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ISOLATION AND PARTIAL CHARACTERIZATION OF SUPERNATANT AND MITOCHONDRIAL SHRIMP MUSCLE MALATE DEHYDROGENASES

J. L. HODNETT, J. E. EVANS,* H. B. GRAY, JR. AND A. H. BARTEL

Department of Biophysical Sciences, University of Houston, Houston, TX 77004, U.S.A.

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Abstract—1. The supernatant and mitochondrial forms of muscle malate dehydrogenase (E.C. 1.1.1.37) isolated from two closely related species of shrimp have molecular weights and hydrodynamic properties similar to those previously reported for the corresponding enzymes of vertebrates.

2. The catalytic properties of these species more closely resemble those of the vertebrate malate dehydrogenases than those of lower eukaryotes.

3. The electrophoretic behavior of the shrimp malate dehydrogenases indicates that they are comprised of two subunits.

4. The subunits of the shrimp enzymes are non-identical and give rise to three electrophoretically distinguishable isoenzymes of each dehydrogenase.

INTRODUCTION

DIFFERENCES in the chemical and physical properties of the supernatant and mitochondrial forms of malate dehydrogenase (MDH†) have been observed for numerous vertebrate species. The kinetic, electrophoretic, and other physical properties of purified malate dehydrogenase isoenzymes of beef heart (Englard & Breiger, 1962), chicken heart (Kitto & Kaplan, 1966), ox kidney (Dupourque & Kun, 1968), tuna heart (Kitto & Lewis, 1967) and salmonid fish muscle (Bailey *et al.*, 1970) have been reported. Few studies have been carried out to date with purified malate dehydrogenases of marine invertebrates. The electrophoretic and kinetic properties of the supernatant and mitochondrial malate dehydrogenases of a marine snail (Meizel & Markeit, 1967) and a sea urchin (Ozaki & Whiteley, 1970) have been determined.

This communication reports the purification and properties of the mitochondrial and supernatant muscle malate dehydrogenase isoenzymes of the white shrimp, *Penaeus setiferus* (Linnaeus) and the brown shrimp, *Penaeus aztecus* (Ives), which are two closely related migratory species inhabiting the Gulf of Mexico. These shrimp were obtained during that portion of their life cycle which occurs in the estuarine system. Our main interest was to adequately characterize the chemical and physical properties of the purified shrimp isoenzymes for comparison with those of the corresponding malate dehydrogenases of various vertebrate species.

* Present Address: Environmental Chemistry Division, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD 21010, U.S.A.

† *Abbreviations used*: NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; EDTA, ethylenediaminetetra-acetic acid; DEAE, diethylaminoethyl; CM, carboxymethyl.

MATERIALS AND METHODS

Materials

Live juvenile and sub-adult shrimp from the Galveston and Trinity bays of Texas were identified and separated according to the descriptions of Moffett (1967). The shrimp were decapitated and the whole tail sections were frozen in plastic containers immersed in acetone–dry ice. The tissue was stored at −20°C until used for experiments.

NAD, NADH, oxalacetate, L-malic acid, D-malic acid, 2-mercaptoethanol, ammonium sulfate, nitro blue tetrazolium, and phenazine methosulfate were purchased from Sigma Chemical Company. Purified acrylamide, N,N'-methylene-bis-acrylamide, DEAE-cellulose, and CM-cellulose were products of Bio-Rad Laboratories. Ultra pure urea and Sephadex G-150 were purchased from Schwartz-Mann and Pharmacia, respectively. All other chemicals were standard reagent grade.

Enzyme assays and kinetic studies

During purification procedures protein was measured spectrophotometrically at 280 nm. Enzyme concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Enzyme assays were performed at approx 25°C using the spectrophotometric procedure of Yoshida (1969). L-malate oxidation and oxalacetate reduction were determined by measuring the increased absorption at 340 nm due to NAD reduction and the decreased absorption at 340 nm due to NADH oxidation, respectively. Specific activities are expressed as μmoles coenzyme utilized/min per mg enzyme protein.

Values of the Michaelis constants (K_m) for L-malate oxidation and oxalacetate reduction were determined from plots of reciprocal reaction velocity vs reciprocal substrate concentration (Lineweaver–Burk plots). Measurements were at approx 25°C in 0·1 M potassium phosphate, pH 7·5. The duplicate determinations made at each substrate concentration were in good agreement and the above plots were linear. The substrate concentrations were kept below values for which substrate inhibition was observed (Figs. 4,5).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed using 7·5% acrylamide gels as described by Dietz & Lubrano (1967). A specific tetrazolium staining procedure described by Van der Helm (1961) was used to localize protein bands with malate dehydrogenase activity. 40 μl protein samples (in 30% sucrose) were layered directly onto the gels. The electrophoresis was performed for 90 min at 2·5 mA/gel. For electrophoresis under conditions of protein denaturation, enzyme samples which had been exposed to 6 M urea and 1 mM 2-mercaptoethanol for 24 hr at room temperature were layered onto 7·5% gels containing the above concentrations of urea and 2-mercaptoethanol.

Detection of malate dehydrogenase from mitochondria

Mitochondria were prepared from shrimp muscle immediately following the sacrifice of the animals. The tissue was minced into ice-cold 0·25 M sucrose and homogenized in a Waring blender for 30 sec. The mitochondria were isolated by differential centrifugation and washed 3 times with 0·25 M sucrose. A mitochondrial suspension was layered onto 28 ml sucrose step gradients consisting of equal vol of 0·75, 1·0, 1·3 and 1·75 M sucrose. The gradients were centrifuged for 1 hr at 20,600 rev/min in a Beckman SW25.1 rotor. The mitochondria, which layered upon the 1·3 M sucrose step, were collected by gentle aspiration and diluted 4-fold with 0·25 M sucrose. The sucrose step gradient was repeated as above. The mitochondria were collected by centrifugation at 20,000 rev/min for 10 min and suspended in 5 mM potassium phosphate (pH 7·5) containing 1 mM EDTA and 1 mM 2-mercaptoethanol. The mitochondrial suspension was subjected to three freeze-thaw cycles using acetone–dry ice and then centrifuged to remove all particulate material. This extract was subjected to polyacrylamide gel electrophoresis and the gels stained using the tetrazolium procedure (Van der Helm, 1961) to determine the electrophoretic behavior of the mitochondrial MDH isoenzymes.

Analytical ultracentrifugation

Ultracentrifugation studies were performed using a Beckman model E analytical ultracentrifuge equipped with a monochromator, photoelectric scanner system, and multiplexer system. Boundary sedimentation velocity experiments were performed at enzyme concentrations of about 0·4 mg/ml in 0·05 M potassium phosphate (pH 7·5) containing 1 mM EDTA and 1 mM 2-mercaptoethanol. Centrifugation was performed at 52,000 rev/min at 20°C with the monochromator adjusted to 265·4 nm. The positions of the sedimenting boundaries were determined from the photoelectric recordings of optical density vs distance from the axis of rotation using the half-height method. The observed sedimentation coefficients were corrected to standard $S_{20,w}$ values by assuming a partial specific vol of 0·740 ml/g as reported for chicken heart MDH by Kitto & Kaplan (1966), and using experimentally determined values for the density (1·0053 g/ml) and viscosity relative to water (1·0209) of the solvent.

Sedimentation equilibrium studies were performed with enzyme solutions of about 0·25 mg/ml in the same solvent. The equilibrium profiles were scanned after centrifugation at 13,000 rev/min at 20°C for 30 hr. Following collection of the equilibrium data, the rotor was accelerated to 44,000 rev/min to obtain a meniscus optical density baseline corresponding to zero protein concentration. Molecular weights were calculated from the least-squares slope of a plot of ln C vs r^2, where C is proportional to the enzyme concentration at a distance r from the axis of rotation, according to the standard equation for sedimentation equilibrium in two-component systems. Correction was made for the non-linear dependence of the displacement of the scanner recording pen upon optical density.

At the low protein concentrations employed in the analytical ultracentrifugation experiments, thermal convection in the sample cell may occur with the use of the standard Beckman rotor temperature control system. This problem has indeed been observed in the instrument used in these studies in attempted sedimentation equilibrium experiments at low protein concentrations (Gray, H. B., Jr., unpublished). Accordingly, the modified temperature control system described by Hearst & Gray (1968) was employed. The small amounts of purified MDH available precluded the use of the schlieren and Rayleigh optical methods in these studies.

RESULTS

Isolation of shrimp muscle supernatant and mitochondrial malate dehydrogenases

For each preparation tissue from only one of the two shrimp species was used. These isolations were performed using about 200 g of the frozen shrimp muscle. All procedures were carried out at 4°C.

Step 1. Tissue extract. The frozen muscle was subjected to three freeze-thaw cycles to rupture mitochondria. The tissue was cleaned of exoskeletal material, weighed, and minced into 2 vol of 5 mM potassium phosphate (pH 7·5) containing 1 mM EDTA and 1 mM 2-mercaptoethanol (buffer A). The tissue was blended in a Waring blender for 1 min. The resulting slurry after stirring in a Waring blender for 1 min was diluted 1:3 with buffer A and homogenized in a large glass-Teflon tissue homogenizer. This homogenate was centrifuged for 30 min at 8,500 g. The supernatant was decanted and used as the source of both supernatant and mitochondrial MDH.

Step 2. Ammonium sulfate fractionation. Successive ammonium sulfate fractionation of the extract showed that the protein precipitate from the 40 to 80% (240–520 g/l) fraction contained about 60% of the original MDH activity and all the isoenzyme electrophoretic components found in the unfractionated tissue extract. The precipitate was dissolved in as small as possible a volume of buffer A and dialyzed to equilibrium against three sequential 2 l vol of the same buffer.

Step 3. DEAE-cellulose chromatography. The dialyzed protein solution was placed on a 2·5 × 40 cm column of DEAE-cellulose (previously equilibrated with buffer A) and eluted with a linear gradient formed using 500 ml vol of buffer A and 0·1 M potassium phosphate (pH 7·5) containing 1 mM EDTA and 1 mM 2-mercaptoethanol. Fractions were assayed for absorption at 280 nm and for MDH activity. A protein peak possessing MDH activity eluted near the beginning of the gradient. A second malate dehydrogenase peak eluting at a phosphate concentration of approx 0·045 M contained electrophoretic components identical to those found in the purified mitochondrial extracts. These two enzyme fractions were pooled separately and concentrated in an Amicon A-50 ultrafiltration cell equipped with a UM-10 filter. The samples were dialyzed to equilibrium against several 1 liter vol of buffer A.

Step 4. CM-cellulose chromatography. The dialyzed enzyme samples were applied to separate 1·5 × 20 cm CM-cellulose columns (previously equilibrated against buffer A). Neither enzyme fraction was absorbed to the CM-cellulose under these conditions,

Table 1. Summary of the purification of supernatant and mitochondrial malate dehydrogenases from the brown shrimp, *Penaeus aztecus*

Fractionation Step	Total Protein (mg)	Total enzyme units (μmoles/min)	Specific activity (units/mg)	Purification Factor
1. Tissue extract	5925	2720	0.46	1
2. Ammonium sulfate precipitation	248	1568	6.3	14
3. DEAE-cellulose chromatography				
supernatant	31.6	305	9.8	21.3
mitochondrial	29.5	330	11.1	24.1
4. CM-cellulose chromatography				
supernatant	20.9	234	11.2	24.4
mitochondrial	18.3	271	14.7	31.9
5. Sephadex G-150 chromatography				
supernatant	2.3	110	49.0	106
mitochondrial	3.2	247	76.2	165

but other proteins were retained by the columns. The enzyme fractions were pooled, concentrated as described above, and dialyzed against several 1 liter vol of 0·05 M potassium phosphate (pH 7·5) containing 1 mM EDTA and 1 mM 2-mercaptoethanol (buffer B).

Step 5. Sephadex G-150 chromatography. The enzyme fractions were layered onto separate 2·5 × 50 cm Sephadex G-150 columns previously equilibrated with buffer B. The fractions having the highest MDH activities were pooled and concentrated as described above to about 0·5 mg/ml.

The results of a typical isolation procedure using 200 g of shrimp muscle are summarized in Table 1.

Electrophoretic properties

Preliminary experiments with the 40–80% ammonium sulfate precipitable fractions of shrimp muscle MDH showed that these isoenzymes have ion-exchange properties on DEAE-cellulose very similar

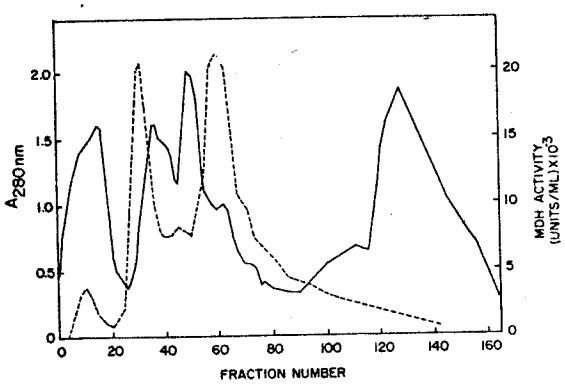

Fig. 1. Elution pattern of malate dehydrogenase obtained in step 2 of the purification procedure (described in the Results and Discussion) from DEAE-cellulose. Fractions 25–40 were pooled as the supernatant enzyme and fractions 50–65 were pooled as the mitochondrial enzyme. The fractions contained about 3 ml each. A_{280} nm (——), malate dehydrogenase activity (-------).

to those of the corresponding malate dehydrogenases of tuna heart (Kitto & Lewis, 1967) or the sea urchin (Ozaki & Whiteley, 1970). The second shrimp muscle MDH peak obtained during the DEAE-cellulose chromatography (Fig. 1) was observed to contain electrophoretic components identical to those found in the purified mitochondrial extract. Therefore, based on electrophoretic evidence, the peak I fraction corresponds to the supernatant isoenzymes and the peak II fraction corresponds to the mitochondrial isoenzymes.

Figure 2 shows the electrophoretic patterns (under non-denaturation conditions) of the supernatant and mitochondrial isoenzymes of the brown shrimp. Similar examination of the white shrimp isoenzymes showed nearly identical MDH isoenzyme electrophoretic patterns. The protein of the supernatant enzyme (Fig. 2 A, B) appears in three bands when stained for either total protein or specifically for MDH activity. The mitochondrial enzyme (Fig. 2 C, D) shows four bands with either the total protein staining or the specific MDH staining and is apparently contaminated by another mitochondrial NAD-dependent dehydrogenase. The three closely spaced anodal bands which show the majority of the staining for the MDH activity must represent the malate dehydrogenase isoenzymes, while the uppermost poorly stained band is presumably due to the minor protein contaminant. One possibility for such contamination is mitochondrial L-3-hydroxyacyl coenzyme A dehydrogenase. Noyes *et al.* (1974) reported that the pig heart mitochondrial enzymes MDH and L-3-hydroxyacyl coenzyme A dehydrogenase are very similar structurally, extraordinarily difficult to separate by chromatographic procedures, and very similar in their substrate specificities.

The shrimp mitochondrial isoenzymes have a slightly greater rate of migration towards the anode which suggests, in accord with the ion-exchange chromatographic behavior, that they are less positively charged compared to the supernatant forms. The relative elution positions in ion-exchange chroma-

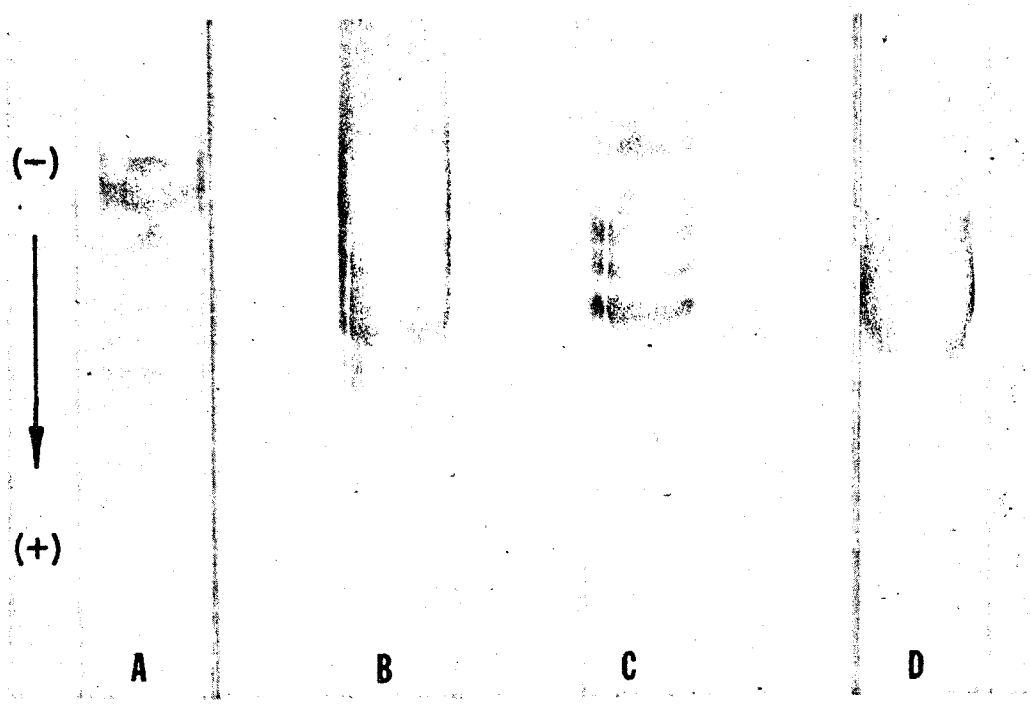

Fig. 2. Electrophoresis of *Penaeus aztecus* muscle supernatant and mitochondrial malate dehydrogenase in 7·5% polyacrylamide gels. Gels A and B are the supernatant isoenzymes; gels C and D are the mitochondrial isoenzymes. Gels A and C were stained with 0·1% amido black; gels B and D were stained using the specific malate dehydrogenase tetrazolium procedure (Van der Helm, 1961).

tography of the supernatant and mitochondrial malate dehydrogenases from the shrimp muscle of these studies, as well as those of the corresponding enzymes from tuna heart (Kitto & Lewis, 1967) and sea urchins (Ozaki & Whitelèy, 1970) are reversed compared to those of most organisms studied to date (e.g. Peak *et al.*, 1972). The same statement with regard to the relative electrophoretic mobilities may be made for

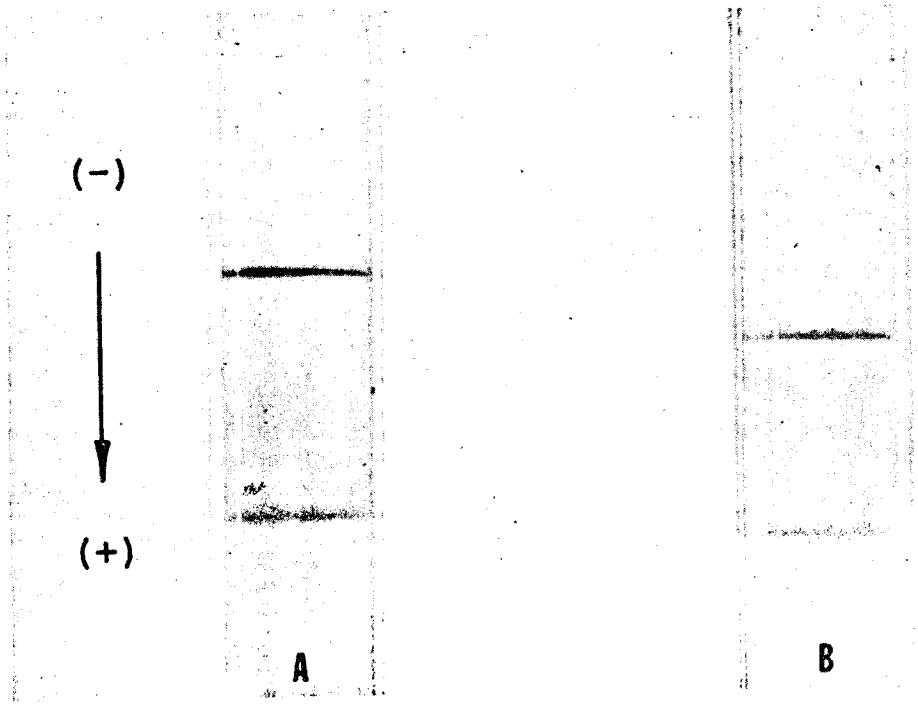

Fig. 3. Electrophoresis of urea-denatured *Penaeus aztecus* muscle supernatant (A) and mitochondrial (B) malate dehydrogenases in 7·5% polyacrylamide gels containing 6 M urea and 1 mM 2-mercaptoethanol. The gels were stained with 0·1% amido black.

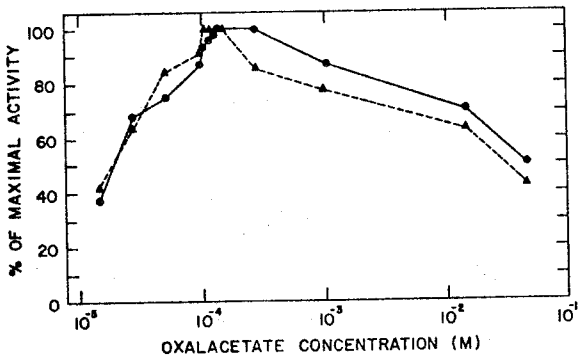

Fig. 4. The effect of oxalacetate on the enzymatic activity of *Penaeus setiferus* muscle supernatant (●—●—●) and mitochondrial (▲----▲----▲) malate dehydrogenase. The assay conditions are described in Materials and Methods.

the above species and also for the marine snail (Meizel & Markeit, 1967) and the teleost, *Fundulus heteroclitus* (Whitt, 1970).

The presence of multiple electrophoretic forms of supernatant and mitochondrial malate dehydrogenases has been observed in numerous vertebrate species (e.g. Bailey *et al.*, 1970). This heterogeneity has been attributed to conformational changes (Kitto *et al.*, 1966), proteolytic degradation (Cassman & King, 1972), desamination of glutamine and asparagine residues (Noyes *et al.*, 1974) and to differences in the amount of covalently bound phosphate (Cassman & Vetterlein, 1974) in mammalian and avian species. Bailey *et al.* (1970) have clearly shown that the multiple electrophoretic components of supernatant MDH of salmonid fish are dimeric proteins composed of distinct *a* and *b* type subunits. The electrophoretic mobilities of the two shrimp isoenzymes following denaturation in buffered 6 M urea and 1 mM mercaptoethanol are shown in Fig. 3. The denatured shrimp supernatant and mitochondrial malate dehydrogenases both show two electrophoretic components with differing relative mobilities. This strongly suggests that the multiple electrophoretic forms found in each of the shrimp isoenzymes is the result of association into dimeric molecules of their constituent nonidentical polypeptide subunits.

Catalytic properties

The catalytic properties of the purified malate dehydrogenases of the two shrimp species are quite similar. These enzymes are not able to use D-malate as substrate. The pH optima for oxalacetate reduction and L-malate oxidation are 7·5 and 10·0, respectively. Figures 4 and 5 show the effects of oxalacetate and L-malate concentrations on the enzymatic activity of the supernatant and mitochondrial isoenzymes. With oxalacetate there is a slightly greater inhibition of the mitochondrial fraction at high substrate concentrations. The mitochondrial MDH of bovine heart (Englard & Breiger, 1962), ox kidney (Dupourque & Kun, 1968), chicken heart (Kitto & Kaplan, 1966) and tuna heart (Kitto & Lewis, 1967) are significantly inhibited by 1×10^{-3} M oxalacetate. A slight oxalacetate inhibition of mitochondrial MDH similar to that observed with shrimp muscle (Fig. 4) has been reported for *Drosophila virilis* (McReynolds & Kitto, 1970), *Euglena gracilis* Z (Peak *et al.*, 1972) and the slime mold, *Physarum polycephalum* (Teague & Henney, 1973). The shrimp supernatant MDH is slightly more sensitive to malate inhibition than the mitochondrial enzyme. Very similar results are found for the homologous isoenzymes of vertebrate species (Kitto & Kaplan, 1966; Kitto & Lewis, 1967).

Heat inactivation studies with the shrimp muscle malate dehydrogenases clearly displayed first-order kinetics. The first order rate constant for inactivation is 5·1 times as large for the mitochondrial MDH as for the supernatant MDH of the brown shrimp at 45°C, compared to 8·6 for this ratio for the corresponding tuna heart malate dehydrogenases heated at 48°C (calculated from Fig. 8 of Kitto & Lewis, 1967). The greater thermal lability of the mitochondrial enzyme was likewise observed for the white shrimp, although detailed kinetic data were not obtained. The apparent K_m values for oxalacetate reduction and L-malate oxidation were determined for the shrimp supernatant and mitochondrial fractions (Table 2). These results compare favorably with those found for the corresponding malate dehydrogenases of both vertebrate (Kitto & Kaplan, 1966; Kitto & Lewis, 1967; Dupourque & Kun, 1968; Bailey *et al.*, 1970) and invertebrate (Ozaki & Whiteley, 1970; McReynolds & Kitto, 1970) species.

Ultracentrifugation studies

The results of ultracentrifugation studies of the supernatant and mitochondrial malate dehydrogenases from the brown shrimp are summarized in Table 3. Both enzymes displayed single symmetrical boundaries in the sedimentation velocity experiments. $S_{20,w}$ values of 4·9 S and 4·7 S were obtained for the supernatant and mitochondrial isoenzymes, respectively. Because these experiments were conducted at concentrations near 0·4 mg/ml using the photoelectric scanner, extrapolation to zero protein concentration is unnecessary; Fig. 2 of Kitto & Kaplan (1966) shows that the observed sedimentation coefficient would be indistinguishable from an S° value at such concentrations in the case of the chicken heart malate dehydrogenases, which are similar in size to the enzymes of these studies. Sedimentation coefficients of 4·3–5·4 S have been reported for the supernatant malate dehydrogenases of beef heart (Cassman & Vetterlein,

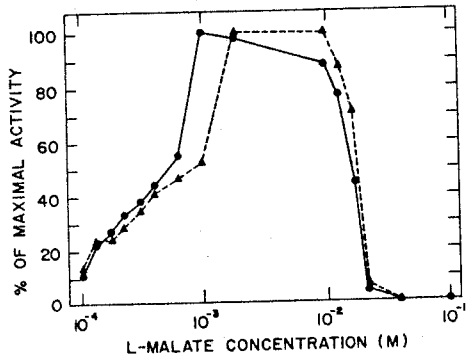

Fig. 5. The effect of L-malate on the enzymatic activity of *Penaeus setiferus* muscle supernatant (● ● ●) and mitochondrial (▲----▲----▲) malate dehydrogenase. The assay conditions are described in Materials and Methods.

Table 2. Comparison of the catalytic properties of the supernatant and mitochondrial malate dehydrogenase fractions of the white shrimp, *Penaeus setiferus*, and the brown shrimp, *Penaeus aztecus*

	White shrimp		Brown shrimp	
	supernatant	mitochondrial	supernatant	mitochondrial
Optimum pH (oxalacetate)	7.5	7.5	7.5	7.5
Optimum pH (malate)	10.0	10.0	10.0	10.0
K_m (oxalacetate)	$1.03 \cdot 10^{-4}$ M	$5.65 \cdot 10^{-5}$ M	$5.77 \cdot 10^{-5}$ M	$6.80 \cdot 10^{-5}$ M
K_m (malate)	$3.98 \cdot 10^{-4}$ M	$4.92 \cdot 10^{-4}$ M	$2.2 \cdot 10^{-4}$ M	$5.88 \cdot 10^{-4}$ M
Oxalacetate inhibition	slight at $5 \cdot 10^{-3}$ M	slight at $2.5 \cdot 10^{-3}$ M	slight at $5 \cdot 10^{-3}$ M	slight at $2.5 \cdot 10^{-3}$ M
Malate inhibition	marked at $1 \cdot 10^{-2}$ M	marked at $1 \cdot 10^{-2}$ M	marked at $1 \cdot 10^{-2}$ M	marked at $1 \cdot 10^{-2}$ M

1974), ox kidney (Dupourque & Kun, 1968), chicken heart (Kitto & Kaplan, 1966), salmonid fish muscle (Bailey *et al.*, 1970) and *Euglena gracilis* Z (Peak *et al.*, 1972), while values of 4·0–4·7 S have been reported for the mitochondrial malate dehydrogenases of pig heart (Consiglio *et al.*, 1970), chicken heart (Kitto & Kaplan, 1966), *Drosophila virilis* (McReynolds & Kitto, 1970) and *Euglena gracilis* Z (Peak *et al.*, 1972).

From the sedimentation equilibrium experiments, mol. wt of 75,800 and 69,300 were calculated for the shrimp supernatant and mitochondrial MDH fractions, respectively, with no evidence of curvature in the plots of ln C vs r^2 and thus no evidence for molecular weight heterogenity. These molecular weights are in the general range of 65,000–76,000 observed in a number of studies in which the supernatant and mitochondrial enzymes have been examined separately (Kitto & Kaplan, 1966; Bailey *et al.*, 1970; Peak *et al.*, 1972; Cassman & Vetterlein, 1974; Consiglio *et al.*, 1970; Wolfenstein *et al.*, 1969). The sole exception among the above is a minor component of pig heart mitochondrial MDH (Consiglio *et al.*, 1970) which is twice the molecular weight of the major species. Where such information has been obtained, the above are dimeric molecules which, for some of these organisms, possess non-identical subunits.

The diffusion coefficients (Table 3) were calculated from the Svedberg equation using the experimentally determined values of the sedimentation coefficients and molecular weights. These values are similar to the experimental values of 5·3 and 5·4 $\times 10^{-7}$ cm^2/sec reported for pig heart (Consiglio *et al.*, 1970) and chicken heart (Kitto & Kaplan, 1966) mitochondrial MDH. Frictional ratios were calculated using the sedimentation and diffusion coefficients according to the procedure suggested by Tanford (1961). These values are very similar to the fractional ratios reported for pig heart (1·32) (Consiglio *et al.*, 1970) and chicken heart (1·4) (Kitto & Kaplan, 1966) mitochondrial MDH.

Table 3. Physicochemical characteristics of *Penaeus azteeus* (brown shrimp) muscle mitochondrial and supernatant malate dehydrogenase

	Mitochondrial	supernatant
Sedimentation coefficient (Svedbergs)	4.69	4.94
Molecular weight from sedimentation equilibrium	69 300	75 800
Diffusion coefficient from $s^*_{20,w}/M$ ($D^*_{20,w} \cdot 10^7$ cm^{2}sec^{-1})	6.27	6.03
Frictional ratio f/f_o from $s^*_{20,w}$ and $D^*_{20,w}$	1.25	1.23

DISCUSSION

The mitochondrial and supernatant forms of shrimp muscle malate dehydrogenases are similar in a number of catalytic properties. Both enzyme fractions were partially inhibited by high L-malate concentrations. The supernatant enzyme is relatively stable at 45°C, but the mitochondrial enzyme is heat labile. The apparent K_m's for oxalacetate reduction and L-malate oxidation for the shrimp enzymes are similar to those found for the corresponding malate dehydrogenases of various vertebrate species (Kitto & Kaplan, 1966; Kitto & Lewis, 1967; Dupourque & Kun, 1968; Bailey *et al.*, 1970).

The molecular weights, hydrodynamic properties, and dimeric structure of shrimp muscle mitochondrial and supernatant malate dehydrogenases described in this work are remarkably similar to those previously reported for the corresponding enzymes of both vertebrate (Kitto & Kaplan, 1966; Dupourque & Kun, 1968; Noyes *et al.*, 1974; Bailey *et al.*, 1970; Consiglio *et al.*, 1970; Wolfenstein *et al.*, 1969) and invertebrate species (McReynolds & Kitto, 1970; Teague & Henney, 1973). These studies reinforce the view (Noyes *et al.*, 1974) that the dehydrogenases of species of diverse phylogenetic origin possess a high degree of structural similarity.

The occurrence of multiple forms of malate dehydrogenases in shrimp may by physiologically significant. Cassman & Vetterlein (1974) recently reported that there are two forms of supernatant malate dehydrogenase in beef heart and that these two enzymatic species may play different roles in metabolic regulation. One possible role for multiple forms of shrimp malate dehydrogenases, assuming that shrimp are true facultative anaerobes possessing aerobic-anaerobic transitions similar to those demonstrated in other invertebrates (Hochachka & Mustafa, 1972), is that they have differences in their enzymatic properties which are required for proper regulation of the various metabolic pathways under conditions of aerobic or anaerobic metabolism that may occur during their migratory behavior. An investigation of the kinetic properties of the individual isoenzyme forms may reveal such differences.

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