Dehydrogenation of D-lactate by a soluble enzyme from kidney mitochondria

Washed-particle preparations from rabbit kidney and liver have been shown to oxidize D-lactate with formation of pyruvate; added DPN did not stimulate the oxidation[1,2]. Oxidation of the D-isomer has also been observed with particles from liver and kidney of the rat[3]. HAUGAARD[4] obtained oxidation of D-lactate by cytochrome-containing particles from *Escherichia coli* and also by acetone powders of these particles; with the powders methylene blue was required as hydrogen acceptor.

In view of the ability of respiratory particles from widely different sources to oxidize D-lactate a study of the enzymes involved seems desirable. In the present work a D-lactate dehydrogenase has been obtained in soluble form from rabbit-kidney mitochondria. The mitochondria from kidney cortex are isolated in 0.25 *M* sucrose, washed twice with 0.125 *M* KCl and made into a thick suspension in 0.25 *M* sucrose. This is added slowly to 15 vol. of acetone at $-10°$; the resulting powder is washed twice with acetone, and dried and stored at 4° over P_2O_5 *in vacuo*. Full activity is retained for several weeks at least. The powder is extracted at 0° with 0.01 *M* tris-(hydroxymethyl)aminomethane–chloride buffer, pH 7.8 (1 ml/20–50 mg). The supernatant after centrifuging at 40,000 × *g* contains the enzyme, and is dialysed overnight against the same buffer.

The dehydrogenase reduces, in presence of D-lactate, a number of one- and two-electron acceptors, *e.g.* 2,6-dichlorophenolindophenol, methylene blue, phenazine methosulphate, ferricyanide, and cytochrome *c*. There is no appreciable oxygen uptake in absence of carrier. Paper chromatography indicates formation of pyruvate. The routine assay system is shown in Fig. 1. Under these conditions the rate is independent of indophenol concentration within the range tested (10^{-5} to 10^{-4} *M*), the unit of activity is defined as the amount of enzyme causing a change of absorbancy at 600 mμ of 0.01/min, and the velocity is proportional to enzyme concentration in the range 0.2–2 units/ml. The optimum pH is about 8.1, while K_m for D-lactate is $3.4 \cdot 10^{-3}$ *M*. The extract, when fully activated (see below), normally contains 15–30 units/ml and has an absorbancy (1 cm) of 5–10 at 280 mμ. 20-fold purification has been obtained using a diethylaminoethylcellulose column.

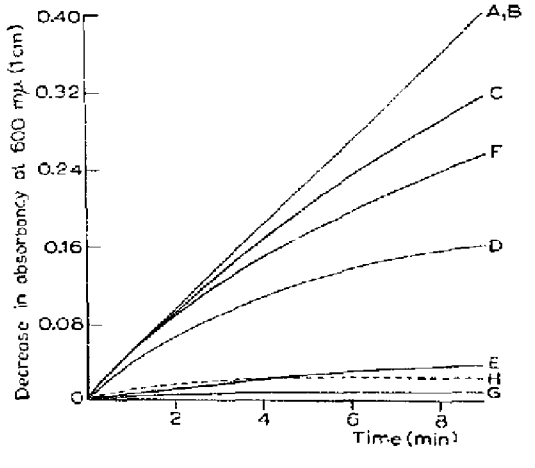

Fig. 1. Reduction of 2,6-dichlorophenolindophenol by D- and L-lactate and effects of chelating agents. Assay system: 0.022 *M* potassium D-lactate; 0.021 *M* tris(hydroxymethyl)aminomethane, pH 8.1; $3.8 \cdot 10^{-5}$ *M* dye; final vol. 3 ml. Dialysed extract, 2.4 mg protein. Temp. 20°. A, no addition; B, 10^{-3} *M* cyanide; C, 10^{-3} *M* o-phenanthroline, D, $5 \cdot 10^{-4}$ *M* cyanide + $5 \cdot 10^{-4}$ *M* o-phenanthroline; E, enzyme pre-incubated with 10^{-3} *M* o-phenanthroline for 3 h at 0°; F, $5 \cdot 10^{-4}$ *M* ethylenediaminetetraacetate. In G, no substrate; in H, substrate was 0.022 *M* L-lactate.

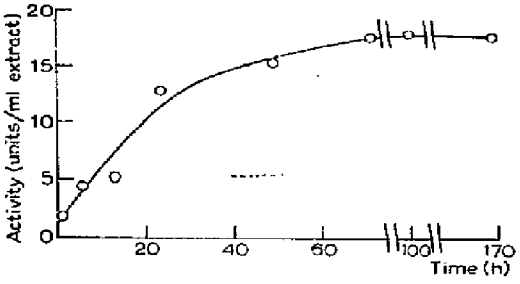

Fig. 2. Effect of storage at 4° after extraction.

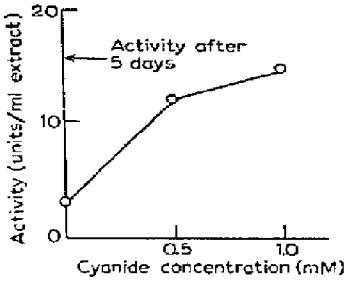

Fig. 3. Effect of cyanide in assay system with fresh enzyme, compared with activation by ageing at 4°.

The activity of extracts is very low immediately after preparation but increases with time (Fig. 2). Dialysis against tris(hydroxymethyl)aminomethane buffer has no effect on the rate of activation, but dilute cyanide raises the activity of fresh extract almost to the maximal value (Fig. 3). An explanation of these effects is being sought; possibly removal of an inhibitory metal is concerned.

After the enzyme has been activated by ageing, cyanide (10^{-3} *M*) inhibits by 0 to 35 %; various chelating agents are strong inhibitors with all acceptors tested. This, and particularly the progressive nature of the inhibition (Fig. 1), suggest the presence of a bound, essential metal. Cyanide ($5 \cdot 10^{-4}$ *M*) greatly potentiates *o*-phenanthroline inhibition. L-Lactate, glycollate, aureomycin (20 μg/ml), DPN and adenosine triphosphate neither inhibit nor stimulate. *p*-Chloromercuribenzoate, Cu^{2+} and Hg^{2+} strongly inhibit.

The physiological significance of this enzyme is not clear; D-lactate, although produced by various bacteria, has not been detected in animals. The lactate formed by glyoxalase is the D-isomer[5], but the extent of this formation *in vivo* is uncertain (see, however, ref. [6]). With the exception of L-lactic, all α-hydroxy fatty acids in animals, such as those occurring in cerebrosides, wool wax and, possibly, butter fat, are of D-configuration[7,8]. It is of interest that a *Lactobacillus casei* mutant requires D-α-hydroxy acids for growth[8].

Further study and purification of the enzyme are proceeding, with a view to investigating the metal and possible flavin components; distribution and substrate specificity are also being examined.

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