Buttner, 1965; Feldman and Weiner, 1972a, Crow *et al.*, 1974) and as a result it has been assumed that aldehyde dehydrogenase is the most important enzyme involved in the removal of acetaldehyde in mammals. Further evidence to support this conclusion comes from the results of studies with the drug disulfiram (antabuse). The administration of this drug results in nausea, flushing, sweating and respiratory difficulties when the recipients ingest ethanol, and it has been shown by Kitson (1975) that the drug's presence causes inhibition of aldehyde

dehydrogenase activity, indicating that the hangover-like symptoms observed are due to a build up of acetaldehyde levels in the blood.

The existence of aldehyde dehydrogenase was first shown by Racker (1949) and has since been purified to homogeneity from a number of mammalian sources including horse liver (Feldman and Weiner, 1972a; Eckfeldt and Yonetani, 1976), bovine liver (Sugimoto et al., 1976), rat liver (Shum and Blair, 1972; Tottmar et al., 1973), sheep liver (Crow et al., 1974; MacGibbon et al., 1979; Hart and Dickinson, 1977) and human liver (Greenfield and Pietruszko, 1977; Kraemer and Deitrich, 1968). Sheep liver aldehyde dehydrogenase has been extensively studied since it was first isolated by Crow et al., (1974). The enzyme has been shown to be present in sheep liver as three distinct isoenzymes, one located in the mitochondria, one in the cytoplasm and the other in the microsomes. The majority of the enzyme being present in approximately equal proportions in the mitochondria and the cytoplasm.

The mechanism of oxidation of aldehydes by the cytoplasmic enzyme is believed to be an ordered Bi Bi mechanism with NAD^+ binding first (MacGibbon et al., 1977a), the rate-determining step in the steady-state being dissociation of NADH from the binary E.NADH complex. The rate determining step in the pre-steady-state was not identified by these workers although, due to the absence of a kinetic isotope effect on the hydride transfer step, they concluded that it occurred prior to this step. The mitochondrial isoenzyme has also been studied by these workers who have suggested that it operates by a mechanism similar to that found for the cytoplasmic enzyme. However a report has been published by Hart and Dickinson (1978) suggesting that the mitochondrial enzyme may operate by a group transfer mechanism rather than an ordered Bi Bi mechanism.

In view of the potential importance of aldehyde dehydrogenase in the metabolism of ethanol, it was decided to carry out further steady-state and pre-steady-state

studies using both the mitochondrial and cytoplasmic isoenzymes in an attempt to resolve some of the unanswered questions which remain about the mechanism of the cytoplasmic isoenzyme and determine which of the proposed mitochondrial mechanisms is the better approximation to the actual reaction mechanism. In particular, studies on proton release during the first enzyme turnover, which have not so far been carried out, may reveal information about the rate determining steps in both the steady-state and pre-steady-state. Also it has been recently reported (Venteicher et al., 1977) that both the mitochondrial and cytoplasmic horse liver isoenzymes are sensitive to the presence of $MgCl_2$, and since the cytoplasm of liver cells contains significant levels of this ion (20 meq/Kg, Soman et al, 1970) it was decided to investigate the effects of $MgCl_2$ on the cytoplasmic sheep liver enzyme. This thesis presents the results of such studies.