Cellular Location and Partial Characterization of the Alanine Aminotransferase in Ribbed Mussel Gill Tissue

KENNEDY T. PAYNE, LEHMAN L. ELLIS, AND STEPHEN H. BISHOP
Department of Zoology, Iowa State University, Ames, Iowa 50011

ABSTRACT  Differential centrifugation of ribbed mussel gill tissue homogenates and extraction of the mitochondrial fraction demonstrated that most (72%) alanine aminotransferase (AAT) activity was mitochondrial. Subsequent characterization of the cytosolic activity demonstrated properties identical to those demonstrated by the mitochondrial enzyme. Both enzyme fractions showed little variation in V_{max} with pH, had low K_m's for ketoacid substrates, and were inhibited by aminoxyacetate (AOA), L-cycloserine, and β-chloro-L-alanine. It appears that the AAT in ribbed mussel gill tissue is strictly mitochondrial and that alanine production during hypoxia or hyperosmotic stress must be mitochondrial.

Amino acids, particularly alanine and glycine, comprise a substantial portion of the osmotically active constituents within the cells of osmoconforming euphausiine bivalves (Lange, '72; Bishop, '76) and ribbed mussels in particular (Baginski and Pierce, '75, '77, '78). In the last few years the processes regulating the cellular concentrations of these amino acids have focused on aspects of the membrane permeability that result in cellular retention of these amino acids (see Pierce, '82) and on the metabolic processes that determine which amino acids accumulate (see Bishop et al., '83). The specific requirement for active transaminase activities in the metabolism of alanine and aspartate was demonstrated in a series of experiments employing transaminase inhibitors with ribbed mussel tissues subjected to hyperosmotic stress (Greenwalt and Bishop, '80; Bishop et al., '81).

The specific properties of the transaminase activities in molluscan tissues have received very little attention. All tissues of all molluscs assayed have both the alanine aminotransferase (AAT) and aspartate aminotransferase (AAT) activities (Bishop et al., '83). Recent studies by Paynter et al. ('84) indicate that ribbed mussel tissues have both cytosolic and mitochondrial AAT isozymes (cAAT and mAAT) and that the activities of these two isozymes differ considerably in terms of kinetic properties, heat stability, and electrophoretic migration. The cAAT had unusually high K_m's for the amino acid substrates, particularly for aspartate at low pHs (150 mM at pH 6.5). The mAAT showed reasonably low K_m's for all substrates (except glutamate) throughout the pH range (6.5-9.5) that were in the range generally reported for AAT activities from mammalian tissues.

In light of the results with the AAT activities it was important to determine the cellular distribution of the AAT activities in ribbed mussel tissues and to compare similarities or differences among isozymes that might be important in the regulation of amino acid accumulation during hyperosmotic stress. Enzymes of AAT with differing properties have been detected in the cytosol and mitochondria of most mammalian tissues (Hopper and Segal, '62; Swick et al., '65; DeRosa and Swick, '75; Ruscak et al., '82). Although AAT activities have been found in both the cytosol and mitochondria of tissue homogenates of some pulmonate snails by Sollock et al. ('79) and in whole body homogenates of oysters (Crassostrea virginica) by Chambers et al. ('75), these authors did not determine the properties of the

Please address correspondence to S.H.B.

Contribution number 213 from the Tallahassee, St. Simons, and Gulf Coast Marine Biological Association.

© 1984 ALAN R. LISS, INC.
AIAT activities and whether or not the cytosolic and mitochondrial activities were associated with different isoforms. Preliminary studies with oyster gill tissue (Burcham et al., '83) indicated that the cytosolic and mitochondrial AIATs were associated with separate and distinct isoforms.

This paper reports the partial purification and some properties of ribbed mussel gill AIAT activities. The major AIAT is within the mitochondrial compartment.

MATERIALS AND METHODS

Ribbed mussels (Modiolus demissus), purchased from Northeast Environmental Laboratories (Monument Beach, Mass.), were kept in artificial seawater (Jungle Laboratories Inc., Sanford, Fla.) and maintained as described by Greenwald and Bishop ('80). Ex-cept where noted all reagents and coupling enzymes were purchased from Sigma Chemical Co., St. Louis, MO. Hexadecyltrimethylammonium bromide (CTAB) and ammonium sulfate (enzyme grade) were obtained from Eastman Organic Chemicals, Rochester, N.Y., and Schwartz-Mann (Orangeburg, N.Y.), respectively. Beta-chloro-L-alanine and vinylglycine were purchased from Calbi-ochem (La Jolla, CA).

The digitonin used in these experiments was recrystallized according to Kun et al., ('79). Digitonin (Sigma grade) was dissolved in absolute ethanol (1 gm/25 ml) at 75°C, then precipitated by chilling the solution on ice for 20 minutes, and collected by centrifugation at 0°C. This procedure was repeated and the precipitate was dried in a desiccator.

Enzyme assay

During purification and standard assay, the AIAT activity was determined spectrophotometrically by measuring pyruvate production as NADH oxidation (340 nm) using a Beckman 3600 recording spectrophotometer in a reaction mixture containing 20 mM alanine, 0.5 mM 2-oxoglutarate, 70 μM NADH, 5 units of lactic dehydrogenase, 50 μM Tris-HCl pH 8.3, and enzyme in 2 ml. The reverse reaction (forming alanine) was measured by coupling the transaminase action to 2-oxoglutarate dehydrogenase and measuring the reduction of NAD (340 nm) in a reaction mixture containing 20 mM glutamate, 0.5 mM pyruvate, 70 μM NAD, 0.5 mM CoA, 0.5 units of 2-oxoglutarate dehydrogenase, 50 mM Tris-HCl, and enzyme in 1 ml at pH 8.3. Control assays without gill extract demonstrated the absence (< 1%) of any contaminating transaminase activity in either the LDH or the 2-oxoglutarate dehydrogenase. Glutamate dehydrogenase (GDH) was assayed in the glutamate-forming direction by the procedure of Reiss et al. ('75) with 1 mM ADP. Incubations were at room temperature (23°C). One unit of activity synthesized one μ mole of product per minute under the conditions specified. Procedural modifications for kinetic experiments are described below. Kinetic constants were determined using computer-assisted analysis of initial rates (Cleland, '79) using least-squares analyses for the best linear fit.

Isozyme determination

Isozyme distribution and purity of electrophor-ems was determined using starch gel electrophoresis. One liter of electrode buffer contained 15.5 gm Tris (Base), 1.5 gm EDTA, 1.15 gm boric acid, and glacial acetic acid to pH 7.0. Electrode buffer was diluted 1:10 to make the gel buffer. To examine tissue distribu-tion samples of whole tissues were homogenized in an equal volume of gel buffer using a Polytron (Brinkman Inst., Westbury, N.Y.) tissue homogenizer with a small probe. The suspension was applied to individual slots in 13/4 (w/v) horizontal starch gels (12 cm × 20 cm × 0.8 cm) and electrophoresis was performed at approximately 35 mamp at 4°C. Progress was marked with Bromphenol blue. Gels were sliced and stained according to the alamine aminotransferase detection procedure of Harris and Hopkinson ('76) using a filter paper overlay and hand-held ultravi-olet light to scan for NADH oxidized (NAD).

Cytosolic and mitochondrial fractions of gill homogenates were prepared using the previously described differential centrifugation procedure (Paynter et al., '84). Protein was determined by modified Lowry procedure (Miller, '59).

RESULTS

Isozyme distribution

Starch gel electrophoresis of samples from the cytosol and unbroken mitochondria suspended in 10 mM phosphate (pH 6.8) indicated electromorphs in both the cytosolic and mitochondrial compartments (Fig. 1, lanes a,b). However, more than half of the mAIA T activity remained at the origin in the wells (Fig. 1, lanes a) and was not released by suspension of the mitochondria in dilute buffer (10 mM). The mAIA T activity from the mito-
Mussel Gill Alanine Aminotransferase

![Diagram of enzyme fractions]

Fig. 1. Distribution of AIAT gill tissue enzymes using starch gel electrophoresis. Four lanes of identical samples were run for each preparation. Mitochondrial (lane a) and cytosolic (lane b) fractions from gill tissue were applied to wells and electrophoresed at pH 6.8. Mitochondrial digitonin extract was applied in lanes c. Cytosolic salt fraction 1 was applied in lanes d and cytosolic salt fraction 2 was applied in lanes e. See text and Table 1 for definition of procedures of electrophoresis and fractions assayed.

Chondria released in dilute buffer had the same electrophoretic mobility as the major cytosolic AIAT, suggesting that this AIAT was mitochondrial in origin. With prolonged staining of the electrophoretic gels (12 hours), a second faintly staining, anodally migrating "AIAT electromorph" was detected in the cytosolic fraction (Fig. 1, lanes e).

It was now critical to extract the remaining AIAT activity from the gill mitochondria and compare the properties of the mAIAT to those of the "cytosolic AIAT." Freezing and thawing of the mitochondria as described previously for the release of the mitochondrial aspartate aminotransferase (Paynter et al., '84) liberated very little additional soluble AIAT activity from the mitochondria. Treatment of 1 ml of suspended mitochondria (3.8 mg protein/ml buffer) with 9 ml of 0.1% CTAB released little or no AIAT and apparently inactivated the AIAT. Treatment with 1 ml of a 0.1% CTAB solution released about 50% of the mAIAT activity with a loss of 50% of total activity. Treatment of these mitochondrial suspensions with 1%, 0.5%, and 0.25% Triton X-114 at 23°C for 30 minutes did not cause a release of additional mAIAT activity and caused in a loss of total activity.

Digitonin has been shown to release tightly bound GDH and AIAT from sea mussel tissue mitochondria (Addink and Veenhof, '75; Reise et al., '77). Treatment of the mitochondria with a series of digitonin treatments released most of the mAIAT activity and did not appear to inhibit the mAIAT activity (Fig. 1, lanes c).

A comparison of the release of AIAT and GDH from these gill mitochondria in a series of digitonin treatment experiments was made in order to determine the relative efficiency of extraction and the relative tightness of binding within the mitochondria. Results of the digitonin extraction are in Table 1 (extraction procedure outlined in footnote). Two successive digitonin extractions liberated 94% of the total mitochondrial AIAT and 88% of the total mitochondrial GDH. Total tissue activities were 772 mUnits/gm wet wt. (AIAT) and 228 mUnits/gm wet wt. (GDH). The major cAIAT activity released during the initial tissue homogenization and mitochondrial preparation procedure had the same electrophoretic migration as the AIAT released from the mitochondria with digitonin treatment; this activity was termed the cytosolic mitochondrial AIAT or cmAIAT. In a series of experiments with gill tissue from 30 individual animals, there was no variation in this single mAIAT-cmAIAT isozyme pattern.

Partial purification of the gill AIAT activities

During typical mAIAT purification, mitochondria from approximately 20 gm gill tissue were resuspended in 20 ml of 2.5 mg/ml newly recrystallized digitonin suspension in 10 mM potassium phosphate buffer (pH 6.8) and stirred for at least 30 minutes at 0°C in a beaker with a magnetic stirring bar. The resulting suspension was centrifuged (10,000g for 20 minutes) and the supernatant fluid containing most of the mAIAT activity (174 mUnits/mg protein) was dialyzed against 10 mM potassium phosphate buffer (pH 6.8) overnight at 4°C. Hydroxylapatite (HAP) (Bio-Rad Laboratories, Richmond, CA) was washed in degassed 10 mM potassium phosphate (pH 6.8), the fine particles were removed, and the slurry was poured into a column to give a 2 x 10 cm bed. After equilibration of this column with degassed dialysate buffer (see above), the preparation from the dialysis bag was added onto the column and the column was washed with 150 ml of degassed dialysate. Most of the protein was eluted from the column by washing with 150 ml of 60 mM potassium phosphate (pH 6.8); the mAIAT activity remained on the column. The mAIAT activity was then eluted as a
TABLE 1. Intracellular distribution of alanine aminotransferase and glutamate dehydrogenase in rabbit mussel gill tissues

<table>
<thead>
<tr>
<th></th>
<th>Total units (mUnits)</th>
<th>Total protein (mg)</th>
<th>Specific activity (mUnits/mg prot)</th>
<th>% Of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIAT</td>
<td>5,400</td>
<td>916</td>
<td>5.70</td>
<td>28</td>
</tr>
<tr>
<td>GDH</td>
<td>550</td>
<td>916</td>
<td>0.60</td>
<td>10</td>
</tr>
<tr>
<td>Mitochondria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIAT</td>
<td>13,900</td>
<td>49</td>
<td>280</td>
<td>72</td>
</tr>
<tr>
<td>GDH</td>
<td>5,150</td>
<td>49</td>
<td>110</td>
<td>90</td>
</tr>
</tbody>
</table>

1 Mitochondrial and cytosolic fractions were prepared as described in text. Mitochondria were sonicated in 10 mM potassium phosphate buffer (pH 6.8) and assayed. The supernatant was then treated with 0.5 mg lysozyme protein on ice with constant stirring for 10 minutes. The supernatant was centrifuged (15,000 g, 15 minutes), the supernatant was added, and the pellet resuspended in the final volume of 10 mM potassium phosphate buffer. Mitochondrial original protein was again added, and the extraction repeated for 30 minutes. The supernatant was centrifuged, supernatant discarded, and solids repeatedly extracted in 10 mM potassium phosphate buffer. Mitochondrial original protein was added, and the mitochondria were extracted for 30 minutes. All supernatants were assayed for AIAT and GDH. The final pellets retained insignificant amounts of either enzyme activity.

The single peak of activity by washing the column with 100 ml of 200 mM potassium phosphate (pH 6.8). The fractions (1 ml) with the highest specific activity (620 mUnits/mg protein) eluting behind the buffer front were pooled. These pooled fractions constituted the final partially purified preparation of mAIAT activity used from the kinetic experiments.

Approximately 20% of the total GDH activity extracted from the mitochondria by the digitonin treatment was eluted from the HAP column with the mAIAT activity (200 mM buffer wash). There was no (<1%) NAD reduction associated with mAIAT activity in the presence of only glutamate, NAD, and enzyme (homogenate or enzyme preparation). GDH activity was dependent on the presence of ADP in the reaction cocktail in that there was a 5 x loss of activity without 1 mM ADP.

The AIAT activity in the cytosolic fraction (Table 1) was partially purified. Addition of 42 mg/100 ml ammonium sulfate to the cytosolic fraction precipitated most (75%) of the AIAT (cytosolic salt fraction 1). Addition of an additional 14 gm/100 ml ammonium sulfate precipitated cytosolic salt fraction 2 of the AIAT (~5% of the total tissue activity). With starch gel electrophoresis, the AIAT in cytosolic salt fraction 1 migrated as the mAIAT (cmAIAT) and the AIAT in cytosolic salt fraction 2 contained approximately equal amounts of the cmAIAT and the anodally migrating, slow-staining AIAT activity described previously (Fig. 1, d,e). The cytosolic salt fraction 1 was dialyzed and chromatographed on hydroxylapatite using the procedure described for the mAIAT activity. This cmAIAT activity eluted with the 200 mM phosphate (pH 6.8) buffer wash in a manner identical to the mAIAT activity and had the same electrophoretic mobility as the mAIAT.

Properties of the mAIAT

The partially purified AIAT activity was characterized with respect to substrate binding, heat stability, and reactivity with inhibitors.

The heat stabilities of the cmAIAT and mAIAT were identical (Fig. 2). The activity in both fractions was lost at temperatures above 50°C. This heat stability was slightly lower than the heat stability reported by Bulos and Handler (65) for the beef heart AIAT activity. The gill AIAT was much more heat sensitive than either the gill mAIAT or cAIAT (see Paynter et al., '84) and apparently not as labile as the mammalian mitochondrial enzyme (Swick et al., '63).

Kinetic analysis of the partially purified mAIAT showed optimal activity over a broad pH range from pH 5.6 to pH 9.5 (Fig. 3). Substrate binding constants (apparent K_m) were determined in the forward (pyruvate forming) and reverse (alanine forming) directions (data not shown). Between pH 6.4 and pH 9.5, the apparent K_m values and relative maximal velocities did not change markedly (Table 2). The K_m's for substrate binding and ratios of rates of the forward vs. the reversed direction at pH 8.3 with the cmAIAT were
Fig. 2. Thermal stability of mAlAT found in the cytosol fraction (Ο) and extracted from the mitochondria (△). Approximately 100 μl of enzyme was incubated in a water bath for 2 minutes at each temperature. One hundred microliters was added to a 1.0 ml reaction cocktail at 23°C and assayed as described in Materials and Methods for pyruvate formation.

The results indicate that most (95%) of the mAlAT in ribbed mussel gill tissue is located in the mitochondria. Although the AlAT activity is found in both the cytosolic and mitochondrial fractions separated from homogenates by differential centrifugation, both the major “cytosolic” form and the mitochondrial form show the same electrophoretic, kinetic, and heat-stability properties, suggesting that this “cytosolic AlAT” is identical to the mitochondrial AlAT and is released from the mitochondria during tissue preparation. In this regard more than half of the mAlAT activity and most of the mGDH activity are cryptic or tightly bound within the mitochondria and released only with successive digitonin (detergent) extractions.

The initial homogenization-differential centrifugation procedure used here was employed by Paynter et al. (84) to separate mitochondrial and cytosolic aspartate aminotransferase (AAT) activities. Very little mitochondrial breakage was apparent (as evidenced by cross mAAT and mAAT isozyme contamination) even though the mAAT is apparently not membrane bound and is easily released by freeze/thaw treatment. The release of a small amount of otherwise tightly bound mAAT and GDH during the homogenization-centrifugation procedure may indicate a binding mode within the mitochondria that differs considerably from the mAAT activity.
TABLE 2. Apparent K_m values for gill tissue mitochondrial (mAlAT) and mitochondriallike cytosolic (cmAlAT) alanine amino transferases*

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Condition</th>
<th>Ala</th>
<th>2-</th>
<th>Oxa</th>
<th>Pyr</th>
<th>Glu</th>
<th>Activity</th>
<th>E/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAAT</td>
<td>6.4</td>
<td></td>
<td>4.2</td>
<td>0.12</td>
<td>0.03</td>
<td>3.7</td>
<td>5.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mAAT</td>
<td>8.3</td>
<td></td>
<td>4.3</td>
<td>0.11</td>
<td>0.09</td>
<td>5.8</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mAAT</td>
<td>9.5</td>
<td></td>
<td>2.3</td>
<td>0.12</td>
<td>0.13</td>
<td>2.7</td>
<td>3.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cmmAAT</td>
<td>8.3</td>
<td></td>
<td>5.5</td>
<td>0.08</td>
<td>0.07</td>
<td>5.0</td>
<td>5.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Kinetic constants were generated by computer analysis (Cleland, '78).
*E/R represents the ratio of the maximal velocities of the forward (E) or pyruvate-forming direction to the reverse (R) or alanine-forming direction. Assays are described in Materials and Methods. Alternate substrate concentrations for the reactions were 20 mM alanine and 10 mM 2-oxoglutarate for the pyruvate-forming direction and 20 mM glutamate and 5 mM pyruvate for the alanine-forming direction. Standard error for all measurements averaged 11%.

Fig. 4. Inhibition of the gill tissue mAAT and cmmAAT activities by aminooxyacetic acid (A) and L-cycloserine (B). Enzyme (125 μl) was incubated for 2 minutes at 23°C with 125 μl of inhibitor. Two hundred microliters of the enzyme-inhibitor mixture was then added to a 1.8 ml reaction cocktail and assayed as described in Materials and Methods.

Fig. 5. Inhibition of the gill tissue mAAT activity by β-chloroalanine (β-Cl-Ala). Enzyme (125 μl) was incubated with indicated concentrations of β-Cl-Ala and assayed (100 μl) after 1 minute and 2 minutes (see inset). Initial rates of inhibition (I_R) were determined from the change in activity during the first minute of incubation (see inset). The I_R values were replotted as a function of β-Cl-Ala concentration.

Although preliminary studies with sea mussel tissues (Addink and Veenhof, '75) indicated that release of the mAAT required digitation treatment of these mitochondria, these authors make no comment on a possible cytosolic or "soluble" mAAT activity in these tissues.

The small amount (~5% of total mAAT) of anodal migrating, faintly staining cmmAAT activity concentrated in the 60-80% ammonium sulfate fraction was resolved with a greater degree of confidence as an mAAT activity (Fig. 1, lanes c). Attempts to purify this activity using the hydroxylapatite column have been unsuccessful. Preliminary results on the mAAT isozyme distribution in the other tissues of the ribbed mussel indicate that all had the same mAAT isozyme as the gill tissue, but it is not at this time certain that all of this activity is located within the mitochondria of all these tissues. Studies on the identity of the minor anodally migrating "cy-
tosolic ALAT," with respect to substrate specificity and distribution in the other tissues, are continuing.

This pattern of ALAT isozyme distribution differs from the pattern in oyster gill (Burcham et al., '83). Most mammalian tissues also show separate ALAT isozymes for both the mitochondrial and cytosolic compartments (Hopper and Segal, '82; Swick et al., '85; DeRosa and Swick, '75; Ruscak et al., '82). On the other hand, chicken heart, liver, and kidney apparently lack the ALAT (DeRosa and Swick, '75). Burton and Feldman ('82,'83) have reported fast- and slow-migrating "soluble" ALAT alleles in heterozygotes and fast- or slow-migrating ALAT alleles in respective homozygotes of marine copepod (T. californicus) populations. The method used for preparation of the copepod homogenates involves a buffered sucrose solution of 600-650 mosmoles (Burton and Feldman, '81) which may not break the mitochondria. Although these results suggest that both these copepod ALAT alleles may be cytosolic rather than mitochondrial, there has been no attempt to identify them as cALAT or mALAT.

For most part, the apparent K_m's for the gill ALAT (Table 2) are somewhat lower than those reported for rat and beef heart and liver cALAT's (Hopper and Segal, '82; Swick et al., '85; Bales and Handler, '85; DeRosa and Swick, '75). The variation in activity with pH (Fig. 3) is very similar to that found by Swick et al. ('85) and Orlicky and Ruscak ('76) for the rat liver and heart mALATs. The small variation with pH in apparent K_m's with the amino acid substrates is similar to that found with the gill mALAT activity (Paynter et al., 1984). The decrease in apparent K_m for pyruvate with decreasing pH (Table 2) may be of particular physiological importance with the lowered intracellular pH that occurs during short-term anebiosis (Ellington, '83).

The mALAT is freely reversible. The ratio of rates with the gill mALAT in forward (pyruvate forming) vs. the reverse (alanine forming) direction (Table 2) is similar to the ratio of 4.4 (in Tris) reported for the rat liver mALAT (Swick et al., '85). Awapara and Campbell ('64), using homogenates of clam (Rangia) and oyster Crassostrea mantle and snail (Otala) hepatopancreas at fixed low substrate concentrations report lower forward-reverse rate ratios of 1.8, 1.1, and 0.88, respectively.

Previous studies using AOA and L-cycloleucine with gill tissue indicate that the metabolism of both aspartate and alanine are transaminase linked (Bishop et al., '81). Paynter et al. ('84) have shown that the kinetic properties of the cALAT and mALAT predict that aspartate synthesis should be favored in the cytosol and that aspartate catabolism should be favored in the mitochondria at normal substrate concentrations. Aspartate metabolism seems to be linked to alanine or alanine-glycine metabolites (stramine, alanosine) accumulation in most bivalve tissues when the animals or tissues are subjected to hypoxic or hyperosmotic stress (Collicutt and Hochachka, '77; deZwaan, '77; Bugnanski and Pierce, '78; Greenwalt and Bishop, '80; Bishop et al., '81; deZwaan et al., '82,'83). This study shows that the alanine amino transferase is mainly mitochondrial. Therefore alanine turnover and coupling of alanine turnover to aspartate metabolism must occur within the mitochondria. During hyperosmotic or hypoxic stress, gill tissue alanine accumulation would require an influx of both amino groups (carried as ammonium, aspartate, or other amino acids) and carbon skeletons for pyruvate production (glycolytic products, gluconeogenic precursors) for subsequent transamination to alanine. The alanine would then exit the mitochondria for accumulation in the cytosol. On the other hand, with hypoxosmotic stress, coupling of the mALAT with the mGDH and an increased rate of oxidative metabolism would result in alanine catabolism in the mitochondria and a disappearance of alanine from the cellular pool. This model predicts a "special" regulation of pyruvate metabolism within the mitochondrion to control shunting to the TCA cycle or to alanine and possibly a regulation of the mitochondrial amino acid transporters. In this regard it is of considerable interest that pyruvate is a very poor substrate for oxidative metabolism by bivalve gill tissue mitochondria showing high degrees of respiratory control (Burcham et al., '83,'84).

ACKNOWLEDGMENTS

We give thanks to R. J. Hoffmann, (ISU, Zoology) for advice on aspects of the electrophoretic procedures, the loan of the electrophoretic apparatus plus the use of some facilities in his laboratory, to Dr. David E. Metzler (ISU, Biochemistry) for helpful discussions, to Dr. J.M. Viles for the use of the Ultraturrax, and to Ms. Mary Nims for assistance in the preparation of the manuscript. This work was supported in part by grants PCM-80-22066 to S.H.B. and DEB-82-05986 to R.J.H. from the National Science...
Foundation and by funds from the Iowa State University Research Foundation.

LITERATURE CITED


