Characterisation of two malic dehydrogenases from rat liver

Differential centrifugation of a rat-liver homogenate has shown that malic dehydrogenase activity is located in both the mitochondrial and supernatant fractions[1]. The mitochondrial and supernatant enzymes have been shown to differ in their oxaloacetate-reduction kinetics[2], and in their electrophoretic mobilities[3]. In the present work a separation and partial purification of the two enzymes has been achieved, and some further comparisons made.

The rat livers were homogenised in 0.25 *M* sucrose and centrifuged. The 600–3,000 × *g* (15 min) precipitate and the 10,000 × *g* (30 min) supernatant were retained, as "mitochondrial" and "supernatant" fractions respectively. To release malic dehydrogenase from the mitochondria, an acetone powder was made, and this powder extracted with 0.05 *M* sodium phosphate, pH 7.0. Use of $(NH_4)_2SO_4$ fractionation, calcium phosphate adsorption and elution, and chromatography on Amberlite IRC 50 resin and DEAE-cellulose effected a 100-fold purification of the mitochondrial and a 250-fold purification of the supernatant enzyme. The two enzymes differ significantly in their chromatographic behaviour. The mitochondrial enzyme is retarded, and the supernatant enzyme unretarded by the Amberlite resin, whereas the reverse is true of the DEAE-cellulose (Fig. 1). The elution patterns are repro-

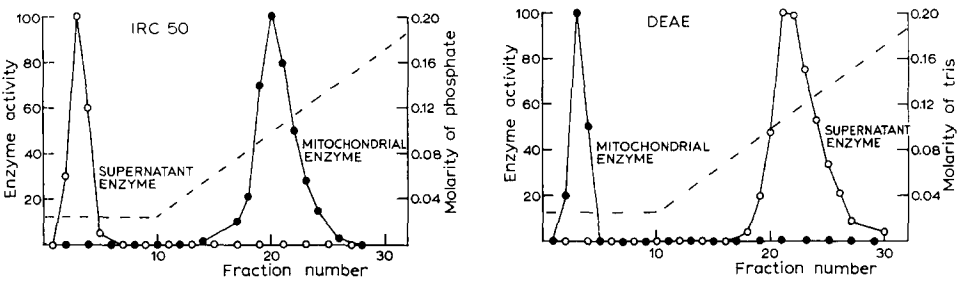

Fig. 1. Chromatography of rat-liver malic dehydrogenases on Amberlite IRC 50 and on DEAE-cellulose. Open circles, supernatant enzyme; solid circles, mitochondrial enzyme; broken line, molarity of eluting buffer (sodium phosphate, pH 7.0 for Amberlite; tris(hydroxymethyl)-aminomethane, pH 8.2 for DEAE-cellulose).

ducible and characteristic, whether the enzymes are run separately or as a mixture, or when a crude rat-liver homogenate is tested. Pig-heart mitochondrial malic dehydrogenase, prepared by a modification of the method of WOLFE AND NEILANDS[4], showed the same elution patterns as the rat-liver mitochondrial enzyme.

The substrate specificities of the enzymes were determined by comparing the rates of oxidation of malate and tartrate, and the rates of reduction of DPN and some of its analogues (a gift from Prof. N. O. KAPLAN) (Table I).

Both rat-liver enzymes reduce oxaloacetate, the rate of reduction being affected by the inhibition due to high substrate concentration. A plot of reaction velocity against oxaloacetate concentration gives a curve with a maximum at a concentration which depends on both the pH and the nature of the buffer used. The position of the maximum, under defined conditions, distinguishes between the two enzymes;

Abbreviations: DEAE, diethylaminoethyl-; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; APDPN, acetylpyridine analogue of DPN; TNDPN, thionicotinamide analogue of DPN; DeDPN, deamino analogue of DPN.

TABLE I

OXIDATION OF MALATE AND TARTRATE, AND REDUCTION OF DPN AND ITS ANALOGUES BY MALIC DEHYDROGENASES FROM DIFFERENT SOURCES

Reaction rates expressed as percentages of the rates with L-malate (20 mM) and DPN (1.2 mg/ml or 0.3 mg/ml, as shown); 0.1 M glycine–NaOH buffer, pH 10.0 (*cf.* ref. 4) used throughout.

Substrate		*Coenzyme*	*Enzyme source*		
			Rat-liver supernatant	*Rat-liver mitochondria*	*Pig-heart mitochondria*
		(1.2 mg/ml)			
L-malate	20 mM	DPN	100	100	100
L-tartrate	100 mM	DPN	0.16	1.54	1.79
meso-tartrate	50 mM	DPN	0.15	1.05	1.01
		(0.3 mg/ml)			
L-malate	20 mM	DPN	100	100	100
L-malate	133 mM	DPN	86	104	103
L-malate	20 mM	APDPN	83	105	90
L-malate	133 mM	APDPN	38	66	56
L-malate	20 mM	DeDPN	41	65	69
L-malate	20 mM	TNDPN	42	16	16
L-malate	20 mM	TPN	0	0	0

in 0.1 M sodium phosphate, pH 7.5, ethylenediaminetetraacetate $2 \cdot 10^{-3}$ M, and DPNH 0.1 mg/ml, it lies at $5 \cdot 10^{-4}$ M oxaloacetate for the supernatant enzyme, and at $2 \cdot 10^{-4}$ M oxaloacetate for the mitochondrial enzyme. On substituting 0.1 M tris(hydroxymethyl)aminomethane chloride, pH 7.6, for the phosphate buffer, the values become $5 \cdot 10^{-4}$ M and $6 \cdot 10^{-5}$ M respectively.

Paper electrophoresis of the two enzymes, in 0.06 M veronal, pH 8.6, for 12 h at 10 V/cm, 4°, confirmed (see ref. 3) that the supernatant enzyme moves toward the anode (5 cm under the conditions used), whereas the mitochondrial enzyme remains at the origin. Pig-heart mitochondrial malic dehydrogenase also remains at the origin.

The malic dehydrogenases of the rat-liver supernatant and of rat-liver mitochondria differ, then, in a number of respects, the latter resembling closely the enzyme of pig-heart mitochondria. Malic dehydrogenases from other sources are currently being investigated.

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