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.

Reynolds, C. H. & McKinley-McKee, J. S. (1969). *Eur. J. Biochem.* **10**, 474.

Reynolds, C. H. & McKinley-McKee, J. S. (1970). *Biochem. J.* **119**, 801.

Reynolds, C. H., Morris, D. L. & McKinley-McKee, J. S. (1970). *Eur. J. Biochem.* **14**, 14.

Theorell, H. & McKinley-McKee, J. S. (1961). *Acta chem. scand.* **15**, 1811.

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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Ma, P. F. & Fisher, J. R. (1968). *Comp. Biochem. Physiol.* **27**, 105.

Ma, P. F. & Fisher, J. R. (1969). *Comp. Biochem. Physiol.* **31**, 771.

Piggott, C. O. & Brady, T. G. (1970). *Biochem. J.* **119**, 6P.

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).

The enzyme was shown to catalyse the oxidation of 3-hydroxypropionaldehyde 3-phosphate in the presence of NAD^+ and arsenate (or phosphate). With phosphate a sixfold decrease in $V_{\max}$ is seen with little change in K_m . Aldehyde substrate inhibition was also observed and this proved to be uncompetitive against NAD^+ . These results suggest that the liberation of products is ordered with NADH being the last product released (Cleland, 1970).

Product inhibition studies showed NADH to be a competitive inhibitor against NAD^+ and non-competitive against the aldehyde and arsenate, thus confirming the order of product release.

3-Phosphoglycerate, inorganic nitrate and α -glycerophosphate were all shown to be effective dead-end inhibitors. The ordered release of products was further confirmed by the observation that 3-phosphoglycerate was an uncompetitive inhibitor no matter what the nature of the variable substrate. The ratio of the apparent inhibition constants [K_i (phosphate)/ K_i (arsenate)] was about 6, correlating with the $V_{\max}$ ratio for the two acyl acceptors. This suggests that with arsenate as the acyl acceptor the rate-limiting step is partly the release of NADH, whereas with phosphate it probably occurs in the quaternary-ternary complex interconversion.

Nitrate was found to be a competitive inhibitor of arsenate but uncompetitive against the aldehyde and NAD^+ . In contrast α -glycerophosphate, although an uncompetitive inhibitor for NAD^+ , was non-competitive towards the aldehyde and arsenate.

The occurrence of uncompetitive effects is characteristic of ordered sequential mechanisms. It is suggested that a compulsory-order mechanism is operating with the order of addition being NAD^+ , aldehyde and then phosphate (or arsenate) followed by the ordered release of products, first acyl phosphate and then NADH. This conclusion is discussed in relation to previous work on the kinetic mechanism of this enzyme (Furfine & Velick, 1965; Keleti & Batke, 1965; Fahien, 1966).

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Cleland, W. W. (1963). *Biochim. biophys. Acta*, **67**, 104.
Cleland, W. W. (1970). In *The Enzymes* 3rd ed., vol. 2, p. 1. Ed. by Boyer, P. D. New York: Academic Press Inc.

Dalziel, K. (1969). *Biochem. J.* **114**, 547.

Fahien, L. A. (1966). *J. biol. Chem.* **241**, 4115.

Fromm, H. J. (1967). *Biochim. biophys. Acta*, **139**, 221.

Furfine, C. S. & Velick, S. F. (1965). *J. biol. Chem.* **240**, 844.

Keleti, T. (1965). *Acta physiol. Hung.* **48**, 19.

Keleti, T. & Batke, J. (1965). *Acta physiol. Hung.* **48**, 195.

The Lactate Dehydrogenase System of the Rudd (*Leuciscus erythrophthalmus*)

By B. M. BUCKLEY and T. G. BRADY. (*Department of Biochemistry, University College, Cork, Co. Cork, Irish Republic*)

The lactate dehydrogenase isoenzyme systems of many vertebrate species have been extensively studied (Latner & Skillen, 1968). Somatic cells of mammals and birds possess five electrophoretically and kinetically distinguishable forms of the enzyme. This corresponds with their possession of two genes coding for monomeric subunits of the tetrameric enzyme. A third type of subunit has been found in mammalian and avian sperm. Fish, however, have been shown to possess from one to 20 isoenzymes, according to species (Markert & Faulhaber, 1965). The explanation of this in molecular terms is more complex, but it is generally thought to be due to polymorphism of individual subunit-producing genes, or to the presence of several distinct genes.

We have studied the properties of the lactate dehydrogenase isoenzyme system in the rudd (*Leuciscus erythrophthalmus*), a freshwater fish and member of the Order Cypriniformes, which includes carp and goldfish. This latter species is the only member of this Order whose lactate dehydrogenase system has been extensively studied. Our investigations concern the chromatographic, electrophoretic and kinetic properties of the system.

Sephadex chromatography of rudd lactate dehydrogenase shows it to be very similar to the mammalian form in size. The rudd system is similar to that of mammals electrophoretically in possessing five major isoenzymes in most tissues. This is a feature uncommon in fish; Markert & Faulhaber (1965) found only three species to be of this type in an extensive survey. Differences exist between rudd and mammalian systems in both the mobilities of their isoenzymes and the tissue-specific predominance of fast-moving and slow-moving forms. Tissue-specific isoenzymes such as the eye form found in most fish, and the sperm form of mammals and birds, do not appear to be present in the rudd. The goldfish, another cyprinid, has a system different from that of the rudd in that many of its tissues possess up to 12 isoenzymes (Hochachka 1965).

Other properties studied in the rudd include specific activities and isoenzyme distribution among the tissues, effects of substrate and product inhibition, temperature, pH and denaturing agents on activity, and variation of the K_m with temperature.

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