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L-Malate dehydrogenase of the sea urchin *Strongylocentrotus purpuratus*

The occurrence of two categories of L-malate dehydrogenase (EC 1.1.1.37) is now widely recognized in a variety of animals[1–8], and more recently also in a sea urchin[9]. One of these categories occurs exclusively in a supernatant fraction, whereas the other is found in a mitochondrial fraction as well. For various species, these categories have been further differentiated with respect to catalytic, physical, and immunological properties. During the course of experiments to explore biosynthesis of L-malate dehydrogenase in echinoderm development, a comparable distinction between supernatant and particulate enzymes has been found in the pluteus larvae of the sea urchin *Strongylocentrotus purpuratus*. The present communication reports biochemical properties of the sea-urchin particulate enzyme.

In this species two distinct electrophoretic components can be separated by disc electrophoresis in acrylamide gel (Table I). Total homogenates prepared in

TABLE I

ELECTROPHORETIC COMPONENTS OF L-MALATE DEHYDROGENASE IN THE SEA URCHIN *S. purpuratus*

Total homogenates, about 60 μg protein, in 0.01 M Tris chloride (pH 7.6) were made in 20% sucrose and analysed by disc electrophoresis[20]. Electrophoretic run was 50 min at 2 mA per tube at room temperature. Each gel was incubated for 10 min at room temperature in 2 ml of a solution containing 0.2 mg phenazine methosulfate, 0.8 mg nitro blue tetrazolium, 70 μmoles glycine–NaOH (pH 10), 1 μmole NAD^+, and 10 μmoles L-malate. Relative mobility is defined as the ratio of the distance from the origin to the migrated enzyme over that to the bromo-phenol blue dye front. The values represent the results of three determinations.

Component	*Av. relative mobility*	*Range*	*Localization*
1	0.56	0.55–0.57	Soluble and particulate fraction
2	0.41	0.40–0.43	Soluble fraction

various media (0.4–0.5 M sucrose, 0.75 M mannitol, distilled water, 0.1 M and 0.5 M KCl) and the supernatant fractions obtained by centrifugation under various conditions give gel preparations with the same two components. In contrast to the two components present in *S. purpuratus*, four components were reported from embryos of the sea urchin *Arbacia punctulata* at the corresponding stage of development[10]. Whether the components of the latter species may also include conformers[11] has not been determined.

To examine the possibility that one of the components in *S. purpuratus* may occur in mitochondria, a particulate fraction, comparable to the sea-urchin mitochondrial fraction of MAGGIO[12] was prepared in the following manner: embryos washed twice with 1.5 M dextrose *plus* 0.001 M EDTA, pH 8.0, were suspended to 15% (w/v) in a homogenizing medium (0.44 M sucrose–0.01 M Tris chloride (pH 7.6)–0.001 M $MgCl_2$) and were blended with a Virtis homogenizer at 30 V for 2 min. The supernate of the initial 750 × *g*, 5 min centrifugation was subjected to another centrifugation at 1700 × *g* for 20 min. The pellet was re-suspended in the homo-

genizing medium with a teflon-glass homogenizer and re-centrifuged. The second pellet was suspended in the medium (0.44 M sucrose–0.01 M Tris chloride (pH 7.6)–0.001 M EDTA) and centrifuged at 750 × *g* for 5 min. 5 ml of the supernate was layered over 45 ml of 1.5 M sucrose *plus* 0.01 M Tris chloride (pH 7.6) *plus* 0.001 M EDTA, gently mixed with the upper two-thirds of the latter solution, and centrifuged in Spinco rotor SW 25.2 at 20 000 rev./min for 3 h. A pellet was recovered from the bottom of the centrifuge tubes. A small portion was immediately fixed for electron-microscopic examination and the rest was suspended in cold 0.01 M Tris chloride (pH 7.6) and sonicated twice at setting 4 on Sonifier Model LS-75 (Branson Instruments, Inc.) for 30 sec with a 1-min interval to release the enzyme. The solution was then cleared by centrifugation at 1700 × *g* for 20 min. This solution, when analysed by disc electrophoresis as above, was shown to contain only Component 1.

Electron-micrographs of the fraction revealed that it contains not only intact mitochondria, but other cellular inclusions such as Golgi apparatus, irregular vesicular bodies, and some nuclei. Therefore, while this enzyme may be located on mitochondria as in other animals, the positive identification of the site is not yet possible.

The mitochondrial enzyme of the vertebrate animals in general is the less anionic species of the two forms and possesses a slower electrophoretic mobility[2–6,8,13–15]. The exception to this rule is the tuna mitochondrial enzyme[7]. The latter enzyme migrates ahead of the supernatant enzyme in starch-gel electrophoresis. The major component of a particulate fraction of *A. punctulata* migrates faster toward the anode by disc electrophoresis than does that of a soluble fraction[9]. The single particulate enzyme of the present species also exhibits a greater mobility in a similar electrophoresis system. Whether the mobility in the present case reflects charge properties alone or selection on the basis of size and shape of the molecule by the gel matrix has not been examined.

Certain kinetic properties were determined with the particulate enzyme prepa-

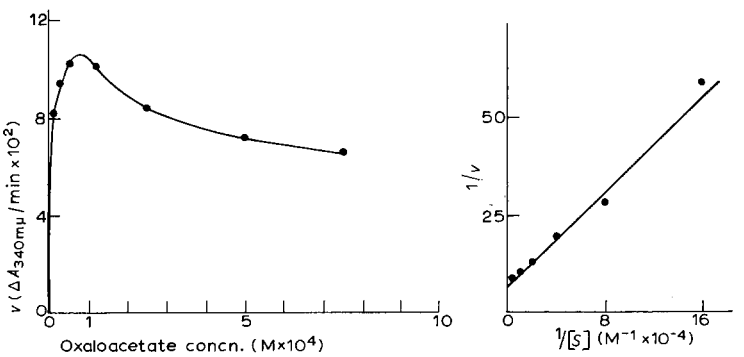

Fig. 1. Substrate–activity relationship of the sea-urchin particulate L-malate dehydrogenase. Enzyme activity (*v*) was assayed by the method of OCHOA[21] which was scaled down to a total of 1 ml vol. containing 25 μmoles Tris chloride (pH 7.4), 0.05 μmole NADH and varying amounts of oxaloacetate. Reduction of absorbance at 340 mμ was measured with a Model 2000 Gilford absorbance recorder at 20°.

Fig. 2. Coenzyme–activity relationship of the sea-urchin particulate L-malate dehydrogenase. Enzyme activity (*v*) was determined as described in the legend for Fig. 1. Varying amounts of NADH ([*S*]) were added to the reaction mixture.

ration. High oxaloacetate concentration is inhibitory for the enzymic reaction (Fig. 1). Such inhibition is known to occur with mitochondrial enzymes of many animals[1,2,6–8,16,17]. The oxaloacetate concentration for the maximal activity is 10^{-4} M, which is the same order of magnitude as in other instances[2,6,7,17]. In the reaction, the K_m for the coenzyme NADH was determined as $5 \cdot 10^{-4}$ M (Fig. 2), which is also similar to the value obtained from a mammalian mitochondrial enzyme[4]. Phosphate and ammonium sulfate act as activators of a mammalian malate dehydrogenase[18]. Moreover, the mitochondrial enzyme alone is activated by phosphate ion[19]. It was found that the sea-urchin particulate enzyme is activated by both of these agents (Table II).

In summary, the present data demonstrate that in the pluteus larvae of the sea urchin *S. purpuratus*, L-malate dehydrogenase is resolved into two forms, one

TABLE II

ACTIVATION BY PHOSPHATE AND AMMONIUM SULFATE OF THE SEA-URCHIN PARTICULATE L-MALATE DEHYDROGENASE ACTIVITY

Enzyme activity (v) was determined as described in the legend for Fig. 1. The control reaction mixture contained 25 μmoles Tris chloride (pH 7.4), 0.05 μmole NADH and 0.25 μmole oxaloacetate in a total of 1 ml. The values are the average of the duplicate determinations.

	Activity ($v \times 10^2$)	% *Activation*
Control	4.1	—
+ 0.05 M potassium phosphate	6.3	55%
+ 0.005 M ammonium sulfate	4.9	21%
+ 0.01 M ammonium sulfate	4.8	20%

occurring only in a soluble fraction of the cell, and the other occurring also in association with a particulate fraction. The latter enzyme is kinetically similar to mitochondrial enzymes of other animals and perhaps is also localized in the mitochondria.

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The disappearance of ^{14}C-labelled isoenzyme 5 of L-lactate dehydrogenase from plasma

In a previous investigation it was observed that intravenously injected isoenzyme 5 of lactate dehydrogenase (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27) disappeared from plasma much more rapidly than isoenzyme 1 (ref. 1). The present experiments were performed using ^{14}C-labelled LDH 5 to find out whether the rapid decline of enzymic activity in plasma following LDH 5 injection represents a genuine disappearance of circulating LDH 5 molecules. Since iodoacetate reacts only with the non-essential thiol groups of lactate dehydrogenase[2], ^{14}C-labelled LDH 5 was prepared by incubating purified sheep muscle LDH 5 with [2-^{14}C]iodoacetate. The radioactive carboxymethylated isoenzyme was injected intravenously into sheep and the rate of disappearance of enzymic activity compared with that for radioactivity.

Preparation of ^{14}C-labelled LDH 5. Total lactate dehydrogenase activity was determined by the method of HENRY *et al.*[3]. The enzyme units are the μmoles of NADH oxidized per min at 25°. LDH 5 was partially purified from minced sheep hind leg muscles as described previously[1]. The remaining impurities were removed by electrophoresis on the vinyl copolymer Pevikon (Shandon Scientific Co. Ltd., London, England) in 0.07 M barbitone buffer, pH 8.6, at 2.5 V/cm for 48 h. The entire purification was performed at 4° in a cold room.

Three batches of purified sheep LDH 5 were incubated at 37° with [2-^{14}C]-iodoacetate, specific activity 7.0 μC/μmole, from The Radiochemical Centre, Amersham, Bucks., England (Table I). The labelled enzyme sedimented in the ultracentrifuge as a single peak ($s_{20,w}$ 7.42) with a trace of a slightly heavier impurity.

Abbreviations: LDH 5, isoenzyme 5 of lactate dehydrogenase; LDH 1, isoenzyme 1 of lactate dehydrogenase.