EFFECTS OF METAL-COMPLEXING AGENTS ON MITOCHONDRIAL D-α-HYDROXY ACID DEHYDROGENASE

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Mitochondria from various mammalian sources contain a dehydrogenase capable of catalysing the oxidation of many D-α-hydroxy acids to the corresponding α-keto compounds; no cofactors are required, even after dialysis of the partially purified enzyme (Tubbs and Greville, 1959; Tubbs, 1960). The activity of this enzyme, normally assayed by following the reduction of 2,6-dichlorophenol-indophenol by D-lactate at pH 8.5, is affected in several ways by reagents which can form metal complexes. An enzyme from yeast, showing many similarities to the present one, is inhibited by chelating agents (Curdel, Naslin and Labeyrie, 1959; Boeri, Cremona and Singer, 1960), and it would be of interest to examine this and other enzymes for various effects reported below.

The soluble enzyme is obtained by extracting acetone-dried rabbit kidney mitochondria with 20mM Tris buffer, pH 7.8. Such extracts, or further purified enzyme, show little activity immediately after preparation; however, the activity increases (up to 8-fold) on storing the enzyme at 4° for 2-3 days. Full activity is shown immediately if cyanide (0.5mM) is included in the assay system. The "spontaneous" activation does not affect the K_M for substrate (1-2mM for D-lactate). Cyanide is a competitive inhibitor: the inhibitory species is CN^- ion, not HCN as found in the case of catalase by Chance (1952), and the K_i for CN^- is 1.9×10^{-5}M over the range pH 7.8-8.8.

With fresh enzyme inhibition is only manifest at cyanide concentrations >0.5mM, due to the conflicting activation effect, but after spontaneous activation much lower concentrations inhibit. The foregoing effects result in the observed enzymic activity being a complicated function of the "age" of the enzyme, the pH, and the concentrations of substrate and of cyanide.

Oxalate is a very potent competitive inhibitor; K_i is 5-10 x 10^{-6}M, close to that reported for the yeast enzyme (Boeri et al., 1960). 1,10-phenanthroline (OP) and ethylenediamine-tetra-acetate (EDTA) inhibit progressively: the inhibition is pseudo first-order, and is prevented by substrate (Fig.1).

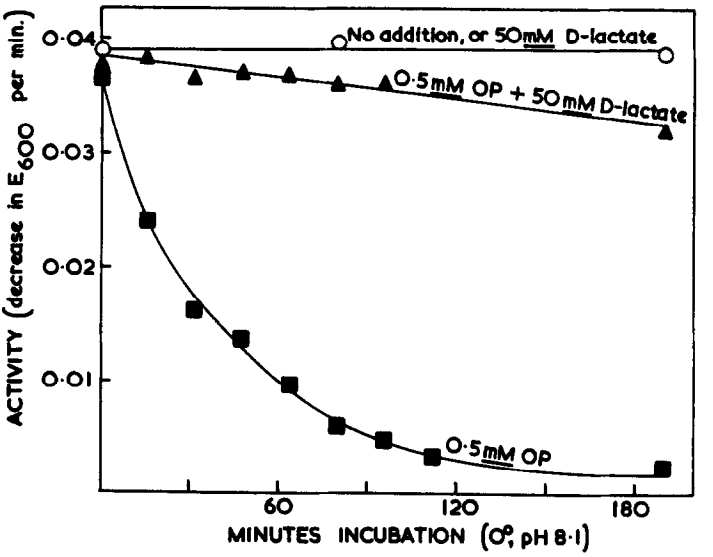

Fig.1. Protection by substrate from 1,10-phenanthroline inhibition.

After incubation as above the enzyme was assayed at 25° in a system containing: Tris-HCl buffer, pH 8.5; 41.5mM; D-lactate, 25mM; 2,6-dichlorophenolindophenol, 3.8 x 10^{-5}M. The reaction was started by addition of enzyme.

The protection is complete at infinite substrate concentration, while the protection constant (Burton, 1951) and the K_M for D-lactate are approximately the same (e.g. 1.25mM at 0° and 2.1mM at 25°, respectively); this suggests that there is a metal component at or near the substrate binding site. This hypothesis is strengthened by the findings that oxalate (ratio $\frac{K_M \text{D-lactate}}{K_i \text{oxalate}}$=350) is more than a hundred times as effective a protective agent as D-lactate, while L-lactate (a weak competitive inhibitor, $\frac{K_M \text{D-lactate}}{K_i \text{L-lactate}}$=0.25) is rather less

effective than the D enantiomorph.

Instead of protecting the enzyme, cyanide potentiates inhibition by OP (Fig.2) and EDTA; the effect is especially marked with the former. No previous

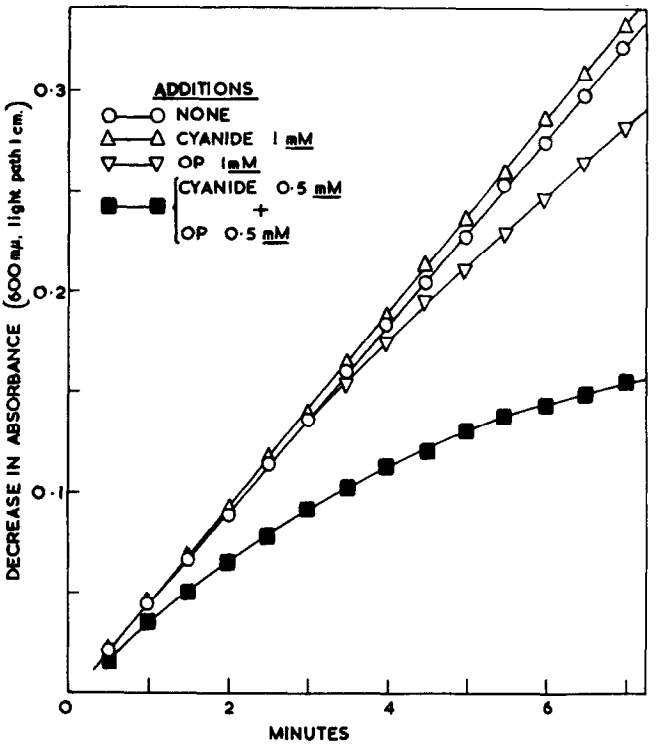

Fig.2. Potentiation of OP inhibition by cyanide.

The assay system contained the components shown, but was otherwise similar to that described for Fig.1.

example of such a potentiation seems to have been reported, although incubation with substrate ($DPN.H_2$) enhances OP inhibition of heart $DPN.H_2$ cytochrome c reductase (Mahler and Elowe, 1954). It seems possible that CN^- may form a complex with the metal component, and subsequently be replaced by the bulky chelating agent to which the metal is almost inaccessible directly; Hope and Prue (1960) have found CN^- to enter a Co(III) complex by substitution even more rapidly than does OH^-, and Schilt (1957) has studied reversible cyanide-OP exchanges in ferrous complexes. No reagent other than cyanide has so far been found to show this potentiation (or to activate fresh enzyme).

After inhibition by EDTA, removal of excess chelating agent by dialysis

does not by itself restore activity. However, after such treatment, various metal ions are capable of this, Zn^{++} being apparently the most efficient (pre-incubation for 30 min. at 0° with 0.5mM Zn^{++} restores almost all the activity). Mg^{++} is only effective in presence of cyanide, and this also assists reactivation by other metals. Indeed, 0.5mM cyanide alone has sometimes caused considerable reactivation, perhaps by a reversal of the suggested potentiation mechanism. Enzyme ostensibly reactivated by Ca^{++} is still fully sensitive to OP, but since Ca^{++} has no stable OP complex it seems likely that an active "Ca-enzyme" is not formed; a possible scheme is:-

(1) Inhibition : Enzyme=metal + EDTA ⟶ Enzyme-metal-EDTA

(2) Reactivation : Enzyme-metal-EDTA + Ca^{++} ⟶ Enzyme=metal + EDTA-Ca

In the case of the yeast enzyme it has been suggested (Curdel et al., 1959; Boeri et al., 1960) that chelating agents actually remove the metal from the enzyme, but it seems more probable, at least with the animal dehydrogenase, that the inhibitors act by binding the metal while leaving this attached to the enzyme.

The ratio $\frac{K_i \text{L-lactate}}{K_i \text{oxalate}}$, about 1500, is similar to the ratio of the dissociation constants of metal mono-lactato and mono-oxalato complexes; indeed the absolute values of the inhibition constants (and the K_M for D-lactate) are comparable to the dissociation constants of the relevant Zn^{++} complexes. The inference from this alone that zinc is the native metal of the enzyme would be completely unjustified, however. The present enzyme does not, of course, belong to the category of DPN-linked dehydrogenases, of which many contain zinc (Vallee, 1955, 1960).

The observations above indicate not only that the dehydrogenase has a tightly bound and somewhat inaccessible metal component, but also that this is situated near to, or actually at, the substrate binding site.

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