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[Short Communication]

Distribution of Pyruvate Oxidoreductases in Three Body Parts of the Intertidal Sipunculid, *Phascolosoma arcuatum*

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ABSTRACT—Distribution of opine dehydrogenases and lactate dehydrogenase (LDH) among different body parts of a sipunculid are reported for the first time. Alanopine dehydrogenase (ADH), octopine dehydrogenase (ODH) and strombine dehydrogenase (SDH) were present in the body wall and the introvert of *Phascolosoma arcuatum*. However, no activity of ADH or SDH was detected in the internal organs of this sipunculid. LDH activities were detected in the three body parts studied. In the body wall and the introvert, the activities of LDH represented only a small fraction of the total pyruvate oxidoreductase activities. In the internal organs, however, the activity of LDH was approximately half that of ODH. The lactate oxidizing:pyruvate reducing ratio of the LDH from the internal organs was greater than those from the body wall and the introvert. The electrophoretic pattern of LDH isozymes from the internal organs was also different from those of the latter body parts. The distribution patterns of pyruvate oxidoreductases in these body parts of *P. arcuatum* were discussed in relation to the physiology of this sipunculid.

INTRODUCTION

The Phylum Sipuncula consists of a group of approximately 320 species of soft bodied animals, sometimes called peanut worms (Barnes, 1980). They are exclusively marine animals. Most of them are bottom dwellers, the majority in shallow water (Barnes, 1980). An atypical example is *Phascolosoma arcuatum*, which lives in the mud of mangrove swamps. The habitat of *P. arcuatum*, compared to those of other sipunculids, is physiologically more stressful with respect to the fluctuation in salinity and the low level of oxygen present. Physiological responses of *P. arcuatum* to changes in salinities (Chew *et al.*, 1994; Peng and Ip, 1994; Peng *et al.*, 1994) and environmental hypoxia/anoxia (Lim and Ip, 1991a, b; Ip *et al.*, 1992, 1994) have been studied recently.

In anoxia, *P. arcuatum* produces succinate via phosphoenolpyruvate carboxykinase and the phosphoenolpyruvate branch point of glycolysis (Lim and Ip, 1991a). Using cellulose acetate electrophoresis and isoelectric focusing techniques, Lim and Ip (1991b) discovered the presence of three distinct pyruvate kinase isozymes in three different body parts of *P. arcuatum*. They further reported that the kinetic properties of the pyruvate kinase isozymes in the body wall and the introvert were altered during anoxia through protein phosphorylation to facilitate succinate formation. However, anoxia did not induce phosphorylation of the pyruvate kinase isozyme in the internal organs of *P. arcuatum* (Lim and Ip, 1991b). Such results indicate that the internal organs of this sipunculid may depend on the lactate/opine pathways to survive anoxia. Therefore, we suspected that the pattern of distribution of pyruvate oxidoreductases in the internal organs would be different from those in other body parts of this sipunculid.

In general, pyruvate oxidoreductases include D- and L-lactate dehydrogenase (D-, L-lactate: NAD oxidoreductase; EC 1.1.1.28;27; D-, L-LDH) and opine dehydrogenases. The latter dehydrogenases catalyze the reductive condensation of pyruvate with an amino acid to form imino acid derivatives known as opines. The three major opine dehydrogenases characterized so far are octopine dehydrogenase [Nα-(D-1-carboxymethyl)-D-alanine: NAD oxidoreductase; EC 1.5.1.11; ODH], strombine dehydrogenase [*N*-(carboxymethyl)-D-alanine: NAD oxidoreductase; EC 1.5.1.22; SDH],and alanopine dehydrogenase [meso-*N*-(1-carboxyethyl)-alanine: NAD oxidoreductase; EC 1.5.1.17; ADH] (Livingstone *et al.*, 1990). Tauropine dehydrogenase and β-alanopine dehydrogenase are also present in some marine invertebrates (Hamman and Fielding, 1993).

To date, no information is available on the distribution of pyruvate oxidoreductases in various body parts of a sipunculid.

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Even for the whole body of sipunculids, there are conflicting results. Livingstone *et al.* (1983) detected activities of LDH and SDH, but not those of ODH and ADH, from *Phascolion strombi*. On the contrary, Portner *et al.* (1984) reported the presence of ODH, SDH and ADH, but were unable to detect LDH activity, in the muscle tissues of *Sipunculus nudus*.

Hence, the present study was undertaken to elucidate the distribution of various pyruvate oxidoreductases, including LDH isozymes, in the body wall, the introvert and the internal organs of *P. arcuatum*.

MATERIALS AND METHODS

P. arcuatum were collected from the mud-flats of the mangrove swamp at Mandai, Singapore. They were maintained at 25°C in the laboratory in plastic aquaria with aerated 50% (15‰ salinity) seawater. No attempt was made to feed them. Experiments were performed 7 days after acclimation of the worms to the laboratory conditions.

Worms were dissected, drained of coelomic fluid and blotted dry. The worm body was dissected into the body wall, the introvert (which included the associated retractor muscles) and internal organs (which comprised the oesophagus, intestine, rectum and rectal diverticulum). These body parts represented 68%, 17% and 18% of the wet weight of the total tissues, respectively (Ip *et al.*, 1996). Each sample of body wall represented tissues from three worms. Tissues from five worms constituted a sample of introvert. For internal organs, tissues from six worms formed a sample.

Samples were homogenized three times in 5 vol (w/v) of an ice-cold buffer containing 50 mM triethanolamine-HCl (pH 7.5) using an Ultra-Turrax homogenizer (Janke and Kunkel Co., Germany) at maximum speed for 15 s each with 10 s-off intervals. The homogenized sample was centrifuged at 27,000 × g and 4°C for 30 min. The supernatant fluid obtained was then passed through a 10 ml column of bio-gel P-6DG gel (Bio-Rad Laboratories, USA) pre-equilibrated with the homogenization buffer to remove metabolites.

Activities of various enzymes were determined by following the oxidation of NADH or reduction of NAD at 340 nm and 25°C using a Shimadzu UV-160 spectrophotometer. Specific activities were expressed as μmol NADH oxidized or NAD reduced per min per g wet weight ± standard deviation.

The pyruvate reducing (PR) activity of LDH was assayed according to Zammit and Newsholme (1976). The incubation medium contained in a final volume of 1.5 ml: 50 mM Tris-HCl (pH 7), 0.15 mM NADH, 1 mM sodium pyruvate, 1 mM KCN and 0.1 ml of enzyme sample. The lactate oxidizing (LO) activity of LDH was assayed in an incubation medium of 1.5 ml: 50 mM Tris-HCl (pH 7), 0.4 mM NAD, 40 mM lactate, 1 mM KCN and 0.1 ml of enzyme sample.

ODH was assayed according to Zammit and Newsholme (1976). The incubation medium contained in a final volume of 1.5 ml: 50 mM Tris-HCl (pH 7), 0.15 mM NADH, 1 mM sodium pyruvate, 5 mM arginine, 1 mM KCN and 50 μl of enzyme sample. The assay was initiated by the addition of arginine after a constant rate of change of absorbance at 340 nm was obtained. The ODH activity was obtained by subtracting the rate in the absence of arginine (PR activity of LDH) from that in the presence of the amino acid (ODH activity plus PR activity of LDH).

ADH and SDH were assayed by procedures similar to that for ODH, except that the reactions were initiated by alanine (200 mM) and glycine (200 mM), respectively, instead of arginine.

To prepare the sample for electrophoresis, the supernatant fluid obtained after centrifugation was concentrated using a Centricon-30 Microconcentrator (Amicon Inc., USA) Concentrated sample was dialyzed against the electrophoresis buffer (Gelman High Resolution Buffer; Gelman Science Inc, USA) for 1 hr in a microdialyser (Pierce, USA). Electrophoresis was performed on cellulose acetate strips (Sepraphore III, Gelman Science Inc., USA) which had been soaked in Gelman High Resolution Buffer (PH 8.8) for 30 min, at 3 mA per strip in a Gelman Deluxe Electrophoresis Chamber for 45 min. The positions of LDH isozymes on the cellulose acetate strips were detected using a Gelman LDH Isozyme Substrate Set. Electrophoresis was performed three times to confirm the observed migration pattern.

RESULTS AND DISCUSSION

Comparable activities of ADH, ODH and SDH were detected in the body wall of *P. arcuatum* (Table 1). Although these three opine dehydrogenases were also present in the introvert, the activity of ODH was greater than those of ADH and SDH by two fold. No activity of ADH or SDH was detected in the internal organs. In this body part, the activity of ODH was approximately 1/17 and 1/9 of those detected in the body wall and the introvert, respectively (Table 1). This is the first report on the differences in distribution patterns of opine dehydrogenases between various body parts of a sipunculid.

Livingstone *et al.* (1983) proposed three factors which might have affected the distribution of pyruvate oxidoreductases in whole bodies of various organisms: (i) the concentration and availability of free amino acids, (ii) the rate of energy production through the specific pathway, and (iii) molecular aspects of the dehydrogenase. As discussed below, our results indicate that factors (i) and (ii) were also essential in determining the distribution of opine dehydrogenases in various body parts of *P. arcuatum*.

A correlation between amino acid concentration and opine

Table 1. Specific activities (μmol NADH oxidized or NAD reduced per min per g wet weight ± SD, n=5) of lactate dehydrogenase (LDH), in the pyruvate reducing (PR) or lactate oxidizing (LO) direction, alanopine dehydrogenase (ADH), octopine dehydrogenase (ODH) and strombine dehydrogenase (SDH) from the body wall, the introvert and internal organs of *Phascolosoma arcuatum*

	LDH		ADH	ODH	SDH
	PR	LO			
Body wall	1.08 ± 0.23	0.113 ± 0.045	31.22 ± 6.71	33.06 ± 9.66	32.75 ± 8.75
Introvert	0.45 ± 0.17	0.048 ± 0.018	8.25 ± 2.34	17.41 ± 5.81	8.63 ± 3.29
Internal organs	0.72 ± 0.21	0.104 ± 0.043	n.d.	1.89 ± 0.87	n.d.

n.d. = not detectable

dehydrogenase activity was observed in the gastropod *Thais haemastoma* (Kapper *et al.*, 1985; Kapper and Stickle, 1987), the abalone *Haliotis lamellosa* (Gade, 1988) and the bivalve *Scapharca broughtonii* (Sato *et al.*, 1988). Informations available indicate that, in the case of ADH and SDH, high concentrations of amino acids are required for appreciable enzyme activity (Livingstone *et al.*, 1983). The apparent Michaelis-Menton constants (Km) of ADH and SDH for glycine and alanine, respectively, are of the order of 10-50 mM (Livingstone, 1983). Ip *et al.* (1992) demonstrated that contents of alanine and glycine in the internal organs of *P. arcuatum* were the lowest among the three body parts studied. Hence, this correlated well with the absence of activities of ADH and SDH in the internal organs of this sipunculid.

The opine and lactate pathways have equally low energetic efficiencies (Livingstone, 1983). Three ATP molecules are produced per glucosyl unit. However, the maximum potential rate of energy production via the lactate pathway is greater than those via the opine pathways, because the operation of the former is limited only by the size of the glycogen pool (Livingstone, 1983). Besides glycogen, the opine pathways require also the presence of amino acids. In this respect, the octopine pathway is likely to realize a higher rate of energy production than the strombine or alanopine pathways (Livingstone, 1983), as the apparent Km values of ODH from various sources for arginine are of the order of 1 mM (Gade, 1980). The higher activities of ODH in the body wall and the introvert, in comparison to that in the internal organs, of *P. arcuatum* support the proposition that a major function of this pathway is to generate a relatively higher rate of energy production. *P. arcuatum* is found 20-30 cm deep in the mud at the upper intertidal zone of the mangrove swamp (Lim and Ip, 1991b). Through the contraction/relaxation of its body wall musculature and the muscle associated with its introvert, burrowing and feeding activities can be achieved. The presence of ADH and SDH in these muscles can prevent the continuous accumulation of a single type of metabolic end-product. In addition, as suggested for *S. nudus*, ADH and SDH may be involved more during environmental hypoxia as compared to functional hypoxia (Portner *et al.*, 1984).

Portner *et al.* (1984) were unable to detect LDH activity in the sipunculid worm *S. nudus*. In this study, however, LDH activities were detected in all three body parts of *P. arcuatum*. This is in agreement with other reports on the distribution of pyruvate oxidoreductases in various phyla, which suggest that all the terminal dehydrogenases, including LDH, were present in the early stages of metazoan evolution (Livingstone, 1983).

The activities of LDH represented only a small fraction of the total activities of pyruvate oxidoreductases from the body wall and the introvert of *P. arcuatum* (Table 1). This is also in agreement with informations obtained from the whole bodies of other organisms. For example, although LDH and ODH can be present together in gastropods, they are quantitatively mutually exclusive as they have similar functions (Livingstone, 1983). In the internal organs of *P. arcuatum*, however, the activity of LDH was approximately half that of ODH. In addition, the LO:PR ratio of the LDH from the internal organs (0.16 ± 0.03) was greater than those from the body wall (0.011 ± 0.004) and introvert (0.010 ± 0.002). Hence, the properties and functions of the LDH from the internal organs might differ from those of the LDH from the body wall and the introvert.

Cellulose acetate electrophoresis revealed the presence of two bands of LDH isozymes from all three body parts of *P. arcuatum* (Fig 1). The LDHs from the body wall and the introvert exhibited similar electrophoretic patterns. In both cases, the less anodic band was more intense than the more anodic band. The more anodic band of LDH from the internal organs was different in electromobility from those from the body wall and the introvert. The two bands of LDH isozymes from the internal organs were of comparable intensity. These data indicate that three different loci for LDH might exist in *P. arcuatum*. Two of these loci acted in the body wall and the introvert, and produce two isozymes of LDH, while one of these loci, together with the third one, acted in the internal organs. Gene expression of different loci of LDH isozymes is also known in different tissues and organs of fishes (Shaklee *et al.*, 1973; Markert, 1984; Coppes, 1992).

To date, no information is available on whether *P. arcuatum* can produce lactate in a lack of oxygen. Portner *et al.* (1984) was unable to detect lactate in *S. nudus* exposed to functional or environmental hypoxia. If indeed the body wall and introvert of *P. arcuatum*, due to its adaptation to a naturally hypoxic habitat, could produce lactate anaerobically, the LDH present in the internal organs might have the important function

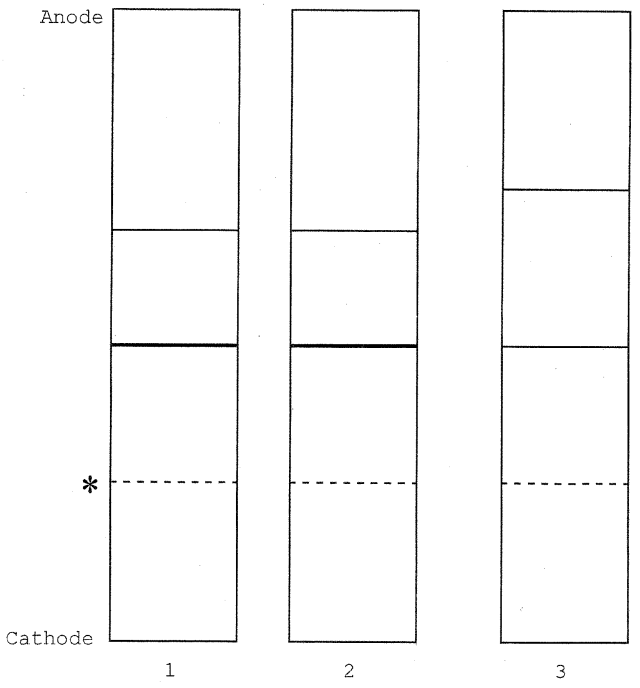

Fig. 1. Composite figure showing electrophoretic resolution on cellulose acetate paper of lactate dehydrogenase isozymes from (1) the body wall, (2) the introvert and (3) internal organs of *Phascolosoma arcuatum*; * indicate origin of sample application.

of removing lactate from the coelomic fluid. Consequently, ODH must be essential for the regeneration of NAD in the internal organs of *P. arcuatum* in anoxia.

REFERENCES

Barnes RD (1980) Invertebrate Zoology, Saunders College Publishing, pp 719–720
Chew SF, Peng KW, Low WP, Ip YK (1994) Comp Biochem Physiol 107A: 141–147
Coppes Z (1992) Comp Biochem Physiol 102B: 673–677
Gade G (1980) Mar Biol Lett 1: 121–135
Gade G (1988) Comp Biochem Physiol 67B: 387–393
Hamman CS, Fielding C (1993) Comp Biochem Physiol 106B: 989–992
Ip YK, Peng KW, Chew SF, Lim RLW (1992) J Exp Mar Biol Ecol 165: 125–132
Ip YK, Peng KW, Chew SF, Lim RLW, Tan GQ (1994) Mar Biol 119: 261–266
Ip YK, Tan GQ, Kuah SSL, Chew SF (1996) J Comp Physiol B (in press)
Kapper MA, Stickle WB (1987) Physiol Zool 60: 159–173
Kapper MA, Stickle WB, Blakeney E (1985) Biol Bull 169: 458–475
Lim RLW, Ip YK (1991a) Zool Sci 8: 673–679
Lim RLW, Ip YK (1991b) J Exp Mar Biol Ecol 152: 123–133
Livingstone DR, de Zwaan A, Leopold M, Martejin E (1983) Biochem Syst Ecol 11: 415–425
Livingstone DR, Stickle WB, Kapper MA, Wang S, Zurburg W (1990) Comp Biochem Physiol 97B: 661–666
Markert CL (1984) Cell Biochem Function 2: 131–134
Peng KW, Ip YK (1994) Zool Sci 11:879–882
Peng KW, Chew SF, Ip YK (1994) Physiol Zool 67: 580–597
Portner HO, Kreutzer U, Siegmund B, Heisler N, Grieshaber MK (1984) Mar Biol 79: 237–247
Sato M, Suzuki S, Yasuda Y, Kawauchi H, Kanno N, Sato Y (1988) Analyt Biochem 174: 623–627
Shaklee JB, Kepes KL, Whitt GS (1973) J exp Zool 185: 217–240
Zammit VA, Newsholme EA (1976) Biochem J 169: 447–462

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