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Acclimation to Sublethal Acidic and Alkaline Media of *Tilapia mossambica* (Peters): Changes in Glycogen Metabolism of Red Muscle

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Freshwater bodies at several parts of the globe are presently undergoing prograssive acidification due to acid precipitation and acid mine drainage. Significant changes under altered pH stress includes reduced primary production of algal biomass, benthic communities and rapid decline in fish populations. Studies dealing with the physiological responses of fish to acidic and alkaline water pollution are very limited. Hence, the studies dealing with the biological impact of acidity and alkalinity on the physiology and biochemistry of freshwater fish has been undertaken.

MATERIALS AND METHODS

Freshwater fish, Tilapia mossambica (Peters) of 12±1 g weight were acclimatized to the laboratoty conditions in large glass aquaria with flowing dechlorinated water (25°C, pH 7.0±0.2 and light period of 12 h). They were fed with commercial fish pellets.

The fish were divided into three groups, each group contain 100 fish. The first group (control) was maintained in normal tap water at pH 7.0±0.2. The second and third groups were acclimated to acidic medium (pH 5.0±0.1) and alkaline medium (pH 9.0±0.1) for 15 days (experimental) respectively.

Control and experimental fishes were sacrificed separately and red muscle tissues were isolated and rapidly chilled by placing them in an ice chamber. These tissues were used for biochemical analysis.

Free glucose (Mendel et al. 1954), glycogen(Carroll et al. 1956), lactic acid (Barker and Summerson 1941) modified by Hukabee (1956) and pyruvic acid (Friedemann and Haugen 1942) were estimated. The activities of phosphorylase a and ab were estimated in the direction of glycogen synthesis (Cori

et al. 1955) by the determination of the amount of inorganic phosphate (Pi) formed (Taussky and Shorr 1953) from glucose-1-phosphate. Protein content was estimated by the method of Lowry et al (1951). Aldolase activity was estimated by the method of Bruns and Bergmeyer (1965). The activity level of glucose-6-phosphate dehydrogenase (NADPH-G-6-PDH) activity was determined by the method of Georg and Waller (1965). The activity levels of lactate dehydrogenase (NAD-LDH) and malate dehydrogenase (NAD-MDH) were estimated by the method of Lee and Lardy (1965). Succinate dehydrogenase (SDH) was estimated by the method of Nachlas et al (1960).

RESULTS AND DISCUSSION

The data are presented in tables 1-3 & Figs 1-3. The red muscle had higher elevated glycogen content than the control on acclimation to acidic stress. The elevated glycogen content might be due to increased glycogenesis or/and decreased glycogenolysis.

Table 1. Level of glycogen and the activity levels of phosphorylases in the red muscle of control and experimental fish.

S. No.	Component	Control	Acclimated (15 days)	
			Acidic medium (pH 5.0)	Alkaline medium (pH 9.0)
(1)	Glycogen (mg/g wet wt)	6.12 ±0.23	7.35 ±0.46*	5.12 ±0.31*
(2)	Phosphorylase "a" (μ mol Pi formed/mg protein/h)	3.12 ±0.17	2.68 ±0.23*	3.89 ±0.35*
(3)	Phosphorylase "b" (μ mol Pi formed/mg protein/h)	13.46 ±0.93	6.96 ±0.38*	17.14 ±1.28*
(4)	Phosphorylase "b" (μ mol Pi formed/mg protein/h)	18.19 ±1.23	10.33 ±1.10*	25.02 ±1.81*

Each value represents the mean of eight individual observations. Mean ± S.D., *P value: 0.001

The activity levels of phosphorylase "ab" (total), "a"(active) and "b" (inactive) were inhibited, suggesting that there was inhibition on the process of glycogenolysis. Such an inhibition on phosphorylase system could have been partly responsible for sparing of glycogen in the tissue. Consequently the free glucose was depleted more than on the normal fish. This could have been due to decreased formation from the glycogen as revealed by the inhibited phosphorylase activity and/or utilization of glucose towards glycogen synthesis and glycolysis.

Table 2. Levels of glucose, pyruvic and lactic acids and activity levels of aldolase in the red muscle of control and experimental fish.

S. No.	Component	Control	Acclimated (15 days)	
			Acidic medium (pH 5.0)	Alkaline medium (pH 9.0)
(1)	Glucose (mg/g wet wt)	4.32 ±0.26	3.64 ±0.17*	3.86 ±0.13*
(2)	Aldolase (μ mol of FDP cleaved/mg protein/h)	42.28 ±3.06	49.42 ±2.41*	23.06 ±1.18*
(3)	Pyruvic acid (μ mol/g wet wt)	0.43 ±0.03	0.38 ±0.035*	0.49 ±0.021*
(4)	Lactic acid (mg/g wet wt)	2.49 ±0.24	1.83 ±0.051*	3.47 ±0.26*

Each value represents the mean of eight individual observations. Mean ± S.D., *P value: 0.001

Since the aldolase activity was elevated, utilization of glucose into glycolysis can be expected. Despite higher aldolase activity the levels of both pyruvic and lactic acids were depleted envisaging the possibility of their active mobilization into oxidative metabolism, probably to reduce the further prevail of metabolic acidosis in the tissue. Besides, G-6-PDH activity was also significantly elevated suggesting

the activation in the operation of HMP pathway. Thus the hexoses seem to be mobilized into hexose mono and di-phosphate pathways in red muscle. Inspite of such an active mobilization of hexoses into metabolic pathways, increased glycogen level of the tissue was suggestive of higher glycogen turnover in response to acclimation process. Since red muscle in fish forms a reserve site for the nutrients of white muscle, increased glycogen content in this tissue suggests the building up of glycogen reserves for general muscular activity. Thus, the red muscle metabolism seems to resemble the hepatic tissue metabolism in having higher glycogen content on one side and the gill metabolism in exhibiting stepped-up glycolysis and oxidative metabolism on the other on acclimation to low pH medium.

Table 3. Activity levels of G-6-PDH, NAD-LDH, SDH and MDH in the red muscle of control and experimental fish.

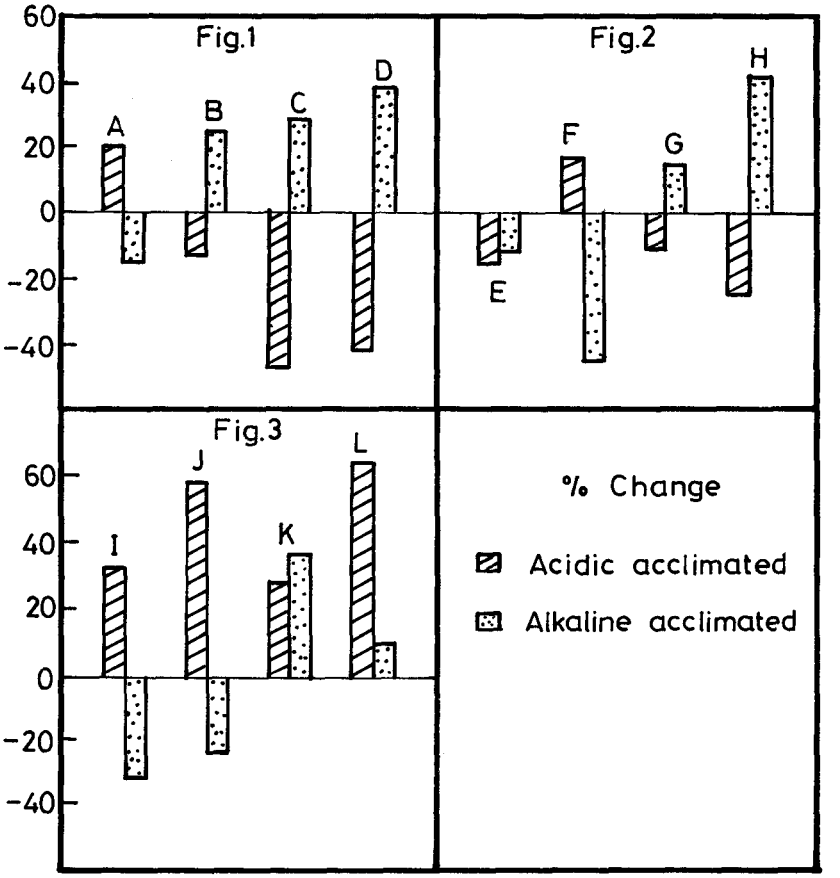
S. No.	Component	Control	Acclimated (15 days) Acidic medium (pH 5.0)	Acclimated (15 days) Alkaline medium (pH 9.0)
(1)	G-6-PDH (μ mol formazan formed/mg protein/h)	0.516 ±0.031	0.682 ±0.042*	0.355 ±0.015*
(2)	NAD-LDH (μ mol formazan formed/mg protein/h)	0.189 ±0.016	0.30 ±0.012*	0.142 ±0.002*
(3)	SDH (μ mol formazan formed/mg protein/h)	0.635 ±0.003	0.81 ±0.01*	0.863 ±0.02*
(4)	MDH (μ mol formazan formed/mg protein/h)	0.031 ±0.003	0.051 ±0.004*	0.034 ±0.003*
(5)	MDH/SDH	0.049 +	± 28.57	-20.41
(6)	SDH/LDH	3.36	2.7 -19.64	6.08 +80.95

Each value represents the mean of eight individual observations. Mean ± S.D.; *P value : 0.001

In view of increased NAD-LDH activity in red muscle on acclimation to acidic medium stepped-up mobilization of glycolytic end products like lactate and pyruvate into TCA cycle can be envisaged. SDH/LDH ratio was lower than the control. MDH/SDH ratio was considerably elevated suggesting the possible operation of gluconeogenesis.

In contrast to the above situation, the red muscle reflects reverse pattern of metabolic events in response to acclimation of fishes to basic medium. Red muscle recorded depleted glycogen content suggesting existence of stepped up glycogen breakdown either through glycogenolysis or glycolysis and decreased glycogenesis. Hence, the activities of phosphorylase and aldolase were estimated. The activities of phosphorylases were significantly elevated in the red muscle. Phosphorylase being a regulatory enzyme of glycogen breakdown, active glycogen degradations can be envisaged, which might be responsible for the depleted glycogen content. However, the aldolase, key enzyme in the glycolysis or hexose-diphosphate pathway was highly inhibited indicating the suppressed glycolysis in the red muscle. The glucose content was decreased than the control. In spite of depleted aldolase activity, there was considerable elevation in the levels of lactic and pyruvic acids and thereby mobilization of other components like glycerol into glycolytic pathway can be envisaged. The operation of glycolysis will facilitate the animal to buffer the alkalosis prevail in the tissues through organic acid production. G-6-PDH activity was significantly inhibited and hence the operation of hexose-monophosphate shunt might have been suppressed. Since NAD-LDH activity was depleted, inhibited rate of mobilization of lactic acid from glycolytic pathway can be envisaged. SDH and MDH activities were elevated suggesting the possible elevation in the operation of citric acid cycle. MDH/SDH ratio was decreased with an elevation in SDH/LDH ratio over the controls.

Thus, the red muscle metabolism was oriented towards lesser production of metabolic acids, increased carbohydrate reserves, with a switching over to aerobic phase during acclimation to sublethal altered pH stress.



A : Glycogen
B : Phosphorylase 'a'
C : Phosphorylase 'b'
D : Phosphorylase 'ab'
E : Glucose
F : Aldolase
G : Pyruvic acid
H : Lactic acid
I : G-6-PDH
J : NAD—LDH
K : SDH
L : MDH

Figures 1–3. % Enzyme activity change in Red muscle

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