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Adaptation to thermal stress in the freshwater eurythermal teleost *Sarotherodon mossambicus*: Lactate dehydrogenase activity

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Abstract. LDH activity decreased in the osmoregulatory organs and increased in the non-osmoregulatory organs from the small and large individuals of *S. mossambicus* on adaptation to both cold and warm temperatures, relative to normals at room temperature. In any size group, the activity was generally higher in the brain and white muscle, lower in the gill and intermediate in the liver, red muscle, kidney and intestine. The enzyme activity was size-dependent, irrespective of adaptation temperature it was higher in the organs from the large individuals than in those from the small ones. However, the variations in the % LDH activity in the organs between the small and large individuals were very slight in magnitude and inconsistent in direction. The metabolic efficiency to adapt to thermal stress decreases with size.

Keywords. Thermal-adaptation; *Sarotherodon mossambicus*; lactate dehydrogenase activity; osmoregulatory organs; non-osmoregulatory organs.

1. Introduction

There are several studies on lactate dehydrogenase activity (LDH) in fish and other non-piscine poikilotherms adapted to thermal stress (Hazel and Prosser 1974; Tsukuda and Ohsawa 1974; Mary *et al* 1976; Tsugawa 1976). However, most of these studies were confined to its isozyme patterns, rather than its activity *per se*. Hence this glycolytic enzyme has been studied in different organs of *S. mossambicus* adapted to cold and warm temperatures to assess the degree and direction of its adaptation to thermal stress in these organs. In view of the importance of osmoregulation in adapting to thermal stress (Umminger 1975; Catlett and Millich 1976) the organs selected included both the osmoregulatory (gill, kidney and intestine) and non-osmoregulatory (brain, liver, red muscle and white muscle) ones. In as much as abilities to adapt to thermal stress in fish are size-dependent (Parvatheswararao 1977) this study was carried out on the animals of two size groups to assume the influence of size on the enzyme adaptation to thermal stress.

2. Materials and methods

The collection, maintenance and adaptation-time of the fish were as described earlier (Radhakrishnaiah and Parvatheswararao 1981). The fish were divided into two size-groups, small (10 g ± 2 g) and large (50 g ± 2 g), and were adapted separately to cold (20°C ± 0·5°C) and warm (35°C ± 0·5°C) temperatures, while those maintained at room temperature (27·5°C ± 0·5°C) alongside served as controls. On completion of adaptation, the gill, kidney, a part of the intestine, brain, liver, some red muscle from the

lateral line region and some white muscle from the anterodorsolateral region of the trunk were dissected out from each fish in a sterilised cold room at 15°C. The LDH activity was estimated in the cytosolic fraction of these organs using the colorimetric method of Srikantan and Krishnamoorthy (1955) as modified by Govindappa and Swami (1965). Protein content was estimated by using folinphenol reagent method (Lowry *et al* 1951) and the enzyme activity was expressed as μg formozan/mg protein/hr.

3. Results

3.1 *Inter-organ differences*

LDH activity in the different organs of *S. mossambicus* varied to different degrees on adaptation to thermal stress and these variations were in opposite directions in the osmoregulatory and non-osmoregulatory organs. Thus, relative to normals at room temperature, on adaptation to both cold and warm temperatures, the activity decreased significantly ($P < 0{\cdot}002$) in the ormoregulatory organs whereas a significant ($P < 0{\cdot}002$) increase was observed in the non-osmoregulatory organs (figure 1A).

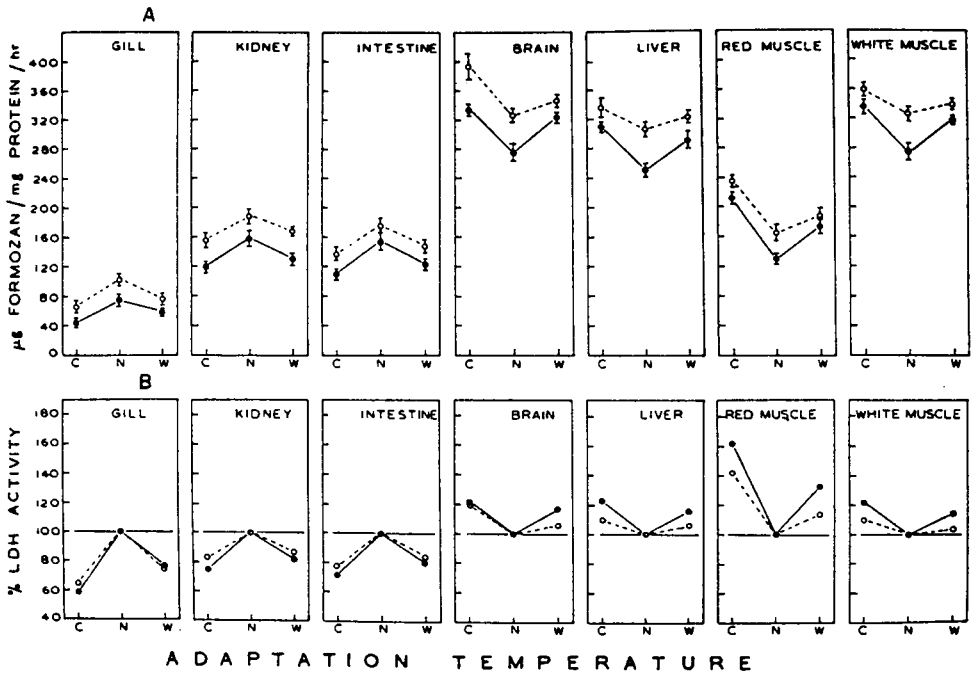

Figure 1. **A.** LDH activity (μg formozan/mg protein/hr) in the different organs from the small (●————●) and large (o--------o) individuals of *S. mossambicus* adapted to cold, normal and warm temperatures. Each point is a mean of six estimations and vertical bars indicate standard deviations. **B.** LDH activity in the different organs studied from the small (●————●) and large (o--------o) individuals of *S. mossambicus* adapted to cold and warm temperatures expressed as % of its normal activity at room temperature, which is fixed at 100. (C: cold (20°C); N: normal (27·5°C); W: warm (35°C))

Further, irrespective of the size, the enzyme activity was maximal in the brain and white muscle, minimal in the gill, and intermediate in the liver, red muscle, kidney and intestine in the sequence indicated. However, there was not much significant difference either in the enzyme activity or its cold- and warm-induced shifts between the brain, white muscle and liver as well as between the kidney and intestine.

3.2 *Influence of body-size*

The LDH activity was size-dependent, irrespective of adaptation temperature, being significantly ($P < 0{\cdot}002$) higher in the organs from the large individuals of the fish than in those from the small ones (figure 1A). Further, the variations in the % LDH activity (the activity at cold- and warm-adapted temperatures was converted as % of its normal activity at room temperature, which is fixed 100) in the organs between the small and large individuals were also compared (figure 1B). These variations were slight in magnitude and inconsistent in direction. However, generally the % LDH activity was higher in most of the organs from the small individuals of the fish than in those from the large ones.

4. Discussion

At enhanced temperatures the anaerobic metabolism through glycolysis increases in fish (Somero 1973) and this appears to hold good in the non-osmoregulatory organs of *S. mossambicus* in which the LDH activity increased on warm-adaptation. Further, there is a suggestion that in fish, when adequate quantity of substrate to the organ is not provided either through the protein or lipid metabolism, stepping up of glycolysis even under decreased temperatures may be considered adaptive (Hazel and Prosser 1974). Perhaps, this may be the reason for the increased LDH activity in the non-osmoregulatory organs of *S. mossambicus* adapted to cold.

In fish, osmo- and iono-regulations play a vital role during thermal-adaptation (Umminger 1975; Catlett and Millich 1976) and this requires a considerable amount of energy to the osmoregulatory organs, the gill, kidney and intestine. So, with the evidence of the pronounced increase in the oxidative metabolism in these organs (Radhakrishnaiah 1983), the decreased LDH activity presently observed suggests that these organs may depend to a greater extent on energetically more efficient oxidative metabolism to meet their higher energy demands during thermal stress and as such, the decrease in the energetically less efficient glycolysis is possible. Further, it is known that the metabolic rate and hence energy expenditure increases in poikilotherms like *S. mossambicus* on adaptation to both cold and warm temperatures (Anantakrishnan and Kutty 1974). Thus the increase in LDH activity in the non-osmoregulatory organs indicates that these organs may be relying more on glycolysis to meet their enhanced energy demands during thermal-adaptation.

Glycolysis and glycolytic enzymes are much more pronounced in the white muscle of fish than in the other organs (Mac Leod 1960; Hazel and Prosser 1974), and the maximal LDH activity in the white muscle is in agreement with this generalisation. Except the suggestion that the white muscle is concerned to improve its tension-temperature relation (Brown 1957), there is no information about the role(s) of this

effector organ during thermal-adaptation of fish. However, what little role it plays involves some energy expenditure which perhaps is mostly met by stepping up glycolysis. This is also evident from the increased LDH isozyme patterns in this organ of some fish during thermal stress (Somero 1973; Bolaffi and Booke 1974). The much lesser LDH activity in the red muscle than that in the white muscle, as observed in the atlantic hagfish, *Myxine glutinosa* (Mellgren and Mathisen 1966) coincides with the suggestion that the red muscle in fish, unlike the white muscle, is predominently oxidative in its metabolic activity (George 1962). However, being involved in many functions during thermal-adaptation such as, slow long-lasting contractions, supply of energy compounds to the white muscle and even thermoregulation, the glycolysis in the red muscle may also be involved to some extent for the supply of the energy compounds. The brain is known to play a very important role in integrating the various physiological processes involved in thermal-adaptation (Lagerspetz 1974). Hence to meet its high energy requirement during thermal-adaptation the glycolysis may be stepped up as indicated by the increased LDH activity in addition to the stepping up of oxidative metabolism (Radhakrishnaiah 1983). Reports on the increased lactate oxidation and LDH isozymes in the liver of fishes during thermal stress (Hochachka 1969; Tsukuda and Ohsawa 1974; Tsugawa 1976) reflect the increase in glycolytic activity as well as lactate cycle for which liver is known to be the centre. Possibly even in *S. mossambicus* the increased LDH activity in the liver on adaptation to thermal stress indicates enhanced activity of glycolysis and/or lactate cycle, hence enhanced metabolic energy production.

Metabolic rate in animals is known to be size-dependent, being higher in the smaller individuals than in the larger ones (cf: Parvatheswararao 1977). Recent studies indicate that such direction in the size-metabolism relation is applicable to oxidative metabolism but not to glycolysis, though this is also size-dependent, has a diametrically opposite direction, being higher in the large individuals than in the small ones (Bashamohideen and Parvatheswararao 1976). Accordingly, at any temperature of adaptation, a higher LDH activity was observed in the organs from the large individuals of *S. mossambicus* than in those from the small individuals. It indicates that with the increase in body size of the fish, probably, the efficiency of oxidative metabolism decreases and that of glycolysis increases and thus the overall metabolic efficiency will be higher in the small individuals of the fish than in the large ones. Further, the more utilisation of energetically less efficient glycolysis by the small individuals of *S. mossambicus*, as evident by the higher % LDH activity in most of the organs, than the large individuals along with the lesser utilisation of energetically more efficient oxidative metabolism (Radhakrishnaiah 1983) indicates that probably the small individuals expend lesser energy to adapt to the imposed thermal stress than the large ones. These results thus coincide with the generalisation that the gross efficiency of the fish in stress media tends to decrease with increasing size and decreasing metabolic rate (Kinne 1964).

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