Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 91, Number 3, May 1982, pp. 235–241.

Branchial protein metabolism of freshwater fish *Tilapia mossambica* (Peters) during acute exposure and acclimation to sublethal alkaline water

M BHASKAR, G VEMANANDA REDDY, V KRISHNA MURTHY, P REDDANNA and S GOVINDAPPA
Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India

MS received 29 December 1980 ; revised 11 March 1982

Abstract. Freshwater fish *Tilapia mossambica* (Peters) were exposed to sublethal alkaline water (pH 9·0) and the branchial protein metabolism was studied on acute exposure and acclimation. Branchial tissue had elevated structural and soluble protein fractions on acclimation which was correlated towards the gill hypertrophy. Proteolytic activity of the tissue was elevated on both acute exposure and acclimation. A/O ratio which forms a measure of ammonia formed per unit O_2 consumption was lesser on acclimation and hence mobilization of tissue free ammonia towards glutamine formation was suggested. The metabolic modulations have been correlated towards the positive survival value of the fish in alkaline waters.

Keywords. pH acclimation ; branchial metabolism ; structural proteins ; soluble proteins ; glutamine ; A/O ratio.

1. Introduction

Fish encounters abnormal pH levels of freshwater due to several factors like environmental pollution, addition of industrial effluents, hot springs, volcanic lakes, mine drainage and geological pattern of natural changes (McKee and Wolf 1963 ; Beamish 1972 ; Cairns *et al* 1972 ; Dovland *et al* 1976 ; Oden 1976 ; Dillon *et al* 1978). These pH changes affect wild fish populations in many freshwater lakes, streams and rivers (Anderson *et al* 1971 ; Beamish and Harvey 1972 ; Jensen and Snekvik 1972 ; Almer *et al* 1974 ; Schofield 1975 ; Karuppasamy 1979).

Studies on fish in altered pH media have been undertaken regarding tolerance levels (Bandt 1936 ; Trama 1954 ; Carter 1964 ; Jordan and Lloyd 1964 ; Beamish 1972 ; Daye and Garside 1975), O_2 consumption (Packer and Dunson 1972 ; Krishna Murthy *et al* 1980) survival and development of embryos and histopathological changes of tissues (Daye and Garside 1976, 1980a, b) and physiological aspects (Packer and Dunson 1970, 1972 ; Lievestad and Muniz 1976).

However there has been little information on tissue metabolism of fish exposed and acclimated to alkaline media. Our previous studies revealed that the pHs 10·5 and 3·5 were lethal limits to the fish *Tilapia mossambica* (Krishna Murthy *et al* 1980) and tissue metabolism was drastically altered (Bhaskara Haranath *et al* 1978).

The animals develop compensatory changes in tissue metabolism under stress conditions (Precht 1958 ; Kanungo and Prosser 1959 ; Das and Prosser 1967 ; Govindappa and Rajabai 1976). Hence it was felt desirable to understand the possible tissue metabolic modulations during acute exposure and acclimation to sublethal alkaline waters. Since the branchial tissue participates in immediate ionic regulations, it will be worthwhile to study the metabolic changes of this tissue under induced alkaline stress.

2. Materials and methods

Freshwater fish, *T. mossambica* (Peters) of 10 ± 1 g weight, were acclimatized in glass aquaria with flowing dechlorinated water to the laboratory conditions (25° C, pH 7·0 ± 0·2 ; and light period of 12 hr). The fish were fed with formulated diet of commercial fish pellets. The test fish were exposed to extreme sublethal alkaline pH medium (pH 9) which was prepared by adding 10^{-1} N NaOH as suggested by Krishna Murthy *et al* (1980) and the pH was checked with pH meter (Elico model LI–10 Hyd.).

The fish were divided into three groups. viz., controls, acute exposed and acclimated. The control fish was maintained in normal tap water at pH 7 ± 0·2 and the second and third groups of fish were exposed to pH 9 ± 0·1 for one day (acute exposed) and for 15 days (acclimated) (Krishna Murthy *et al* 1980). The fish were sacrificed separately, the gill was isolated, rapidly chilled and employed for biochemical analysis.

Total, soluble and structural proteins were estimated by the method of Lowry *et al* (1951) and protease activity (neutral) and free amino acid levels by the method of Moore and Stein (1954). The rate of tissue respiration was measured using the conventional Warburg constant volume respirometric apparatus (Umbreit *et al* 1959). Glutamate dehydrogenase (E.C. 1·4·1·3) activity was estimated by the method of Lee and Lardy (1965) as modified by Reddanna and Govindappa (1979). Free ammonia, urea and glutamine levels were estimated by the methods described by Bergmeyer (1965), Natelson (1971) and Colowick and Kaplon (1957) respectively. A/O ratios were calculated by dividing free ammonia contents with tissue O_2 consumption.

3. Results

Data are presented in tables 1 and 2. On acute exposure to sublethal alkaline medium the branchial tissue had depleted total protein (TP) content (table 1). The soluble protein (SP) fraction was considerably elevated while structural protein (StP) was depleted. Protease activity was highly elevated with significant increase in free amino acid content. The ratios of soluble proteins to structural

Table 1. Levels of total, soluble and structural proteins, free amino acid content, protease activity and ratios of SP/TP, StP/TP, SP/StP in the branchial tissue of control, acute exposed and acclimated fish.

Parameter (mg/g/ wet wt)	Control		Acute exposed		Acclimated
Total proteins (TP)	94·60 ±8·21	−11·24 $P < 0·001$	83·97 ±6·4	+17·92 $P < 0·001$	111·55 ±8·95
Soluble proteins (SP)	32·15 ±2·85	+34·06 $P < 0·001$	43·10 ±3·84	+31·01 $P < 0·001$	42·12 ±4·18
Structural proteins (StP)	62·45 ±4·86	−31·56 $P < 0·001$	40·87 ±4·21	+11·18 $P < 0·01$	69·43 ±5·62
Protease μmol tyrosine/mg protein/hr	0·0064 ±0·00071	+509·4 $P < 0·001$	0·039 ±0·0028	+275 $P < 0·001$	0·024 ±0·0016
Free amino acids	17·91 ±1·84	+40·93 $P < 0·001$	25·2 ±2·32	+92·91 $P < 0·001$	34·65 ±3·85
SP/TP	0·340	+50·20	0·51	+18·81	0·38
StP/TP	0·66	−25·86	0·49	− 6·09	0·62
SP/StP	0·515	+105·28	1·055	+17·74	0·606

The values are mean of 6 observations; Mean ± S.D.; + and − indicate per cent increase and decrease respectively on the control values. *P* denotes statistical significance.

proteins (SP/StP) and soluble proteins to total proteins (SP/TP) were higher and StP/TP was lesser than controls. The tissue oxygen consumption was considerably high (table 2). A/O ratio was slightly lesser and GDH activity was highly inhibited. Free ammonia and urea contents had non-significant changes while glutamine content was depleted. However, on acclimation, the levels of total, soluble and structural proteins were significantly elevated. Tissue protease was activated and free amino acid content was elevated. The SP/TP and SP/StP

Table 2. Levels of O_2 consumption, ammonia/O_2 ratio, glutamate dehydrogenase, free ammonia, urea and glutamine and ratios of urea/ammonia, glutamine/ammonia in the branchial tissue of control, acute exposed and acclimated fish.

Parameter	Control		Acute exposed		Acclimated
Tissue oxygen consumption µl of O_2/g.wt./hr	309·15 ±25·12	+28·73 $P<0·001$	397·96 ±29·85	+ 4·82 NS	324·05 ±26·52
A/O ratio	0·0082	− 6·1	0·0077	−32·95	0·0055
GDH µ mole of formazan/mg protein/hr	0·029 ±0·001	−58·62 $P<0·001$	0·012 ±0·001	+13·79 $P<0·001$	0·033 ±0·002
Free ammonia µ moles/g. wt.	2·55 ±0·35	+ 3·94 NS	2·65 ±0·28	+21·96 $P<0·01$	3·11 ±0·34
Urea µ moles/g. wt.	4·15 ±0·18	− 1·44 NS	4·11 ±0·25	− 25·86 $P<0·001$	2·81 ±0·14
Glutamine µ moles/g. wt.	113·83 ±10·25	− 8·83 $P<0·001$	96·57 ±2·19	+26·74 $P<0·01$	134·24 ±13·12
Urea/Ammonia	1·486	+ 8·51	1·612	−39·33	0·904
Glutamine/Ammonia	44·64	−15·23	37·87	− 3·33	43·16

Each value is mean of 6 observations; Mean ± S.D.; + and − indicate per cent increase and decrease respectively from controls. *P* denotes statistical significance and 'NS' is non-significant.

ratios were considerably higher while that of StP/TP were lower than controls. Tissue oxygen consumption had non-significant change with low A/O ratio as compared to control. GDH activity level was elevated. While the free ammonia and glutamine levels were significantly increased, urea content was depleted. The urea/ammonia ratio was considerably lesser than the control value.

4. Discussion

The branchial protein metabolism showed differential pattern during acute exposure and acclimation to sublethal alkaline waters.

Acute exposure of fish to sublethal alkaline waters depleted total protein content of the gill. In view of highly elevated protease activity, depleted protein content in the tissue can be envisaged. However, soluble protein fraction was elevated while the structural protein fraction was depleted, suggesting possible alterations in the solubility properties of the proteins in the tissue. Higher SP/TP and SP/StP ratios indicate that the soluble protein fraction was elevated, probably a prerequisite for proteolysis in the tissue. Consequently, the structural proteins of the tissue depleted suggesting proteolysis at structural level of organization of gill. These observations agree with earlier reports where high tissue proteolysis was recorded in liver and muscles of fish exposed to alkaline waters (Bhaskara Haranath *et al* 1978) and histopathological changes in tissues of fish exposed to extreme pH of the medium (Daye and Garside 1976). Since protease activity was high, the tissue free amino acid content increased considerably. The oxygen consumption was elevated with lesser A/O ratio suggesting possible suppression of oxidations of protein components in the tissue. In view of the highly inhibited GDH activity, which forms an index of amino acid oxidations, the decreased mobilization of amino acids into oxidations can be envisaged. Consequently, free ammonia and urea levels had non-significant change from the controls.

However, on acclimation the branchial total protein content was significantly elevated suggesting the onset of either enhanced protein biosynthetic mechanisms, or decreased proteolysis in the tissue. Since protease activity was also elevated, increased protein content might be due to stepped-up protein biosynthesis, with active turnover of tissue proteins. In the light of widely reported mucification and hypertrophy of the branchial tissue in altered pH media (Daye and Garside 1980b) active protein synthesis can be visualised. Since both soluble and structural protein fractions were elevated, accumulation of proteins at structural and dynamic levels of organization of gill can be expected. Free amino acid content was elevated, which may be due to increased proteolysis.

Tissue oxygen consumption had non-singificant change, while A/O ratio was far lower than the control, suggesting lesser mobilization of protein components into oxidations or mobilization of ammonia into other components. However, GDH activity was considerably elevated indicating the involvement of amino acids in oxidative reactions. Free ammonia content was increased due to high oxidative deamination reactions in the tissue. Glutamine content was considerably high with decrease in urea, suggesting the mobilization of tissue ammonia towards the formation of glutamine, which may be responsible for the lower A/O ratio.

In general it can be concluded that the branchial tissue, on acclimation in sublethal alkaline waters, accumulates proteins leading to hypertrophy of the tissue which might provide positive survival value for the fish in imposed alkaline stress.

Acknowledgements

The authors express their gratitude to the University Grants Commission for financial assistance.

References

Almer B, Dickson W, Ekstrom C, Hornstrom E and Miller U 1974 Effects of acidification of Swedish lakes ; *Ambio* **3** 30-36

Anderson G, Gustafson K J and Lindstrom T 1971 Rodingen I Rosjoarna Pa Fulufjall. Drottningholm : *Freshwater Research Laboratory Sweden Information* **8** 9

Bandt H J 1936 Der Fur Fische "todliche pH Wert" in alkalischem Bereich ; *Z. Fisch. Deren Hilfwiss* **34** 359-361

Beamish R J 1972 Lethal pH for the white sucker *Catastomus commersani* (Lacepede) ; *Trans. Am. Fish Soc.* **101** 355-358

Beamish R J and Harvey H H 1972 Acidification of the Lacloche mountain lakes, Ontario and resulting fish mortalities ; *J. Fish. Res. Board Can.* **29** 1131-1143

Bergmeyer H U 1965 *Methods of biochemical analysis* (New York and London : Academic Press)

Bhaskara Haranath V, Reddanna P and Govindappa S 1978 Effects of exposure to altered pH media on tissue proteolysis and nitrogenous and products in a freshwater fish *Tilapia mossambica* (Peters) ; *Indian J. Exp. Biol.* **16** 1088-1090

Cairns J, Dickson K L and Crossman J S 1972 The response of aquatic communities to spills of hazardous materials ; *Proc.* 1972 *Natl. Conf. Hazardous materials spills* pp. 179-197

Carter L 1964 Effects of acidic and alkaline effluents on fish in sea water ; *Effluent water Treat. J.* **4** 484-486

Colowick S P and Kaplan N O 1957 *Methods in enzymology* (New York : Academic Press) **3** 501

Das A B and Prosser C L 1967 Biochemical changes in tissues of gold fish acclimated to high and low temperatures. I. Protein synthesis ; *Comp. Biochem. Physiol.* **21** 449-467

Daye P G and Garside E T 1975 Lethal levels of pH for brook trout, *Salvelinus fontinalis* (Mitchill) ; *Can. J. Zool.* **53** 639-641

Daye P G and Garside E T 1976 Histopathological changes in surfacial tissues of brook trout, *Salvelinus fontinalis* (Mitchill) exposed to acute and chronic levels of pH ; *Can. J. Zool.* **54** 2140-2155

Daye P G and Garside E T 1980a Structural alterations in embryos and alevins of the Atlantic salmon, *Salmo salar* L. induced by continuous or short-term exposure to acidic levels of pH ; *Can. J. Zool.* **58** 27-43

Daye P G and Garside E T 1980b Development, survival and structural alterations of embryos and alevins of Atlantic salmon, *Salmo salar* L. continuously exposed to alkaline levels of pH from fertilization ; *Can. J. Zool.* **58** 369-377

Dillon P J, Jeffries D S, Snyder W, Reid R, Yan N D, Evans D, Moss J and Scheider W A 1978 Acidic precipitation in South-Central Ontario : recent observations ; *J. Fish. Res. Board Can.* **35** 809-815

Dovland H, Joranger E and Semb A 1976 Deposition of air pollutants in Norway ; in *Impact of acidic precipitation on forest and freshwater ecosystems in Norway* ; (ed) F H Breekke SNSF Project FR 6/76 14-35

Govindappa S and Rajabai B S 1976 Some aspects of protein metabolism in crab *Paratelphusa hydrodromus* (Herbt) during cold acclimation ; *J. Anim. Morphol. Physiol.* **23** 76-84

Jensen K W and Snekvik E 1972 Low pH levels wipe out salmon and trout population in southernmost Norway ; *Ambio* **1** 223-225

Jordan D H M and Lloyd R 1964 The resistance of rainbow trout [*Salmo gairdneri* (Richardson)] and roach (*Rutilis rutilus* L.) to alkaline solutions ; *Int. J. Air Water Pollut.* **8** 405-409

Kanungo M S and Prosser C L 1959 Physiological and biochemical adaptation of gold fish to cold and warm temperatures. I. Standard and active oxygen consumptions of cold and warm acclimated gold fish at various temperatures ; *J. Cell Comp. Physiol.* **54** 259-263

Karuppasamy P 1979 Pollution and fish mortality in Chaliyar river, Mavoor (near Calicut) from 7-3-1979 to 16-3-1979 ; *Marine Fisheries Information Service* **7** 11-13

Krishna Murthy V, Reddanna P and Govindappa S 1980 Hepatic carbohydrate metabolism in *Tilapia mossambica* (Peters) acclimated to low environmental pH; *Can. J. Zool.* **59** 400–404

Lee Y L and Lardy H A 1965 Influence of thyroid hormones on L-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat ; *J. Biol. Chem.* **240** 1427–1432

Lievestad H and Muniz I P 1976 Fish Kill at low pH in a Norwegian river ; *Nature* (*London*) **259** 391–392

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin phenol reagent ; *J. Biol. Chem.* **193** 265–275

McKee J E and Wolf H W 1963 *Water quality criteria* (Sacramento, Calif. : Water Quality Control Board Publ. 3–A)

Moore S and Stein W H 1954 A modified ninhydrin reagent for the photometric determination of aminoacids and related compounds ; *J. Biol. Chem.* **211** 907–913

Natelson S 1971 *Techniques in clinical chemistry* (Illinois : Charles C. Thomas, Publishers) pp. 146

Oden S 1976 The acidity problem—an outline of concepts : *Proc. 1st Int. Symp. on acid Precip. Forest Ecosystems* ; in special issue ; *J. Water Air Soil Pollut.* pp. 1–36

Packer R K and Dunson W A 1970 Effects of low environmental pH on blood pH and Na^+ balance of brook trout ; *J. Exp. Zool.* **174** 65–72

Packer R K and Dunson W A 1972 Anoxia and sodium loss associated with death of brook trout ; *Comp. Biochem. Physiol.* **A41** 17–26

Precht H J 1958 Concept of temperature adaptation of unchanging reaction systems of cold blooded animals ; In *Physiological adaptation* (ed.) C L Prosser (Washington D.C. : American Physiological Society)

Reddanna P and Govindappa S 1979 Effects of *in vivo* muscular stimulations. II. Influence on hepatic carbohydrate metabolism ; *J. Anim. Morphol. Physiol.* **26** 156–161

Schofield C L 1975 Lake acidification in the Adirondack Mountains of New York : Causes and consequences ; *Proc. 1st Int. Symp. Acid Precip. Forest Ecosystems* ; Ohio State University

Trama F B 1954 The pH tolerance of the common bluegill (*Lepomis machrochirus* Rafinesque) ; *Not. Nat. Acad. Nat. Sci. Philadelphia* **256** 1–13

Umbreit W W, Burris R H and Stauffer J F 1959 *Manometric techniques* (Minneapolis ; Burgess Publishing)