The Significance of Alternative Forms of Xanthine Oxidase in Different Mouse Tissues

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Sackler (1965) has shown that at least two forms of xanthine oxidase exist in the rat. On starch-gel electrophoresis the liver enzyme has a slower mobility (more cathodal) than that of the duodenum. The liver xanthine oxidase activity may be influenced by a number of epigenetic factors. Spandoni & Ticca (1968) report that the injection of xanthine into developing rats brings about a premature appearance of the liver enzyme and that continued administration of xanthine induces a 73% increase in activity over the control animals. Investigations by Stirpe & Della Corte (1969) have suggested that a dehydrogenase is the predominant form of the enzyme in rat and human liver *in vivo*, but that it may be converted *in vitro* into the oxidase type by various treatments. Reported here are preliminary investigations on the mouse xanthine oxidase system and the attempts that have been made to clarify the relationships between different tissue types.

Polyacrylamide-gel electrophoresis and starch-gel electrophoresis have revealed that at least two forms of xanthine oxidase also exist in mouse tissues, with the liver enzyme again being more cathodal than the duodenal form. Most of the liver enzyme was precipitated out between 30 and 50% saturation with ammonium sulphate whereas the duodenal form was precipitated out between 40 and 60% saturation with ammonium sulphate. K_m determinations with the substrates xanthine and 2-amino-4-hydroxypteridine revealed little difference, however, between the different enzyme forms. When the electrophoretic gels were stained for xanthine dehydrogenase activity rather than the oxidase activity, it was noted that the kidney enzyme gave a much more intense reaction. Further, the incorporation of phenazine methosulphate into the assay system caused a 600% increase in activity of the kidney enzyme, 300% for the liver and 200% for the duodenal form. The significance of these results is not clear at present, although the possibility that the kidney enzyme is predominantly a dehydrogenase cannot be excluded.

In order to clarify the possible relationship between these enzyme forms various attempts were made to interconvert them. When the liver enzyme was incubated at 0° or 37°C with high concentrations of a crude commercially obtained (BDH Chemicals Ltd., Poole, Dorset, U.K.) trypsin preparation, its electrophoretic mobility was changed so that it behaved like the duodenal

form. Incubation at 37°C, but not at 0°C, with a duodenal extract also converted the liver into the duodenal form. When duodenal xanthine oxidase is prepared in the presence of soya-bean trypsin inhibitor, it subsequently appears to be very similar, electrophoretically, to the liver enzyme. These results may indicate that the different tissue forms may be due to an artifact of preparation, and the significance to other systems is self-evident.

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Conformation Changes in Chemically Modified Alcohol Dehydrogenase

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Various types of modified alcohol dehydrogenases have been prepared by reaction of enzyme thiol and amino groups and have been found to have interesting properties. Thus iodoacetate was found to inactivate liver alcohol dehydrogenase by formation of a reversible complex, followed by irreversible alkylation of a single thiol in each polypeptide chain. The latter occurred by either an internal or an external mechanism (Reynolds & McKinley-McKee, 1969; Reynolds, Morris & McKinley-McKee, 1970). The inactivation kinetics in the presence of other ligands (e.g. nucleotides, coenzymes etc.) enabled their binding to be studied. The experiments supported the hypothesis that the phosphate of AMP and of the AMP moiety of the oxidized and reduced coenzymes were bound by a positively charged lysine residue. Further evidence for this was proved by enzyme modified with pyridoxal 5'-phosphate. Although iodoacetate seems to bind reversibly to the positive lysine residue, the covalently bound carboxymethyl group may not be near this site, particularly if alkylation is external.

In this connexion the carboxymethyl-enzyme proved interesting in having residual activity (Reynolds & McKinley-McKee, 1970). It also bound the oxidized and reduced coenzymes. Although the dissociation of the binary enzyme-coenzyme complexes may not be the rate-limiting steps as with the native enzyme, the binding properties of the carboxymethyl-enzyme were comparatively similar. Binding is certainly not altered as much as would be expected from introducing an extra anionic group into the active site. This suggests that carboxymethylation does not

take place at the active centre, but that alkylation is external by free iodoacetate in solution. Thus many ligands bound at the active site, including iodoacetate, can protect against alkylation presumably by an induced change in enzyme conformation.

What is the function of the reactive thiol group? The fact that carboxymethylation drastically decreases catalytic activity does not necessarily mean that the thiol group has any positive function at all. Previously inhibition by *p*-chloromercuribenzoate was considered evidence that thiol groups were involved in binding and/or catalysis at the active centre. Criticizing this, it was then suggested that the thiol groups maintained native structure and that reaction with *p*-chloromercuribenzoate disrupted this (Theorell & McKinley-McKee, 1961). However, a reactive thiol group that enables the formation of active carboxymethyl-enzyme need not necessarily be involved in binding, catalysis or maintenance of tertiary structure. It may only be that, when it is modified, a change in the interactions of the polypeptide chain occurs and a much less active enzyme conformation results. Thus for enzyme action so-called 'essential amino acids' may only be essential and have a function when modified.

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Purification of the High-Molecular-Weight Form of Adenosine Deaminase from Rabbit Intestine

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Purified adenosine deaminases from most sources hitherto investigated have been shown to have molecular weights in the region of 35 000. However, recent work has shown that enzymes of higher molecular weight exist. Ma & Fisher (1968) found three adenosine deaminases in the frog having molecular weights of 200 000, 110 000 and 35 000, and these workers have also reported that the enzyme of molecular weight 200 000 is present in the livers of a wide variety of amphibians and mammals (Ma & Fisher, 1969). As yet these high-molecular-weight enzymes have not been characterized.

In this communication a method is presented

for the purification of the high-molecular-weight adenosine deaminase found in rabbit intestine (mol.wt. 260 000). The purification of a low-molecular-weight form of the enzyme (mol.wt. 35 000), which is also present in this tissue, has already been described (Piggott & Brady, 1970).

The initial stages of the purification are essentially those used in the purification of the low-molecular-weight enzyme: extraction with acetate buffer, pH 5.5, precipitation and fractional re-extraction with ammonium sulphate, and chromatography on DEAE-cellulose at pH 5.8. At the DEAE-cellulose step it was observed that the high-molecular-weight enzyme was eluted over a large number of fractions. This necessitated the duplication of all subsequent steps. The enzyme was further purified on Sephadex G-200. The final preparation, which exhibited two protein bands corresponding to enzymic activity on polyacrylamide-gel electrophoresis, had a specific activity of 30 units/mg. This is about ten times less than the value of 300 units/mg obtained with the purified low-molecular-weight form (Piggott & Brady, 1970).

The relationship between the two forms is being investigated. Attempts to convert the high-molecular-weight form into the low-molecular-weight form with urea, detergents and high salt concentrations have not been successful.

Differences have been found in the K_m values and the activation energies of the two forms.

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Inhibition Kinetics of D-Glyceraldehyde 3-Phosphate Dehydrogenase

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The kinetics of ternary-reactant enzymes have been treated theoretically by a number of authors (Cleland, 1963; Dalziel, 1969). In particular Fromm (1967) has suggested the use of inhibitors for determining the kinetic mechanism of such enzymes. This communication describes the inhibition kinetics of rabbit muscle D-glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12).