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# PURIFICATION AND PROPERTIES OF ALANOPINE DEHYDROGENASE ISOZYMES FROM THE CHANNELED WHELK, *BUSYCOTYPUS CANALICULATUM*

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**Abstract**—1. Three tissue specific isozymes of alanopine dehydrogenase (ADH: alanopine: NAD oxidoreductase) were purified to homogeneity from foot muscle, gill and hepatopancreas of the channeled whelk, *Busycotypus canaliculatum*.

2. Muscle ADH showed properties, in particular the effect of decreasing pH in lowering apparent $K_m$'s for pyruvate and L-alanine, which appear to gear this isozyme for alanopine synthesis as an end product of anaerobic glycolysis.

3. The hepatopancreas isozyme, with a high affinity for *meso*-alanopine and NAD, appears suited for a role in alanopine oxidation *in vivo*.

4. The properties of gill ADH are intermediate between those of the other two forms.

## INTRODUCTION

While lactate dehydrogenase functions as the terminal dehydrogenase of glycolysis in vertebrate and many invertebrate species, enzymes of a family of imino acid dehydrogenases (pyruvate + amino acid + NADH + $H^+ \rightleftharpoons$ imino acid + $NAD^+ + H_2O$) have recently been shown to catalyze the same function in the tissues of many marine invertebrates. Octopine dehydrogenase has been well studied (Regnouf and Thoai, 1970; Haas *et al.*, 1973; Storey and Storey, 1979a; Gade, 1980). Amongst cephalopod molluscs the enzyme occurs in tissue specific isozymic forms, the mantle muscle and brain isozymes showing properties functionally analogous to those of M and H type lactate dehydrogenase, respectively (Storey and Storey, 1979a). Muscle octopine dehydrogenase, like M type lactate dehydrogenase, is geared to a role in cytoplasmic redox regulation during glycolytic function; brain octopine dehydrogenase and H type lactate dehydrogenase are poised, instead, for the oxidation of product as an aerobic fuel (Storey and Storey, 1979b).

In 1976 Fields identified a second imino acid dehydrogenase, alanopine dehydrogenase: (L-alanine + pyruvate + NADH + $H^+ \rightleftharpoons$ *meso*-alanopine + $NAD^+ + H_2O$) occurring in the tissues of the oyster. Alanopine and strombine (utilizing glycine) dehydrogenases have subsequently been identified in other marine bivalve and gastropod molluscs (Dando *et al.*, 1981), sponges (Barrett and Butterworth, 1981), sea anemones (Ellington, 1979) and polychaete worms (Storey, 1983). The kinetic and physical properties of alanopine dehydrogenase have been studied in two species, the bivalve, *Crassostrea gigas* (Fields and Hochachka, 1981) and the gastropod, *Littorina littorea* (Plaxton and Storey, 1982a). In recent studies of the tissue distribution of alanopine dehydrogenase in the channeled whelk, *Busycotypus canaliculatum*, we demonstrated the presence of tissue specific isozymic forms of alanopine dehydrogenase (Plaxton and Storey, 1982b). Three forms, specific for muscle, gill/kidney and hepatopancreas, were separable by polyacrylamide gel electrophoresis and isoelectrofocusing (pI = 5.9, 5.6 and 5.7, respectively).

In the present study we have purified and characterized the three isozymes of alanopine dehydrogenase from foot muscle, gill and hepatopancreas of *B. canaliculatum* in order to investigate the tissue specific functions of the enzymes.

## MATERIALS AND METHODS

### *Chemicals and animals*

All biochemicals were purchased from Sigma Chemical Co. except for *meso*-alanopine which was synthesized by the method of Abderhalden and Haase (1931). Chromatofocusing materials, Sephadex G-100 and molecular weight marker proteins were from Pharmacia Ltd. Specimens of the channeled whelk, *B. canaliculatum*, were purchased from the Marine Biological Laboratory, Woods Hole, MA and held in recirculating seawater at 12°C without feeding until use.

### *Enzyme assay and kinetic studies*

Alanopine dehydrogenase was assayed at 24°C by monitoring NAD(H) utilization at 340 nm using a Gilford recording spectrophotometer. Standard assay conditions for alanopine dehydrogenase were: 50 mM imidazole–HCl buffer, pH 7.5, 2.0 mM pyruvate, 50 mM L-alanine and 0.15 mM NADH in the forward direction and 50 mM Tris–HCl buffer, pH 8.5, 40 mM *meso*-alanopine and 2.0 mM NAD in the reverse direction in a final volume of 1 ml. Assays were started by the addition of enzyme preparation. Standard assay conditions for lactate dehydrogenase were: 50 mM imidazole–HCl buffer, pH 7.5, 2.0 mM pyruvate and 0.15 mM NADH. One unit of enzyme activity is defined as the amount of enzyme utilizing 1 μmol of NADH per min at 24°C.

Michaelis constants (apparent $K_m$'s) for substrates were determined from Hanes plots [S]/V vs [S], using constant co-substrate concentrations: 2.0 mM pyruvate, 50 mM L-alanine, 0.15 mM NADH, 40 mM *meso*-alanopine or 2 mM NAD. Product inhibition characteristics were evaluated at subsaturating substrate conditions: 0.2 mM pyruvate, 7.5 mM L-alanine 0.035 mM NADH in 50 mM imidazole buffer, pH 7.0 for the forward direction and 5 mM *meso*-alanopine, 0.12 mM NAD in 50 mM imidazole buffer, pH 8.0 for the reverse direction.

*Purification of alanopine dehydrogenase*

Foot muscle (1:6 w/v), gill (1:4 w/v) or hepatopancreas (1:3 w/v) was minced, blotted, weighed and homogenized in ice-cold 50 mM imidazole–HCl buffer, pH 7.5 containing 15 mM 2-mercaptoethanol using a Polytron PT 10-35 homogenizer. For hepatopancreas, 3 mM PMSF (phenylmethylsulfonyl fluoride), a protease inhibitor, was included in the homogenizing buffer. Homogenates were centrifuged at 27,000 *g* for 30 min at 4°C and pellets discarded. Each supernatant fraction was adjusted to 2.05 M (50% saturation) with ammonium sulphate, stirred at room temperature for 30 min and then centrifuged as above. Pellets were discarded and supernatants were then adjusted to 3.28 M (80% saturation) with ammonium sulphate and stirred and centrifuged as above. The pellets, containing alanopine dehydrogenase, were resuspended in 25 mM imidazole buffer, pH 7.5 containing 10 mM 2-mercaptoethanol and then dialyzed against this buffer for 2.5 hr at 4°C. Enzyme preparation was then layered onto a column (50 × 0.7 cm) of PBE 94 chromatofocusing exchanger equilibrated in the dialysis buffer. The column was eluted at 20 ml/hr with 160 ml Polybuffer 74 (diluted 1:8) adjusted to pH 4 and 1.5 ml fractions were collected. Peak fractions were pooled and applied to a column (90 × 1.5 cm) of Sephadex G-100 equilibrated in 25 mM imidazole buffer, pH 7.3 containing 10 mM 2-mercaptoethanol and 0.04% sodium azide and eluted with this same buffer. Peak fractions were combined and stored at 4°C for use in kinetic studies. The purified enzymes were stable, with no significant loss of activity or change in kinetic parameters, for at least 2 weeks.

*Molecular weight determination*

Molecular weight determinations were made on a column (90 × 1.5 cm) of Sephadex G-100 using 25 mM imidazole buffer, pH 7.3 containing 10 mM 2-mercaptoethanol and 0.04% sodium azide as the elution buffer. One ml fractions were collected and assayed for alanopine dehydrogenase activity and absorbance at 280 nm. Molecular weight was determined from a plot of $K_{av}$ vs log $M_r$ for the standard proteins: ribonuclease ($M_r = 13{,}700$), chymotrypsinogen ($M_r = 25{,}000$), ovalbumin ($M_r = 45{,}000$) and bovine serum albumin ($M_r = 67{,}000$).

*Electrophoresis*

SDS-polyacrylamide gel electrophoresis of the purified enzyme preparations was carried out using 7.5% tube gels containing 1% SDS with 1.5 mA constant current applied per tube for 6 hr at 24°C. Tris/glycine (46 mM/35 mM), pH 8.3 containing 1% SDS was used as the electrode buffer with bromophenol blue as the tracker dye. Prior to application, enzyme samples were pre-incubated in 1% SDS for 10 min at 80°C. Following electrophoresis gels were fixed overnight in a solution of 40% isopropanol and 10% glacial acetic acid followed by staining for protein using Coomassie Brilliant Blue G by the method of Blakesley and Boezi (1977).

*Protein assay*

Protein was measured by the method of Bradford (1976) using the Bio-Rad prepared reagent and bovine gamma globulin as the standard.

## RESULTS

*Enzyme activities*

*B. canaliculatum* foot muscle contained three dehydrogenase activities acting at the pyruvate branchpoint, alanopine dehydrogenase (47 units/g wet wt), octopine dehydrogenase (41 U/g) and lactate dehydrogenase (0.4 U/g). Hepatopancreas and gill showed no lactate dehydrogenase activity but both alanopine dehydrogenase (26 and 10 U/g, respectively) and octopine dehydrogenase (2 and 1 U/g, respectively) were present.

*Enzyme purification*

Final specific activities of the purified enzymes were 340, 387 and 318 U/mg protein for foot muscle, gill and hepatopancreas representing purifications of about 300-, 2700- and 700-fold, respectively. All three enzyme preparations were judged to be homogeneous by SDS-polyacrylamide gel electrophoresis.

*Molecular weight determination*

Sephadex G-100 gel filtration yielded molecular weights of $43{,}000 \pm 2000$, $41{,}900 \pm 1600$ and $41{,}000 \pm 2500$ ($n = 2$ for each) for foot muscle, hepatopancreas and gill alanopine dehydrogenase, respectively, similar to mol. wt of 38,000 to 47,000 reported for octopine dehydrogenase (Olomucki *et al.*, 1972; Fields *et al.*, 1976), alanopine dehydrogenase (Fields and Hochachka, 1981; Plaxton and Storey, 1982a) and strombine dehydrogenase (Dando, 1981) monomers from other sources.

*pH Effects*

In the forward direction pH optima of the foot and hepatopancreas enzymes varied with changing substrate concentrations. The pH optimum of the foot muscle enzyme decreased from 7.05 at high (saturating) substrate concentrations (50 mM L-alanine, 2 mM pyruvate) to 6.45 at low substrate levels (35 mM L-alanine, 1.4 mM pyruvate).

A similar effect was noted for *L. littorea* foot muscle alanopine dehydrogenase (Plaxton and Storey, 1982a). The pH optima of the hepatopancreas enzyme responded oppositely, however, increasing from 6.5 at high substrate to 7.1 at low substrate concentrations. The pH optimum of the gill isozyme (6.55–6.60) was not affected by substrate concentration.

In the reverse direction differential interactions between substrate levels and pH optima were not apparent. All three isozymes showed pH optima of about 9 at high substrate levels (40 mM *meso*-alanopine, 2 mM NAD). At low substrate levels (15 mM *meso*-alanopine, 1.2 mM NAD), all three pH optima decreased, the foot and gill optima to pH 8.5 and 8.6 respectively, and the hepatopancreas to 8.1.

*Michaelis constants*

Apparent $K_m$'s for pyruvate and L-alanine were strongly dependent upon co-substrate (L-alanine or pyruvate) concentration; for all three isozymes apparent $K_m$ decreased with increasing co-substrate concentration. This effect also occurs for alanopine

and octopine dehydrogenases from other sources (Fields and Hochachka, 1981; Plaxton and Storey, 1982a; Storey and Storey, 1979a; Fields *et al.*, 1976).

Apparent $K_m$'s for substrates for the three isozymes are shown in Table 1. As intracellular pH drops markedly during anaerobiosis in marine invertebrates (de Zwaan, 1977), apparent $K_m$'s were determined at two pH's, 6.5 and 7.5. Apparent $K_m$'s for L-alanine and pyruvate of foot muscle alanopine dehydrogenase were strongly dependent upon pH, decreasing by 31 and 55% at pH 6.5 compared to pH 7.5. Similarly, the apparent $K_m$'s for L-alanine and pyruvate of the gill enzyme also decreased at the lower pH, by 14 and 49%, respectively. This pH effect on the apparent $K_m$'s for the substrates of the forward reaction could function to promote alanopine synthesis as the product of anaerobic glycolysis in foot and gill during periods of anoxia stress. By contrast, the apparent $K_m$'s for substrates of hepatopancreas alanopine dehydrogenase responded differently. Apparent $K_m$ for pyruvate was affected only slightly by pH while the response of the apparent $K_m$ for L-alanine was opposite to that seen for the gill or muscle isozymes; $K_m$ for L-alanine increased by 93% with the pH drop from 7.5 to 6.5. Apparent $K_m$'s for NADH were similar for all three isozymes and were not affected by pH.

In the reverse direction, hepatopancreas alanopine dehydrogenase showed the lowest apparent $K_m$'s for both *meso*-alanopine and NAD. Muscle showed the highest apparent $K_m$'s, 2.2- and 2.9-fold greater than hepatopancreas apparent $K_m$'s for *meso*-alanopine and NAD, respectively, at pH 7.5 while apparent $K_m$'s for the gill enzyme were intermediate between those of the other two isozymes. Apparent $K_m$ for *meso*-alanopine decreased with decreasing assay pH for all three isozymes; the same result was seen for *L. littorea* foot muscle alanopine dehydrogenase (Plaxton and Storey, 1982a). Apparent $K_m$ for NAD of the hepatopancreas isozyme was not affected by pH but $K_m$ increased with decreasing pH for the other two isozymes.

Table 1. Michaelis constants (apparent $K_m$) for substrates of alanopine dehydrogenase isozymes from foot muscle, gill and hepatopancreas of *B. canaliculatum* at high and low pH

| | Foot muscle | | Gill | | Hepatopancreas | |
|---|---|---|---|---|---|---|
| | pH 6.5 | pH 7.5 | pH 6.5 | pH 7.5 | pH 6.5 | pH 7.5 |
| L-Alanine (mM) | 16.1 ± 0.28 | 23.3 ± 0.60* | 19.8 ± 1.03 | 22.9 ± 0.75** | 20.5 ± 0.61 | 10.6 ± 0.44* |
| Pyruvate (mM) | 0.29 ± 0.006 | 0.66 ± 0.026* | 0.36 ± 0.019 | 0.71 ± 0.077* | 0.30 ± 0.013 | 0.34 ± 0.014* |
| NADH (μM) | 7.6 ± 0.4 | 7.1 ± 0.4 | 8.3 ± 0.3 | 6.8 ± 0.5 | 7.7 ± 0.4 | 6.8 ± 0.3 |
| | pH 7.5 | pH 8.5 | pH 7.5 | pH 8.5 | pH 7.5 | pH 8.5 |
| *meso*-Alanopine (mM) | 8.3 ± 0.18 | 12.1 ± 0.35* | 5.9 ± 0.30 | 15.0 ± 0.42* | 3.7 ± 0.13 | 7.9 ± 0.55* |
| NAD (μM) | 175 ± 6.0 | 146 ± 6.0* | 128 ± 5.0 | 83 ± 3.0* | 61 ± 2.0 | 60 ± 2.0 |

Apparent $K_m$'s were determined at constant co-substrate concentrations given in Materials and Methods. Data are means ± SEM for $n = 3$ determinations on each of 2 preparations of each enzyme.
*Significantly different from value at the lower pH, $P < 0.01$; **$P < 0.10$.

### *Substrate inhibition*

All three isozymes of alanopine dehydrogenase showed substrate inhibition by high levels of either pyruvate or L-alanine when assayed at saturating co-substrate concentrations. $I_{50}$ for pyruvate was 10, 9 and 11 mM for foot, gill and hepatopancreas alanopine dehydrogenase, respectively, at pH 6.5, values similar to $I_{50}$'s of about 10 mM seen for pyruvate substrate inhibition of H type lactate dehydrogenase (Everse and Kaplan, 1973) or the brain specific isozyme of octopine dehydrogenase (Storey and Storey, 1979a). At pH 7.5, $I_{50}$'s for pyruvate substrate inhibition were 12–15 mM. L-Alanine substrate inhibition was also similar amongst the three isozymes, $I_{50}$ for L-alanine being 250 mM at pH 7.5 and 300–350 mM at pH 6.5. When assayed at subsaturating co-substrate concentrations (0.35 mM pyruvate or 10 mM L-alanine), however, little or no substrate inhibition was found at elevated pyruvate or L-alanine concentrations, a situation which was also seen for foot muscle alanopine dehydrogenase from *L. littorea* (Plaxton and Storey, 1982a).

Table 2. A comparison of the product inhibition characteristics of alanopine dehydrogenase isozymes from *B. canaliculatum*

| | Percentage inhibition by | | | |
|---|---|---|---|---|
| | *meso*-Alanopine 7.5 mM | NAD 0.25 mM | L-Alanine 50 mM | Pyruvate 1 mM |
| Foot muscle | 47.4 ± 3.2 | 12.6 ± 0.1 | 37.2 ± 2.0 | 29.4 ± 2.9 |
| Gill | 48.2 ± 0.9 | 9.4 ± 0.1 | 11.8 ± 0.2 | 12.5 ± 0.1 |
| Hepatopancreas | 64.8 ± 4.6 | 36.2 ± 1.03 | 3.9 ± 0.1 | 3.5 ± 0.1 |

Product inhibitions were measured using subsaturating substrate conditions given in Materials and Methods. Data are means ± SEM for duplicate determinations on two preparations of each enzyme.

*Product inhibition*

Table 2 summarizes the product inhibition characteristics of the three alanopine dehydrogenase isozymes. In the forward direction, the hepatopancreas enzymes showed the strongest product inhibition. Inhibition by *meso*-alanopine was significantly greater than that seen for the foot muscle or gill enzymes (which show equivalent levels of inhibition) while inhibition by NAD was 3- and 4-fold greater, respectively, than NAD inhibition of the foot muscle or gill enzymes. In the reverse, alanopine oxidizing, direction, however, hepatopancreas alanopine dehydrogenase showed very little product inhibition by pyruvate and L-alanine. The greatest product inhibition of the reverse direction was seen for the foot muscle enzyme, where inhibition was 10-fold greater than that found for the hepatopancreas enzyme. Gill alanopine dehydrogenase showed product inhibition characteristics intermediate between those of the foot muscle and hepatopancreas enzymes.

*NHxDH substrate analogue studies*

$M_4$ and $H_4$ isozymes of lactate dehydrogenase, as well as the brain and muscle isozymes of octopine dehydrogenase, show differential responses to the hypoxanthine derivative of NADH. Dawson *et al.* (1964) found activity ratios (NHxDH/NADH) of 0.5 for $M_4$ and 3.0 for $H_4$ lactate dehydrogenase while octopine dehydrogenase showed activity ratios of 0.63 and 2.43 for the muscle and brain isozymes (at high pyruvate concentrations), respectively (Storey and Storey, 1979a). Activity ratios, NHxDH/NADH for the three alanopine dehydrogenase isozymes of *B. canaliculatum*, measured at two pyruvate concentrations, are shown in Table 3. With increasing pyruvate concentration, the activity ratio decreased for the foot muscle enzyme similar to the findings for muscle octopine dehydrogenase (Storey and Storey, 1979a). Both gill and hepatopancreas alanopine dehydrogenase showed opposite reactions, the activity ratios increasing significantly with increased pyruvate levels.

Table 3. Utilization of NHxDH, the hypoxanthine derivative of NADH, by muscle, gill and hepatopancreas isozymes of alanopine dehydrogenase from *B. canaliculatum*

| | Activity ratio NHxDH/NADH | |
|---|---|---|
| | At 2 mM pyruvate | At 15 mM pyruvate |
| Foot muscle | 0.98 ± 0.006* | 0.92 ± 0.014** |
| Gill | 0.94 ± 0.007 | 1.11 ± 0.009** |
| Hepatopancreas | 0.94 ± 0.008 | 1.05 ± 0.006** |

Assay conditions: 50 mM imidazole–HCl buffer, pH 7.5, 50 mM L-alanine, 0.15 mM coenzyme and pyruvate as shown. Data are means ± SEM, $n = 4$ determinations each on 2 separate preparations of each enzyme.

*Significantly different from values for gill and hepatopancreas enzymes at 2 mM pyruvate, $P < 0.01$.
**Significantly different from corresponding value at 2 mM pyruvate, $P < 0.01$.

## DISCUSSION

Alanopine dehydrogenase occurs in three tissue specific isozymic forms in the tissues of the whelk. Muscular tissues, including ventricle, proboscis, mantle and foot contain one form, gill and kidney exhibit a second form and hepatopancreas shows the third form of the enzyme (Plaxton and Storey, 1982b). The isozymes were distinguished by isoelectric point and by migration on polyacrylamide gels and also showed differential utilization of glycine as an alternative amino acid substrate (Plaxton and Storey, 1982a). The ratios of the apparent $K_m$'s glycine:alanine were 132, 192 and 70 for muscle, gill/kidney and hepatopancreas isozymes, respectively.

Data in the present study indicate that alanopine dehydrogenase from hepatopancreas shares a number of kinetic features in common with the H type lactate dehydrogenase. These include: (1) low apparent $K_m$'s for the substrates of the reverse reaction, a property which would favour alanopine oxidation *in vivo*, (2) low product inhibition of the reverse reaction by alanine and pyruvate, again favouring alanopine oxidation by the enzyme and (3) a high product inhibition of the forward reaction by alanopine and NAD, a feature which could limit alanopine accumulation *in vivo* as the product of anaerobic glycolysis. Muscle type alanopine dehydrogenase, however, like M type lactate dehydrogenase (Everse *et al.*, 1971; Everse and Kaplan, 1973) or muscle type octopine dehydrogenase (Storey and Storey, 1979a) shows kinetic properties which would gear the enzyme for alanopine production *in vivo* as the product of anaerobic glycolysis. Compared to hepatopancreas alanopine dehydrogenase, then, the muscle isozyme shows: (1) a much lower affinity (higher apparent $K_m$'s) for the substrates of the reverse reaction, alanopine and NAD, (2) lower product inhibition of the forward reaction by alanopine and NAD, thus allowing continued alanopine synthesis in muscle despite an accumulation of tissue alanopine and (3) much higher product inhibition of the reverse reaction by alanine and pyruvate. These features, as first postulated for lactate dehydrogenase (Everse *et al.*, 1971; Everse and Kaplan, 1973) would enhance the formation of alanopine under conditions of high gly-

colytic flux but are believed to limit the reversibility of the enzyme *in vivo* and the potential for re-oxidation of accumulated end product in muscle. The gill isozyme of alanopine dehydrogenase has properties which are intermediate between those of the muscle and hepatopancreas forms. Product inhibition of the forward reaction is low, similar to that seen for the muscle enzyme, suggesting a good capacity for alanopine accumulation during anaerobic function. However apparent $K_m$'s for the reverse reaction and product inhibition characteristics of this direction are both intermediate between the hepatopancreas and foot muscle kinetics suggesting that the enzyme also has a good capacity for reversing the reaction *in vivo* and utilizing alanopine as an aerobic substrate for gill oxidative metabolism.

Apparent $K_m$'s for both pyruvate and L-alanine of foot muscle alanopine dehydrogenase are strongly influenced by pH, both decreasing when pH is lowered from 7.5 to 6.5. A fall in intracellular pH during anaerobiosis is well documented amongst marine invertebrates (de Zwaan, 1977). The production of alanopine as the end product of anaerobic glycolysis in muscle would be favoured, then, by two effects, both lowering the apparent $K_m$'s for substrates: (1) decreased intracellular pH and (2) increased co-substrate concentrations arising from increased glycolytic flux and the accumulation of alanine as an anaerobic end product. This pH effect on the apparent $K_m$'s for pyruvate and L-alanine is less pronounced for the gill enzyme while hepatopancreas alanopine dehydrogenase shows only a slight decrease in $K_m$ pyruvate with decreasing pH. Apparent $K_m$ for L-alanine of the hepatopancreas enzyme reacts oppositely to decreasing pH, doubling with the pH drop from 7.5 to 6.5. This property coupled with the high product inhibition of the enzyme by *meso*-alanopine and NAD suggests that enzyme function during anaerobiosis could be limited.

Alanopine dehydrogenase isozymes also differed in their utilization of the hypoxanthine analogue of NADH. Muscle alanopine dehydrogenase behaved like the muscle isozyme of octopine dehydrogenase, the activity ratio, NHxDH/NADH, decreasing when pyruvate substrate concentration was increased. Gill and hepatopancreas isozymes, which were similar in this characteristic, showed an analogous behaviour to that seen for brain type octopine dehydrogenase (Storey and Storey, 1979a), the activity ratio increasing at higher pyruvate concentrations.

The three isozymes of alanopine dehydrogenase in *B. canaliculatum* appear to be geared, therefore, by differences in their kinetic properties for tissue specific functions in the metabolism of alanopine *in vivo*. pH and co-substrate effects on the apparent $K_m$'s for pyruvate and L-alanine of muscle alanopine dehydrogenase suggest that the enzyme may have its major function as the cytosolic dehydrogenase responsible for redox regulation during muscle glycolytic function. The kinetic characteristics of the hepatopancreas isozyme, however, particularly the low apparent $K_m$'s for the substrates of the reverse reaction, suggest that the role of the enzyme is chiefly in the oxidation of alanopine. Hepatopancreas could make effective use of blood alanopine (derived from alanopine release from other tissues or from dietary intake) as an oxidative or gluconeogenic substrate. The kinetics of the gill isozyme suggest that the enzyme may have a bidirectional function. As the major terminal glycolytic dehydrogenase activity in the tissue, the enzyme should play a major role in redox regulation during anaerobiosis; the kinetic properties of the forward direction (pH effects on apparent $K_m$'s, low product inhibition) would promote this function. However, the low apparent $K_m$'s for substrates of the reverse reaction also imply a good capacity for the oxidation of alanopine as an aerobic substrate just as vertebrate heart and brain can make effective use of blood lactate as an aerobic fuel.

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