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Tissue-specific alanopine dehydrogenase from the gill and strombine dehydrogenase from the foot muscle of the cherrystone clam *Mercenaria mercenaria* (Linn.)

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Abstract: Tissue-specific forms of alanopine and strombine dehydrogenases were detected in the bivalve mollusc, *Mercenaria mercenaria* (Linn.), by isoelectrofocusing. Alanopine dehydrogenase had a pI of 5.0–5.25 and predominated in the gill and mantle while strombine dehydrogenase had a pI of 4.75–4.80 and predominated in the adductor and foot muscles. The enzymes from gill and foot muscle were partially purified, about 20-fold each, to final specific activities of 3 and 37 μmol · min^{-1} · mg protein^{-1}, respectively. The alanopine dehydrogenase from gill had a much higher affinity for alanine (apparent K_m = 28 ± 2.1 mM) than for glycine (apparent K_m = 291 ± 40 mM) and showed substantial activity with a variety of other neutral L-amino acids. The strombine dehydrogenase from foot muscle had similar affinities for alanine and glycine (apparent K_m values were 242 ± 11 and 173 ± 1.3 mM, respectively) but apart from these two amino acids and L-2-aminobutyrate showed a restricted use of other L-amino acids. Affinities for pyruvate were comparable for both enzymes (K_m values were 0.38 ± 0.05 mM and 0.32 ± 0.04 mM for the gill alanopine dehydrogenase and foot muscle strombine dehydrogenase, respectively).

Both enzymes function as the terminal dehydrogenase of glycolysis in their respective tissues and have roles in maintaining energy production under the stresses of environmental (low-tide exposure) or functional (burst muscle work) anoxia. The distribution of the two enzyme types, coupled with the glycine (8.0 and 2.9 μmol · g wet wt^{-1} in foot and gill) and alanine (18.9 and 3.8 μmol · g^{-} in foot and gill) contents of the two tissues, accounts for the dominance of alanopine in soft tissues of the clam and the appearance of near equal amounts of the two imino acids in muscles.

Key words: Alanopine dehydrogenase; Strombine dehydrogenase; *Mercenaria mercenaria*; Anaerobic metabolism

Introduction

Tissues of many marine invertebrates contain imino acid dehydrogenases, enzymes which catalyze the reductive condensation of pyruvate with an amino acid to form an imino acid (sometimes called an opine). The enzymes act as alternatives to lactate dehydrogenase as the terminal enzyme of anaerobic glycolysis.

Alanopine dehydrogenase (EC 1.5.1. ×) catalyzes the reaction: $NAD^+ + H_2O$ + *meso*-alanopine $\leftarrow\cdots\cdots\rightarrow$ NADH + H^+ + L-alanine + pyruvate.

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The enzyme can often use other neutral amino acids as alternative substrates (glycine is the poorest of these) although alanine is the primary (or sole) physiological substrate in vivo.

Strombine dehydrogenase (EC 1.5.1. ×) catalyzes the reaction: $NAD^+ + H_2O +$ D-strombine $\longleftrightarrow$ $NADH + H^+ +$ glycine + pyruvate. Strombine dehydrogenase (originally called alanopine dehydrogenase) was first discovered in the adductor muscle of the oyster, *Crassostrea gigas* (Fields *et al.*, 1980). The enzyme can catalyze both of the preceding reactions equally well, and has similar affinities for alanine and glycine (Fields & Hochachka, 1981) but generally utilizes glycine as its primary substrate in vivo. Strombine dehydrogenase has subsequently been described from the adductor muscle of other bivalves (Dando *et al.*, 1981; De Zwaan & Zurburg, 1981; Nicchitta & Ellington, 1984), as well as from polychaetes (Storey, 1983) and sponges (in sponges the enzyme reacts only with glycine) (Barrett & Butterworth, 1981). Alanopine dehydrogenase has been found in gastropod molluscs, polychaetes, and cnidarians (Ellington, 1979; Dando *et al.*, 1981; Plaxton & Storey, 1982a,b; Storey, 1983). Both enzymes have a function analogous to that of lactate dehydrogenase (EC 1.1.1.37) in maintaining redox balance for glycolysis, often during environmental anoxia or recovery from anoxia (Fields *et al.*, 1980; De Zwaan & Zurburg, 1981; De Zwaan *et al.*, 1983; Eberlee *et al.*, 1983; Korycan & Storey, 1983; Poertner *et al.*, 1985).

Evidence of tissue specific forms of the enzymes has recently been accumulated. Isozymic forms of alanopine dehydrogenase have been reported in tissues of the whelk, *Busycotypus* (*Busycon*) *canaliculatum* (Plaxton & Storey, 1982b; 1983) while the polychaete *Aphrodite aculeata* was found to have an alanopine dehydrogenase in the body wall muscles and a strombine dehydrogenase in the pharynx (Storey, 1983). Analysis of the tissues of *Mytilus edulis* showed a strombine dehydrogenase in adductor muscle and an alanopine dehydrogenase in foot and various soft tissues (Dando, 1981; Dando *et al.*, 1981).

A recent study of anaerobic metabolism in the cherrystone clam *Mercenaria mercenaria* showed tissue specific differences in the relative accumulation of alanopine and strombine; during environmental anoxia, foot and adductor muscles accumulated approximately equal amounts of the two compounds while gill and mantle showed about ten-fold higher levels of alanopine than strombine (Korycan & Storey, 1983). The pattern of end product accumulation suggested that there might be different forms of alanopine and/or strombine dehydrogenase in muscle and soft tissues (gill and mantle) of this species, forms which support the specific physiological functions of the tissues. The present study reports the partial purification and kinetic characterization of the enzymes from gill and foot muscle of *M. mercenaria* (Linn.) demonstrating the presence of tissue-specific forms of these enzymes, alanopine dehydrogenase in the gill and strombine dehydrogenase in the foot muscle.

MATERIALS AND METHODS

Cherrystone clams were purchased from a local seafood retailer and held in aerated artificial sea water at 15 °C without feeding for at least 1 wk (up to 3 wk) until used. The gills and foot muscle were dissected from freshly killed animals; the gills were used immediately for preparation of alanopine dehydrogenase while the foot muscle was frozen at − 80 °C for future use. All biochemicals were obtained from Sigma Chemical Co., St. Louis or Boehringer Mannheim Corp., Montreal; DEAE-Sephadex and Sephadex G-100 were purchased from Pharmacia Fine Chemicals; other chemicals were of reagent grade. *Meso*-Alanopine was synthesized by the method of Abderhalden & Haase (1931).

ASSAY OF ALANOPINE AND STROMBINE DEHYDROGENASE

The enzymes were assayed spectrophotometrically with either a Unicam SP 8-100 or a Unicam SP-1800 recording spectrophotometer. The decrease in A_{340} due to the oxidation of NADH, or the increase in A_{340} due to the reduction of NAD^+ was measured. Routine assay conditions for the gill enzyme were 200 mM L-alanine, 2.0 mM pyruvate, 0.1 mM NADH, 50 mM imidazole–HCl, pH 7.0; for the muscle enzyme they were 600 mM glycine, 2.0 mM pyruvate, 0.1 mM NADH, 50 mM imidazole–HCl, pH 7.0. One unit of enzyme activity was defined as the amount catalyzing the oxidation of 1 μmol NADH $\cdot$ min^{-1}.

PROTEIN ASSAY

The concentration of protein in solutions was assayed by Coomassie blue binding (Bradford, 1976).

PARTIAL PURIFICATION OF ALANOPINE DEHYDROGENASE FROM GILL

Gills were dissected from about 12 clams and homogenized in 5 vol. (w/v) of cold 100 mM imidazole–HCl, pH 7.5, containing 2 mM EDTA, and 7 mM 2-mercapto-ethanol. The homogenate was centrifuged at 30 000 × g for 15 min at 2 °C. The supernatant was treated with ammonium sulphate to 40% saturation, stirred for 30 min and then centrifuged as above. The supernatant from this centrifugation was further treated with ammonium sulphate to 70% saturation. This suspension was stirred for 30 min and then centrifuged as above. The pellet, which contained enzyme activity, was dissolved in a minimal volume (about 5 ml) of 25 mM imidazole–HCL, pH 7.5, containing 7 mM 2-mercaptoethanol (buffer A). The enzyme was dialyzed for 2 h against 2 l of buffer A, and then applied to a column of DEAE–Sephadex A-50 (1.5 × 10 cm) that had been equilibrated with buffer A. Alanopine dehydrogenase was eluted from the column with a gradient of 0–1 M KCl in buffer A at 40 ml/h. The fractions (1 ml) containing enzyme were pooled and directly applied to a column of

hydroxyapatite (1.5 × 10 cm) that had been equilibrated with buffer A. Enzyme was eluted from this column (speed and fraction size as above) with a gradient of 0–200 mM potassium phosphate, pH 7.5, containing 7 mM 2-mercaptoethanol.

PARTIAL PURIFICATION OF STROMBINE DEHYDROGENASE FROM FOOT MUSCLE

Frozen foot muscle was thawed and homogenized in 5 vol. (w/v) of ice cold 50 mM imidazole–HCl, pH 7.0, containing 15 mM 2-mercaptoethanol with a Polytron PT-10 homogenizer. The homogenate was centrifuged at 30000 × *g* for 20 min at 2 °C. The crude supernatant was removed and ammonium sulphate was added to 45% saturation; the suspension was stirred for 30 min and then centrifuged as above. The supernatant was removed, ammonium sulphate was added to 65% saturation; the suspension was then stirred and centrifuged as above. The pellet, containing enzyme activity, was resuspended in 2 ml of 50 mM imidazole–HCl, pH 7.0, containing 15 mM 2-mercaptoethanol and chromatographed on a Sephadex G-100 column (1 × 50 cm) equilibrated with the same buffer at 20 ml · h^{-1}. Fractions (1 ml) containing strombine dehydrogenase were pooled and used for kinetic studies.

ISOELECTROFOCUSING

Tissues (gill, mantle, foot muscle or adductor muscle) were homogenized and centrifuged as above to prepare crude extracts. These were loaded onto a LKB 8101 column (100 ml) with pH 4–6 ampholines and focused at 400 V for 14 h at 4 °C. The column was then drained and 1 ml fractions were collected and assayed for enzyme activity. Three samples (from individual animals) were run for each tissue.

AMINO ACID ANALYSIS

Foot muscle and gill were dissected out of aerobic animals taken directly from the sea-water tank. Tissues were immediately frozen in liquid nitrogen. Acid extracts of tissues were prepared as described by Storey *et al.* (1981) and free amino acids were measured using Beckman 119BL amino acid analyzer.

KINETIC ANALYSIS

K_m values were determined from Hanes plots or were computer fitted using the method of Cleland (1979). I_{50} values were determined by the method of Job *et al.* (1978). All I_{50} values given are the average of $n = 2$ determinations on different preparations of enzyme. For K_m determinations $n = 6$ to 8 substrate concentrations were used; for I_{50} determinations $n = 4$ inhibitor concentrations were used.

RESULTS

ISOELECTROFOCUSING

The foot muscle showed a major peak of strombine dehydrogenase activity (activity ratio was 5 : 3 for glycine versus alanine at 300 mM of each) with a pI of 4.75–4.80 and a minor peak, less than 10% of the total activity, with a pI of 5.0–5.15 and equal activities with alanine and glycine. The pattern for the adductor muscle was similar. Gill and mantle both showed a major peak of alanopine dehydrogenase activity (activity ratio was 3 : 1 in the gill and 2 : 1 in the mantle for alanine versus glycine at 300 mM of each) with a pI value of 5.15–5.25 and a minor peak with pI values of 4.85–4.95 (activity ratios about 4:3 for alanine versus glycine, each at 300 mM). The minor peaks in the soft tissues accounted for about 33% of the total activity.

PURIFICATION

The activity of strombine dehydrogenase in the adductor muscle was 54 U · g wet wt^{-1}, and that of alanopine dehydrogenase in the gill was 4.1 U · g wet wt^{-1}. The enzyme from the foot muscle was purified ≈26-fold to a specific activity of 37 U · mg $protein^{-1}$; that from the gill was purified ≈25-fold to a specific activity of 3 U · mg $protein^{-1}$. For gill, only the major enzyme form (see isoelectrofocusing data above) was saved during purification, DEAE–Sephadex and hydroxylapatite separation combining to eliminate > 95% of the activity of the minor peak.

ENZYME KINETICS

The relative activities of the foot and gill enzymes with a variety of keto acids and amino acids are summarized in Table I. Of the keto acids, both enzymes showed the highest activity and the lowest apparent K_m with pyruvate. Both reacted with 2-oxobutyrate and glyoxylate to some extent, but the apparent K_m values were at least four-fold greater and the apparent V was less than 50% that observed with pyruvate. Both enzymes showed very low activity with 2-oxovalerate and hydroxypyruvate. In contrast, the enzymes from the two tissues showed very different activity profiles with the amino acids. Foot muscle enzyme had higher activity with glycine as substrate as compared to L-alanine, and a slightly lower apparent K_m for glycine than for L-alanine. In contrast, the gill enzyme showed much higher activity with L-alanine than with glycine, and the apparent K_m for L-alanine was 10-fold lower than the apparent K_m for glycine. For both enzymes, the apparent K_m for L-2-aminobutyrate was the lowest for any amino acid. For the gill enzyme, however, the apparent V was 94% that observed with alanine, while for the foot muscle enzyme it was only about 50% that observed with glycine. The gill enzyme also showed substantial activity with L-cysteine, L-threonine and L-valine whereas the foot muscle enzyme showed only low activity with these amino acids.

TABLE I

Apparent K_m (mM ± SE) and relative V values for *Mercenaria mercenaria* strombine dehydrogenase from foot muscle and alanopine dehydrogenase from gill: assay conditions, all assays were conducted in 50 mM imidazole–HCl, pH 7.0, at 23 °C; for foot muscle strombine dehydrogenase the amino acids were tested at 0.15 mM NADH and 2.0 mM pyruvate, and the keto acids were tested at 0.15 mM NADH and 600 mM glycine; for the gill alanopine dehydrogenase the amino acids were tested at 0.1 mM NADH and 2.0 mM pyruvate, and keto acids were tested at 0.1 mM NADH and 200 mM L-alanine.

	Strombine dehydrogenase		Alanopine dehydrogenase	
	Apparent K_m	Relative V (%)	Apparent K_m	Relative V (%)
Amino acids				
Glycine	173 ± 1.3	100	291 ± 40	69
L-Alanine	242 ± 11	76	28 ± 2.1	100
L-Serine	693 ± 75	82	247 ± 19.5	90
L-2-Aminobutyrate	44 ± 3.9	48	15 ± 2.2	94
L-Cysteine	–	< 10	23 ± 1.2	90
L-Valine	–	< 10	20 ± 0.8	65
L-Threonine	–	< 10	110 ± 12.5	62
Keto acids				
Pyruvate	0.61 ± 0.06	100	0.48 ± 0.02	100
2-Oxobutyrate	2.46 ± 0.20	30	2.50 ± 0.44	22
Glyoxylate	4.18	20	11.9 ± 1.0	44

Absolute K_m values for the primary amino acid and keto acid substrates for both enzymes are shown in Table II. Foot muscle enzyme had a comparable affinity for alanine and glycine. The affinity for pyruvate was slightly lower when glycine was the cosubstrate than when L-alanine was used as cosubstrate. The gill enzyme had a comparable K_m for pyruvate to that of the foot muscle enzyme, but a much lower K_m for L-alanine. This enzyme was not studied with glycine because of the relatively low activity with this amino acid.

The affinities for *meso*-alanopine were comparable for both enzymes. Apparent K_m

TABLE II

Absolute K_m values (mM ± SE) for foot muscle strombine dehydrogenase and gill alanopine dehydrogenase of *Mercenaria mercenaria*: [a] glycine as cosubstrate; [b] L-alanine as cosubstrate; absolute K_m values for amino acids and keto acids were determined using apparent K_m determinations at three concentrations of keto or amino acid cosubstrate; other assay conditions; 50 mM imidazole–HCl buffer, pH 7.0, and 0.15 mM NADH; for strombine dehydrogenase, $n = 3$ determinations on separate preparations of the enzyme were made; for alanopine dehydrogenase, $n = 2$.

Substrate	Strombine dehydrogenase	Alanopine dehydrogenase
Glycine	83.7 ± 3.6	–
L-Alanine	98.4 ± 7.5	15.1 ± 4.2
Pyruvate[a]	0.32 ± 0.04	–
Pyruvate[b]	0.50 ± 0.03	0.38 ± 0.05

values (at 1 mM NAD) were 5.23 ± 0.39 mM (n = 3) (50 mM Tris buffer, pH 9.0) for strombine dehydrogenase and 7.4 ± 2.7 mM (n = 2) (50 mM Tris buffer, pH 8.5) for alanopine dehydrogenase. Affinities for D-strombine were not determined.

Several metabolites were tested for their effects on the foot muscle and gill enzymes. Although both enzymes were weakly inhibited by succinate, L-lactate, D-lactate, and *meso*-alanopine, distinct differences in the responses of the two enzymes to inhibitors were seen. In general, succinate, D-lactate, and L-lactate were better inhibitors of the gill enzyme than of the foot muscle enzyme. Thus, at subsaturating concentrations of pyruvate (0.5 mM for the muscle enzyme; 0.4 mM for the gill enzyme) and saturating concentrations of amino acid (400 mM glycine for the foot muscle enzyme; 100 mM L-alanine for the gill enzyme) the I_{50} values for succinate, D-lactate, and L-lactate were 60, 49, and 9 mM, respectively, for the gill enzyme and >100, >100, and 60 mM, respectively, for the foot enzyme. When amino acid was subsaturating (100 mM glycine for foot, 30 mM alanine for gill) and pyruvate saturating (2 mM), the gill enzyme was again more strongly inhibited by L-lactate (I_{50} = 9 mM) than was the foot enzyme (I_{50} = 22 mM). Under these conditions, however, I_{50} values for D-lactate were similar for both enzymes (42 mM for foot and 40 mM for gill) but succinate was a stronger inhibitor of the foot enzyme (I_{50} = 38 mM) than of the gill enzyme (I_{50} = 74 mM). The foot muscle enzyme was inhibited by D-octopine (I_{50} values of 20 mM with subsaturating amino acid and 32 mM with subsaturating pyruvate) but octopine did not affect the gill enzyme. I_{50} for *meso*-alanopine for the gill enzyme was 18 mM at subsaturating amino acid and 15 mM at subsaturating pyruvate.

TISSUE AMINO ACID LEVELS

Levels of six neutral amino acids in foot and gill of aerobic clams are shown in Table III. In gill, alanine and glycine occur in similar amounts while in foot, alanine levels are about twice as high as those of glycine. Levels of serine, threonine, valine, and cysteine are very low in both tissues such that it is unlikely that these could be physiological substrates for the enzymes in vivo. Levels of alanine in the two tissues agree well with values for the amino acid in control clams reported by Korycan & Storey (1983).

TABLE III

Levels of some free amino acids in foot muscle and gill of aerobic *Mercenaria mercenaria*: results are μmol · g wet wt^{-1}, means ± SEM, n = 4.

	Foot muscle	Gill
Glycine	8.0 ± 1.8	2.9 ± 0.3
Alanine	18.9 ± 3.1	3.8 ± 0.5
Serine	0.8 ± 0.2	<0.05
Threonine	0.4 ± 0.1	<0.05
Valine	<0.05	<0.05
Cysteine	0.3 ± 0.01	0.30 ± 0.01

Discussion

The present study provides a comparison of the imino acid dehydrogenases in foot muscle and gill of *M. mercenaria* and is the first kinetic study of the enzymes from a non-muscle tissue in bivalves. Two distinct, tissue-specific forms of enzyme were found. A strombine dehydrogenase predominated in foot and adductor muscles while an alanopine dehydrogenase was the major form in soft tissues (gill and mantle).

Foot muscle strombine dehydrogenase showed similar affinities for glycine and alanine as substrates but had a very limited ability to use other neutral amino acids. This is generally similar to the characteristics of the enzyme from muscle tissue of other bivalves (Dando, 1981; Fields & Hochachka, 1981; De Zwaan & Zurburg, 1981; Nicchitta & Ellington, 1984). Gill alanopine dehydrogenase showed very different amino acid substrate specificities. Glycine was a poor substrate but the enzyme reacted well with alanine and several other neutral amino acids. Poor reactivity with glycine is generally true of alanopine dehydrogenases from other sources although the relative reactivities with other neutral amino acids, aside from alanine, can vary widely with some enzymes being highly specific for alanine alone (such as the *Littorina littorea* enzyme) while others show wide reactivity with serine, valine, threonine, and cysteine (such as the *Aphrodite aculeata* and *Mytilus edulis* enzymes) (Dando, 1981; Plaxton & Storey, 1982a; Storey, 1983). Substrate affinities for alternative amino acids for these enzymes are probably modulated in response to several factors such as composition of the free amino acid pool and the physiological function of the enzyme in vivo. Thus, for octopine dehydrogenases, reactivity with arginine alternatives (lysine, ornithine) decreased dramatically in species (e.g. cephalopods, scallops) in which the enzyme had a very clearly defined and major role in muscle glycolysis (Storey & Dando, 1982). For strombine and alanopine dehydrogenases, strong or predictable patterns to the amino acid specificities have really not emerged, as yet, except for the tendency for strombine dehydrogenases to appear in muscle tissues with high glycine contents (e.g. in *Aphrodite aculeata* glycine content of proboscis muscle is nearly 100-fold higher than alanine) (Zurburg *et al.*, 1982; Storey, 1983; Nicchitta & Ellington, 1984).

However, even this rule does not hold up for *Mercenaria mercenaria*. Like other bivalves, the strombine dehydrogenase (with similar affinities for glycine and alanine) occurs in muscle tissues but the glycine content of foot is somewhat lower than that of alanine. The result of this is that strombine and alanopine are produced in similar amounts of *M. mercenaria* muscle tissues (Korycan & Storey, 1983), unlike the case in other bivalve species where strombine is the only product accumulated in muscle (De Zwaan & Zurburg, 1981; Zurburg *et al.*, 1982). Gill tissue in *M. mercenaria*, however, contains predominantly alanopine (levels 10-fold higher than strombine) (Korycan & Storey, 1983); this would result from the much higher affinity of gill alanopine dehydrogenase for alanine versus glycine (K_m for alanine 10-fold lower than for glycine) despite equal tissue levels of alanine and glycine. Thus, the relative levels of strombine versus alanopine in tissues of *M. mercenaria* are consistent with and predictable from the interaction of enzyme amino acid affinities with tissue free amino acid levels.

Unlike the situation in many other marine molluscs (Dando *et al.*, 1981), alanopine and strombine dehydrogenases are the only terminal glycolytic dehydrogenases present in significant amounts in *M. mercenaria* tissues; octopine dehydrogenase is absent and lactate dehydrogenase activities are negligible (Korycan & Storey, 1983). Both enzymes, therefore, must assume the primary support of the needs for anaerobic glycolysis in their respective tissues. In soft tissues, this would largely be a function in the survival of environmental anoxia when the tide recedes and leaves clams in sand which becomes progressively anoxic. The metabolic response of these tissues to environmental anoxia is a strong depression of metabolic rate (shutting down nonessential processes to conserve energy in the anoxic state) coupled with a reorganization of energy metabolism to increase ATP yield from substrate fermentations (Storey, 1985). In these tissues, an alanopine dehydrogenase finds a role in glycolytic energy production and utilizes as a substrate the alanine which is a primary product of anaerobic glycolysis during the early hours of environmental anoxia (Korycan & Storey, 1983). In muscle tissues of marine bivalves, the situation is different. Muscle tissues not only have a much lower aerobic potential overall (lower numbers of mitochondria and activities of mitochondrial enzymes, higher activities of glycolytic enzymes) than do tissues such as gill but are faced with two types of low oxygen stresses, the environmental anoxia brought about by intertidal life as well as the functional anoxia incurred when active muscle work (e.g. digging in *M. mercenaria*) overruns the limited capacities of these tissues for rapid aerobic ATP synthesis. Strombine dehydrogenase in foot muscle of *M. mercenaria* must support glycolytic energy production under both of these circumstances. The requirement for burst muscle work (creating functional anoxia) may be the pressure which has promoted the appearance of strombine dehydrogenases in active muscles of marine invertebrates (e.g. the adductor and foot muscles of bivalves, the proboscis muscle of *Aphrodite aculeata* (Dando *et al.*, 1981; De Zwaan and Zurburg, 1981; Storey, 1983; Nicchitta and Ellington, 1984)). Thus, a constant and often very large, pool of muscle glycine can be maintained to be utilized instantly whenever muscle energy demands require a rapid activation of glycolytic energy production. However, tissue alanine pools, because they fluctuate many-fold in concentration in response to environmental oxygen availability (high during anaerobiosis, low under well-oxygenated conditions) are not suitable for this purpose because they cannot provide a stable substrate reserve to support the activation of glycolysis during burst muscle work. Our data suggest that *M. mercenaria* has not become overly specialized in this regard since both glycine and alanine pools are substantial in aerobic foot and both strombine and alanopine accumulate under anoxia (the product(s) of muscle work may be different). However, as the need for burst muscle work supported by glycolytic ATP production becomes more specialized (e.g. the proboscis muscle of *Aphrodite aculeata*), strombine dehydrogenase activity is increased, amino acid specificity is focused on glycine, and tissue glycine contents are greatly elevated (Storey, 1983).

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