

Glucose-6-Phosphate Dehydrogenase (G6PDH) and Lactate Dehydrogenase (LDH) Activities in two Air-breathing and two Gill-breathing Species of Fish—A Comparative Study

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The activities of G6PDH and LDH were studied in the brain, gill and muscle tissues of two species each of the air-breathing and the gill-breathing fishes. In all cases LDH activities were significantly higher than those of G6PDH. G6PDH activities between the two groups of fishes indicate that the primary gill-breathers had higher activities in comparison to the air-breathing species. The highest activity of G6PDH was seen in the gills and lowest in the muscle whereas the LDH levels were in the reverse order. These enzymatic variations between the tissues and species are possibly due to the physiological, metabolic and behavioural differences.

Key Words: G6PDH, LDH, Adaptation, Glucose metabolism

Introduction

The main route of glucose metabolism in most of the tissues is by Embden-Meyerhof-Parnas (EMP) pathway, when glucose is converted to pyruvate. The reversible reduction of pyruvate to lactate is the terminal step that characterizes glycolysis in vertebrates. A secondary pathway of glucose metabolism is the Hexose monophosphate (HMP) shunt which diverges from glycolysis at the level of glucose-6-phosphate. It has been shown earlier that the presence of glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) is a strong evidence for the HMP shunt (Hellman 1964,

Kauffman et al. 1969) and the presence of lactate dehydrogenase (LDH, E.C. 1.1.1.27) indicates the capacity for anaerobic glycolysis (Hochachka 1973). Hochachka (1961) reported that during aerobic metabolism both EMP and HMP pathways were operative whereas exclusive EMP participation for glucose metabolism is seen during anoxic condition.

A shortage of dissolved oxygen has been shown to be the primary environmental factor which stimulated the development of air-breathing mechanism in fishes (Carter & Beadle 1931). Purely gill-breathing fishes live

in well aerated water and are more active whereas the air-breathing fishes usually live in oxygen-deficient water and less active. This habitat difference is expected to have some impact on their metabolism.

Therefore, a comparative work has been carried out on the two key enzymes, LDH and G6PDH in gill, brain and muscle tissues of two species of surface-dwelling, active and purely gill-breathing fishes (*Puntius shalynius* and *Danio dangila*) and two species of bottom dwelling, sluggish and air-breathing fishes (*Heteropneustes fossilis* and *Channa orientalis*) to find out, if there exists, any difference in their glucose metabolism.

Materials and Methods

The fishes were collected from natural sources and were acclimatized to laboratory conditions for three weeks. They were kept in aquaria at $20 \pm 2^\circ\text{C}$ with 12 hours' dark and light conditions. Food was given on alternate days and aeration was done only for the gill-breathing fishes. The weight range of different species of fishes used was: 1.2-2.0 g (*P. shalynius*), 1.0-1.6 g (*D. dangila*), 4.0-6.0 g (*C. orientalis*) and 8.0-12 g (*H. fossilis*). Eight matured fishes from each species were decapitated at a fixed time of the day and tissues (brain, gill and muscle)

were removed, washed in ice-cold sucrose 0.25 M, blotted dry and were immediately deep-frozen at -15°C until estimations were carried out. All estimations were completed within a week of sacrifice.

Enzyme assays: A known percentage of homogenate for each tissue (10% for muscle and gill and 5% for brain) was prepared in 0.25 M ice-cold sucrose and centrifuged at $14,000 \times g$ for 20 min at $0 \pm 2^\circ\text{C}$. The supernatant collected was used for enzyme assays. Both G6PDH and LDH activities were measured spectrophotometrically at 340 nm according to the methods of Langdon (1966) and Kornberg (1955) respectively. The protein contents were estimated according to Lowry et al. (1951). The enzyme activity is expressed both in terms of total activity (units/g wet wt.) of tissues and specific activity (units/mg protein). All biochemicals used were purchased from Sigma Chem. Co., USA and other chemicals were of analytical grade from BDH.

Results and Discussion

The levels of G6PDH activity in the different tissues studied showed a general pattern of gill > brain > muscle in all the four species of fishes. The level of G6PDH was extremely low in the muscle (table 1).

Table 1 (A) Total activities (units/g wet wt.) $\times 10^3$ and (B) Specific activities (units/mg protein $\times 10^3$) of G6PDH and LDH in brain, gill and muscle of four different species of fishes (Data has been expressed as mean \pm S.D. calculated from 8 experiments)

Species of fish		Brain		Gill		Muscle	
		G6PDH	LDH	G6PDH	LDH	G6PDH	LDH
A	<i>H. fossilis</i>	13.3 \pm 4.3	77.8 \pm 7.9	18.9 \pm 4.4	31.3 \pm 7.4	0.9 \pm 0.2	146.4 \pm 60.3
	<i>C. orientalis</i>	11.8 \pm 5.6	102.8 \pm 5.8	18.2 \pm 6.8	62.9 \pm 23.4	0.8 \pm 0.3	66.3 \pm 22.2
	<i>P. shalynius</i>	18.7 \pm 9.2	54.8 \pm 17.9	25.9 \pm 4.8	44.1 \pm 9.8	0.8 \pm 0.3	66.7 \pm 17.1
	<i>D. dangila</i>	27.2 \pm 4.9	76.6 \pm 16.5	27.7 \pm 6.0	36.9 \pm 5.6	2.9 \pm 0.9	116.9 \pm 38.5
B	<i>H. fossilis</i>	37.9 \pm 6.7	230.1 \pm 48.7	116.3 \pm 20.2	154.6 \pm 27.9	4.9 \pm 1.3	584.2 \pm 98.0
	<i>C. orientalis</i>	27.2 \pm 4.7	291.4 \pm 60.2	93.2 \pm 45.6	312.2 \pm 98.9	7.0 \pm 3.4	391.5 \pm 25.9
	<i>P. shalynius</i>	51.6 \pm 7.1	150.3 \pm 30.2	103.6 \pm 11.6	175.7 \pm 33.7	3.4 \pm 0.3	270.5 \pm 83.2
	<i>D. dangila</i>	48.6 \pm 12.1	131.7 \pm 13.8	93.4 \pm 21.1	144.2 \pm 9.7	15.3 \pm 4.6	593.8 \pm 75.6

The specific activity of LDH is significantly high in muscle in comparison to brain and gill in all the species. However, the total activity of LDH is significantly high only in the muscle of *H. fossilis*. This higher activity of LDH in muscle may be due to the higher rate of anaerobic glycolysis taking place in muscle. Hochachka (1969) has shown that the energy requirement in the muscle tissue usually arises suddenly and is supplied by rapid glycolysis resulting in increased formation of pyruvate. In an anaerobic tissue like muscle, the higher activity of LDH is necessary to convert the accumulated pyruvate into lactate utilizing the NADH produced during pyruvate formation and allow the anaerobic glycolysis to continue. On the other hand, the brain and gill do not face such sudden energy requirements. Besides, the oxygen is more readily available to those two tissues enabling aerobic metabolism.

The higher activity of G6PDH in gill and brain in comparison to muscle indicate the existence of efficient HMP shunt in addition to that of glycolytic pathway. It is known that the HMP pathway is predominantly found in red blood corpuscles (Harper et al. 1977). The gill tissue having high network of blood circulation and circulating red blood corpuscles for oxygen uptake should show a correspondingly higher G6PDH activity.

The higher level of G6PDH activity observed in the brain may be due to the increased rate of pentose production for nucleic acid synthesis and a higher rate of phospholipid synthesis. These results are similar to those reported by Farnararo et al. (1977) in two other species, *Anguilla anguilla* and *Ameiurus rebulosus*. The negligible amount of G6PDH in muscle does not necessarily mean that the muscle cannot synthesize pentoses or nucleotides. Ribose could be synthesized in the muscle by a reversal of the HMP shunt utilizing fructose-6-phosphate, glyceraldehyde-3-phosphate and the enzymes transketolase and transaldolase

(Harper et al. 1977). Mayers (1977) has proposed that in insect larvae the HMP shunt serves as a by-pass from glucose-6-phosphate to produce triose phosphates which again enter glycolytic pathway. This process could save the ATP which is needed for converting fructose-6-phosphate to fructose-1-6-diphosphate if ATP is in short supply. The negligible amount of G6PDH activity indicating a near absence of HMP shunt in the anaerobic tissues like muscle and the presence of HMP shunt and glycolytic pathway in the aerobic tissues like brain and gill of these four species of fishes are in keeping with the findings of Hochachka (1961).

Our studies show that the G6PDH activities in all cases are much lower in comparison to that of LDH. Perhaps the HMP shunt is a secondary pathway of glucose metabolism in fishes, unlike that of some snail hepatopancreas where the activity of G6PDH has been shown to be much higher than that of mammalian liver (Mark et al. 1977). However, our studies indicate that the anaerobic glycolysis perhaps operates at a much higher rate than of HMP shunt for glucose metabolism in the four species of fishes investigated. Further studies using radioactive substrates can confirm this hypothesis.

It is also found that the G6PDH activities in all the tissues of the primary gill-breathers, are generally higher in comparison to those of the amphibious species. G6PDH activity were reported to increase in cold acclimated brook trouts (Yamaguchi et al. 1975, Hochachka & Hochachka 1973) and the phenomenon was probably associated with increase in lipogenesis during cold acclimation (Fried & Levin 1973). The purely gill-breathing species of fishes like *D. dangila* and *P. shalynius* used in our experiments are basically cold-water species and normally available at high altitudes (Yazdani & Talukdar 1975), compared to those of the sluggish air-breathing fishes. Thus, the tissue specific and

species specific differences observed in these two enzyme activities in the two groups of fishes are mainly in keeping with the physiological status of the tissues and in turn reflects their adaptation and behaviour to their respective habitat.

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