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

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ABSTRACT Differential centrifugation of ribbed mussel gill tissue homogenates and extraction of the mitochondrial fraction demonstrated that most (72%) alanine aminotransferase (AlAT) 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 aminooxyacetate (AOA), L-cycloserine, and β-chloro-L-alanine. It appears that the AlAT 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 euryhaline 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 (AlAT) 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 AlAT 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. Isoenzymes of AlAT 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 AlAT 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

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AlAT activities and whether or not the cytosolic and mitochondrial activities were associated with different isozymes. Preliminary studies with oyster gill tissue (Burcham et al., '83) indicated that the cytosolic and mitochondrial AlATs were associated with separate and distinct isozymes.

This paper reports the partial purification and some properties of ribbed mussel gill AlAT activities. The major AlAT 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 Greenwalt and Bishop ('80). Except 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 Calbiochem (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 (1gm/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 dessicator.

Enzyme assay

During purification and standard assay, the AlAT 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, 10 mM 2-oxoglutarate, 70 μM NADH, 5 units of lactic dehydrogenase, 50 mM Tris-HCl 9pH 8.3, and enzyme in 2 ml. The reverse (alanine forming) direction was measured by coupling the transaminase reaction 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 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. ('77) with 1 mM ADP. Incubations were at room temperature (23°C). One unit of activity synthesized one μmole 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 electromorphs 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 distribution 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% (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 alanine aminotransferase detection procedure of Harris and Hopkinson ('76) using a filter paper overlay and hand-held ultraviolet 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 mAlAT 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 mAlAT activity from the mito-

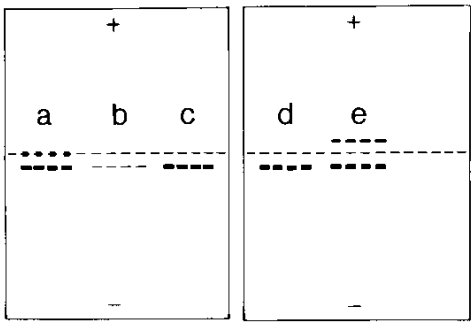

Fig. 1. Distribution of AlAT gill tissue isozymes using starch gel electrophoresis. Four lanes of identical samples were run for each preparation. Mitochondrial (lane a) and cytosolic (lane b) fractions from gill tissue were applied to wells and electrophoresed at pH 7.0. Mitochondrial digitonin extract was applied in lanes c. Cytosolic salt fraction 1 was appled 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 AlAT, suggesting that this AlAT was mitochondrial in origin. With prolonged staining of the electrophoretic gels (12 hours), a second faintly staining, anodally migrating "AlAT electromorph" was detected in the cytosolic fraction (Fig. 1, lanes e).

It was now critical to extract the remaining AlAT activity from the gill mitochondria and compare the properties of the mAlAT to those of the "cytosolic AlAT." 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 AlAT 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 AlAT and apparently inactivated the AlAT. Treatment with 1 ml of a 0.1% CTAB solution released about 50% of the mAlAT 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 mAlAT activity and caused in a loss of total activity.

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

A comparison of the release of AlAT 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 AlAT and 86% of the total mitochondrial GDH. Total tissue activities were 772 mUnits/gm wet wt. (AlAT) and 228 mUnits/gm wet wt. (GDH). The major cAlAT activity released during the initial tissue homogenization and mitochondrial preparation procedure had the same electrophoretic migration as the AlAT released from the mitochondria with digitonin treatment; this activity was termed the cytosolic mitochondrial AlAT or cmAlAT. In a series of experiments with gill tissue from 30 individual animals, there was no variation in this single mAlAT-cmAlAT isozyme pattern.

Partial purification of the gill AlAT activities

During typical mAlAT 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 mAlAT activity (174 mUnits/mg protein) was dialyzed against 10 mM potassium phosphate buffer (pH 6.8) overnight at 4°C. Hydroxyl-apatite (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 × 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 mAlAT activity remained on the column. The mAlAT activity was then eluted as a

TABLE 1. Intracellular distribution of alanine aminotransferase and glutamate dehydrogenase in ribbed mussel gill tissues[1]

	Total units (mUnits)	Total protein (mg)	Specific activity (mUnits/mg prot)	% Of total activity
Cytosol				
AlAT	5,400	946	5.70	28
GDH	550	946	0.50	10
Mitochondria				
AlAT	13,900	49	280	72
GDH	5,150	49	110	90

[1]Mitochondrial and cytosolic fractions were prepared as described in text. Mitochondria were resuspended in 10 mM potassium phosphate buffer (pH 6.8) and assayed. The suspension was then treated with 0.5 mg Digitonin/mg protein on ice with constant stirring for 10 minutes. The suspension was centrifuged (15,000g for 15 minutes), the supernatant set aside, and the pellet resuspended in the original volume of 10 mM potassium phosphate buffer. Digitonin (1 mg/mg original protein) was again added and the extraction repeated for 30 minutes. The suspension was centrifuged, supernatant decanted and set aside, and the pellet once more resuspended in 10 mM potassium phosphate buffer. Digitonin (1 mg/mg original protein) was added and the mitochondria were extracted for 30 minutes. All supernatants were assayed for AlAT and GDH. The final pellets retained insignificant amounts of either enzyme activity.

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 AlAT activity (620 mUnits/mg protein) eluting behind the buffer front were pooled. These pooled fractions constituted the final partially purified preparation of mAlAT 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 mAlAT activity (200 mM buffer wash). There was no (<1%) NAD reduction associated with mAlAT 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× loss of activity without 1 mM ADP.

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

Properties of the mAlAT

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

The heat stabilities of the cmAlAT and mAlAT 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 AlAT activity. The gill AlAT was much more heat sensitive than either the gill mAAT or cAAT (see Paynter et al., '84) and apparently not as labile as the mammalian mitochondrial enzyme (Swick et al., '65).

Kinetic analysis of the partially purified mAlAT 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 cmAlAT were

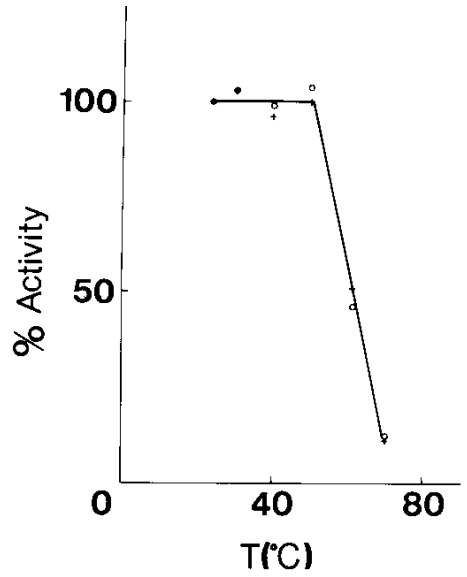

Fig. 2. Thermal stability of AlAT found in the cytosol fraction (○) and extracted from the mitochondria (+). Approximately 150 μl of enzyme was incubated in a water bath for 2 minutes at each temperature. One hundred microliters was added to a 1.9 ml reaction cocktail at 23°C and assayed as described in Materials and Methods for pyruvate formation.

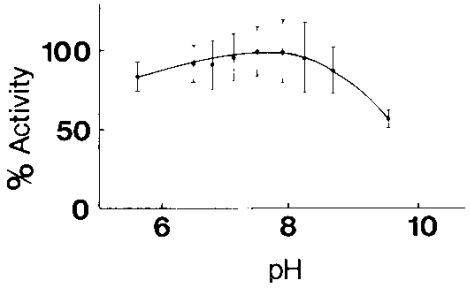

Fig. 3. Relative velocity of mAlAT at different pH values. Activity was assay in the pyruvate-forming direction using the standard assay substrate concentrations (see Materials and Methods) with 50 mM potassium phosphate instead of Tris.

essentially identical to these values with the mAlAT (Table 2).

AOA inhibited the mAlAT with an I_{50} of approximately 7×10^{-8} M and L-cycloserine inhibited the mAlAT activity with an I_{50} of 0.18 mM (Fig. 4). These values were in general agreement with those reported by Greenwalt and Bishop ('80) for the AlAT in ribbed mussel tissue homogenates.

L-serine-O-sulfate (10 mM) and vinylglycine (10 mM) did not inhibit enzyme activity under the conditions described in Figure 4. Beta-chloro-L-alanine (β-CA) inhibited the mAlAT in a time-concentration-dependent fashion (Fig. 5). The apparent K_i (β-CA) generated from the replot in Figure 5 was 25 μM, which was in general agreement with the value of 75 μM found for the pig heart AlAT (Golichowski and Jenkins, '78). Using the standard assay (pyruvate formation) there were no apparent inhibitory stimulatory effects on the observed velocity by NaCl, KCl, glycine, or succinate (up to 250 mM); taurine was a poor noncompetitive inhibitor (K_i 290 mM; data not shown). These results agree with those reported by Gilles ('69) on the effects of various salts on aminotransferases in some crustacean tissues.

DISCUSSION

The results indicate that most (95%) of the AlAT 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 cAAT 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 mAlAT 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[1]

AlAT	pH Condition	Apparent K_m (mM) Ala	2-Oxo	Pyr	Glu	Activity ratio F/R[2]
mAlAT	6.4	4.2	0.12	0.01	3.7	5.47
mAlAT	8.3	4.3	0.11	0.05	5.8	5.61
mAlAT	9.5	2.3	0.12	0.13	2.7	3.45
cmAlAT	8.3	5.5	0.08	0.07	5.0	5.51

[1]Kinetic constants were generated by computer analysis (Cleland, '79).
[2]F/R represents the ratio of the maximal velocities of the forward (F) 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%.

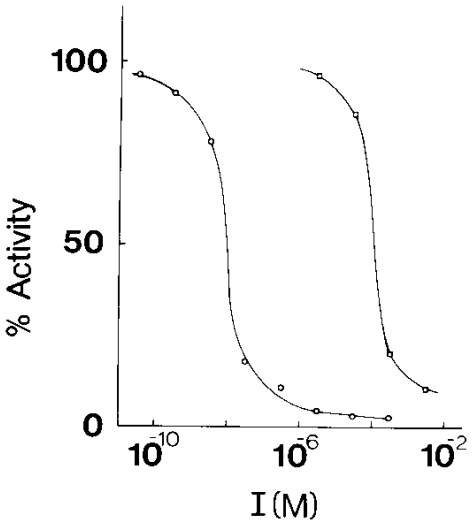

Fig. 4. Inhibition of the gill tissue mAlAT and cmAlAT activities by aminooxyacetic acid (—o—o—) and L-cycloserine (—□—□—). Enzyme (125 μl) was incubated for 2 minutes at 23°C with 125 μl of inhibitor. Two hundred microliters of the enzyme-inhibitor mixture was then added to a 1.8 ml reaction cocktail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

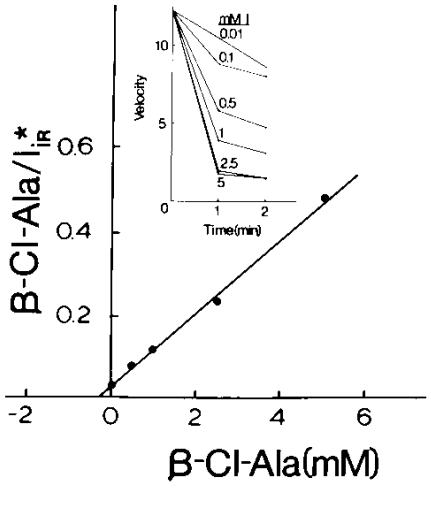

Fig. 5. Inhibition of the gill tissue mAlAT activity by β-chloro-alanine (β-Cl-Ala). Enzyme (125 μl) was incubated with indicated concentrations of β-Cl-Ala and assayed (100 μl) after 1 minute and 2 minutes (see inset). Initial rates of inhibiton (I_{iR}) were determined from the change in activity during the first minute of incubation (see inset). The I_{iR} values were reploted as a function of β-Cl-Ala concentration.

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

The small amount (~5% of total AlAT) of anodal migrating, faintly staining cAlAT activity concentrated in the 60–80% ammonium sulfate fraction was resolved with a greater degree of confidence as an AlAT activity (Fig. 1, lanes e). Attempts to purify this activity using the hydroxyl-apatite column have been unsuccessful. Preliminary results on the AlAT isozyme distribution in the other tissues of the ribbed mussel indicate that all had the same mAlAT 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, '62; Swick et al., '65: DeRosa and Swick, '75: Ruscak et al., '82). On the other hand, chicken heart, liver, and kidney apparently lack the cAlAT (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 cAlATs (Hopper and Segal, '62; Swick et al., '65; Bulos and Handler, '65: DeRosa and Swick, '75). The variation in activity with pH (Fig. 3) is very similar to that found by Swick et al. ('65) and Orlicky and Ruscak ('76) for the rat liver and heart mAlATs. The small variation with pH in apparent K_ms with the amino acid substrates is similar to that found with the gill mAAT 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 anerobiosis (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., '65). 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-cycloserine 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 cAAT and mAAT 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 metabolite (strombine, alanopine) accumulation in most bivalve tissues when the animals or tissues are subjected to hypoxic or hyperosmotic stress (Collicutt and Hochachka, '77; deZwaan, '77; Baginski 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 ammonia, 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 hypoosmotic stress, coupling of the mAlAT with the mGDH and an increased rate of oxidative metabolism would result in alanine catabolism in the mitochondrion 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).

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