STRUCTURAL AND FUNCTIONAL PROPERTIES OF SHEEP LIVER ALDEHYDE DEHYDROGENASE

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ABSTRACT

When aldehyde dehydrogenase reacts with disulfiram (a drug used in alcoholism therapy) a thiol group (A) is initially modified causing enzyme inactivation, and the label is subsequently displaced by a second thiol group (B). In Chapter 3 the effects of various other thiol-modifying agents on the dehydrogenase activity of mitochondrial aldehyde dehydrogenase were examined, and it was found that 4,4'-dithiodipyridine and 5,5'-dithiobis(1-methyltetrazole) are good activators. This implies that modification is at group B and not with the disulfiram-sensitive thiol, group A. On the other hand, methyl diethylthiocarbamyl disulfide and methyl 5-(1-methyltetrazolyl) disulfide are good inhibitors, just as they are of the cytoplasmic form. 2,2'-Dithiodipyridine has no effect on activity of the mitochondrial enzyme and suggests the involvement of groups other than groups A and B. Methyl 5-(1-methyltetrazolyl) disulfide may be the compound responsible *in vivo* for the unpleasant physiological response to alcohol (like that caused by disulfiram) observed during therapy with certain β-lactam antibiotics which contain a 1-methyltetrazole-5-thiol sidechain.

The remainder of this thesis concerns studies of the cytoplasmic isozyme. In Chapters 4 and 5 the research focuses on the esterase and dehydrogenase activities exhibited by this enzyme. Traditionally, these activities have presumed to be mediated by a common active site. However, in the literature over the past 10 years evidence has been presented which suggest they occur at two positionally distinct sites, called P1 and P2 for the dehydrogenase and esterase activities respectively. In the present study support for a common active site was provided by findings which suggest that modification of a certain thiol group by various reagents always leads to a concerted reduction in both these activities (Chapter 4). The intermediacy of a common active site was also supported by these findings, namely: (1) [^{14}C]acetaldehyde is produced from a reaction mixture containing enzyme, NADH, and 4-nitrophenyl [^{14}C]acetate (Chapter 5); and (2) the same catalytic nucleophile appears to be involved in the hydrolysis of *trans*-4-N,N-dimethylaminocinnamoyl imidazole and the oxidation of *trans*-4-N,N-dimethylaminocinnamaldehyde (Chapter 7).

Another area investigated in this study concerns the identity of the disulfiram-sensitive group. Disulfiram is a reagent which potently inhibits the cytoplasmic enzyme, and is inferred to react with Cys-302 on the basis that pre-modification

of the enzyme with disulfiram blocks the incorporation of [^{14}C]iodoacetamide. (The latter has been shown to label Cys-302 specifically.) In Chapter 6 the reaction between [^{14}C]iodoacetamide and enzyme previously modified with various thiol reagents was monitored. With a 2-fold excess of either disulfiram, methyl diethylthiocarbamyl disulfide, or methyl 2-pyridyl disulfide, the reaction rate with [^{14}C]iodoacetamide was slowed compared to the reaction rate with native enzyme. However, in all these cases (including the native enzyme) the total amount of bound radioactivity was the same after 24 hours. Only with a 4-fold excess of disulfiram or methyl diethylthiocarbamyl disulfide was the amplitude reduced significantly over this period. These results suggest that the disulfiram-sensitive group is not Cys-302.

Finally, a true dehydrogenase acyl intermediate was trapped using *trans*-4-N,N-dimethylaminocinnamaldehyde. The denatured species was digested and the active site peptide purified by gel filtration and HPLC. The chromophore was associated almost exclusively with a peptide identified as T5 in the known primary structure. Although, on chemical grounds, only Ser-74 (from T5) has the ability to act as a nucleophile, no direct evidence was found to prove a covalent link between this amino acid and the chromophore. Arguments for and against Ser-74 being the catalytic nucleophile are discussed.

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