J. Biosci., Vol. 1, Number 4, December 1979, pp. 427–432. © Printed in India.

Adaptation to salinity by fish. Macromolecular changes in mitochondria and microsomes of the gill

S. MEENAKSHI, C. RAJAMANICKAM and J. JAYARAMAN
Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021

MS received 5 May 1979; revised 8 September 1979

Abstract. Mitochondria isolated from the gill tissues of the fish *Sarothredon mossambica* were analysed for their macromolecular content, following transfer from freshwater to media of higher salinity. The results suggest a breakdown of mitochondria during the initial phases of the stress and a regeneration during continued exposure. Also all the synthetic machineries, in general, seem to be triggered in gill tissue during continuous exposure to hyperosmotic media.

Keywords. Salinity adaptation; gill mitochondria; macromolecules.

Introduction

It has been shown (Sulochana *et al.,* 1977) that when the freshwater fish *Tilapia mossambica* (now renamed as *Sarothredon mossambica*) is exposed to enhanced salinity (iono-osmotic stress), the effect is reflected even at the level of mitochondria isolated from the muscle tissue. These studies further revealed three distinct phases: (a) an immediate response, where the bioenergetic functions are markedly altered; (b) an adaptive phase of slow restoration to normalcy and (c) a phase where the functional capacity of the cells reach a maximum level.

Physiologically, the gill tissue is more active than the muscles in the iono-osmo regulation in fish. It was, therefore, of interest to investigate the behaviour of mitochondria from the gill tissue during adaptation to higher salinity. The bioenergetics of gill mitochondria are somewhat similar to those of muscle mitochondria. In addition, amino acid incorporation studies have provided evidence for the possible breakdown of the organelle during the response period and its regeneration during the adaptive phase. The present study deals with changes at the macromolecular levels in the gill mitochondria when the fish is subjected to osmotic stress.

Materials and methods

Animals

The collection, maintenance and conditions of transfer to higher salinity (25% sea water) have been described previously (Sulochana *et al.,* 1977).

Publication No. 041 from the Department of Biochemistry.

Differential centrifugation

All operations were carried out at 0–5° C, unless otherwise mentioned. The fishes were killed by decapitation and gills excised out immediately. The gill bars were scraped off without any cartilage and after washing with 0·15 M KCl solution were homogenised in 0·25M sucrose containing 0·002 M tris-HCl, pH 7·6 and 0·002 M EDTA in a Potter-Elvehjem homogeniser to yield a 20% homogenate. The homogenate was then centrifuged at 600 g for 10 min, in a Janetzki K-24 refrigerated centrifuge to remove nuclei and cell debris. The supernatant was again centrifuged at 10,000 g for 10 min to obtain mitochondrial pellet. The pellet was washed three times and finally suspended in the same medium.

The microsomal pellet was obtained by centrifuging the post-mitochondrial supernatant at 100,000 g for 60 min in a Vac 60 ultracentrifuge. The pellet was suspended in the homogenising medium.

Nuclease treatment of mitocnondria

Nuclease treatment of mitochondria was carried out as described by Storrie and Attardi (1972). Washed mitochondria were suspended in 0·25 M sucrose containing 0·05 M NaCl, 0 005 M $MgCl_2$, 0·005 M tris-HCl, pH 7·4 at a concentration of 23 mg protein/ml. They were treated with 25μg DNAse/ml or 25μg RNAse/ml for 30 min at 27° C. The digestion mixture was diluted with 10 ml of cold 0·25 M sucrose containing 10 mM EDTA and 0 002 M tris-HCl buffer pH 7·4 and the mitochondria pelleted out by centrifugation. The pellet was washed once again with the sucrose buffer.

Estimation of DNA, RNA and protein

The mitochondrial or microsomal suspension was treated with perchloric acid to a final concentration of 0·2 N in a total volume of 5 ml and kept at 0° C for 2 h. The precipitate was collected by centrifugation and washed twice with 0·2 N cold perchloric acid. The washed pellet was treated with 0·3 N KOH and incubated at 37° C for 1 h to hydrolyse the RNA. The RNA in the supernatant was measured by the method of Fleck and Berg (1965). For estimation of DNA, the perchloric acid precipitated pellet was incubated with a known volume of 0·4 N perchloric acid at 70° C for 20 min. The supernatant was collected and the pellet was reincubated under the same condition as before. The supernatants were pooled and estimated for the DNA content by the diphenylamine colour reaction.

Protein was estimated by the method of Lowry *et al.* (1951).

Estimation of heme

Total heme was extracted according to the procedure of Levin *et al.* (1972) and estimated by Rieske's (1967) method. The tissue homogenate or mitochondrial suspension was extracted successively with cold acetone, chloroform-methanol (2:1 v/v) mixture and again with cold acetone. The final residue free of lipids was suspended in water and shaken with 10 volumes of a mixture of ethyl acetate: acetic acid (4 :1 v/v). The precipitate was filtered off and the extract was washed with 3 N HCl to remove porphyrins. The ethyl acetate layer was evaporated to

dryness and the residue dissolved in a minimal volume of a mixture of pyridine, 0·2 N KOH (1:1 v/v). The absorbancy was recorded in the oxidised and reduced (with dithionite) states at 550, 556, 587 nm. The concentrations of the hemes were calculated as follows:

a- m , mo s m $_{24}$ (A_{87}) 000

b - m , om s m $_{30}$ (A – A o) 000

– m , mo s m $_{9}$. (A_{0} – A_{0} o 000

Results

Macromolecular composition of gill mitochondria and microsomes from freshwater fishes

The macromolecular composition of mitochondria and microsomes isolated from the gill tissue of freshwater fishes is given in table 1. The amount of mitochondrial DNA appears to be fairly high compared to the values reported for other animal tissue mitochondria. Contamination due to nuclear DNA was ruled out by treatment of isolated mitochondria with deoxyribonuclease. Mitochondria used in these experiments show normal ADP/O ratios and respiratory control (Suresh Narayan, Personal communication).

Table 1. DNA, RNA and, protein content of gilJ mitochondria and microsomes from the freshwater fish *Tilapia mossambica.*

	Protein	RNA	DNA
	mg/g fresh wt	μg/mg protein	
Mitochondria	3·6±0·3	12·25±0·4	19·1±0·76
Microsomes	5·6±0·28	29·57±1·09	..

The results are from three experiments with two samples in each experiment. Five fishes were pooled for each sample.

Variation during adaptation to 25% *sea water*

Mitochondria : An immediate response to enhanced environmental salinity is a decrease in the DNA and protein levels within 24 h. After 24 h, the levels come back to the normal value and after the 6th day there is a slow but steady increase (figure 1) .

The mitochondrial RNA levels however show a different pattern (figure 1). The level increases during the first 3 days, but comes down considerably below normal on the 6th day. Thereafter, there is a steady increase till the 18th day.

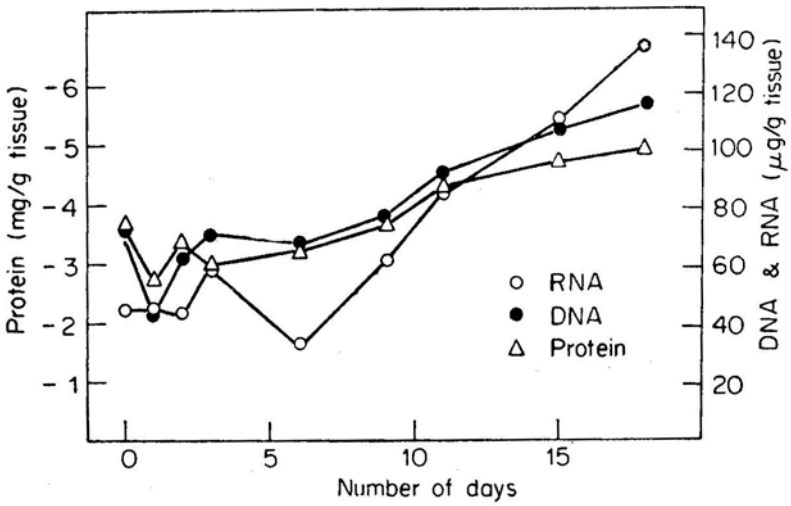

Figure 1. Changes in the levels of mitochondrial DNA, RNA and protein expressed per g gill tissue of fishes exposed to a continuous salinity stress.

Microsomes : Microsomal protein does not show significant variation during the early period, but after the 6th day it also shows a steady increase (figure 2). The pattern of microsomal RNA is almost similar to that of mitochondrial RNA.

Heme content : A casual observation on the colour of the gill tissue of fishes during stress conditions, prompted us to analyse the heme contents. The data indicate

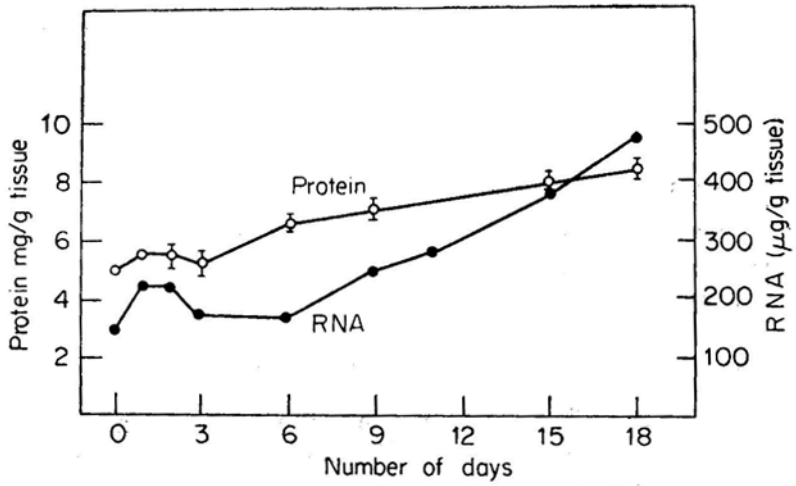

Figure 2. Changes in the levels of microsomal protein and microsomal RNA expressed per g gill tissue of fishes exposed to a continuous salinity stiess. O—-O Protein mg/g tissue; ●—● RNA μg/g tissue.

a drastic reduction of the various heme contents during the initial response phase and their subsequent recovery during the adaptation phase (table 2).

Table 2. Levels of different hemes in gill tissue (total heme) and gill mitochondria from fishes exposed to 25% sea water.

	Freshwater	25% sea water	
		7 days	18 days
Total heme nmol/g tissue			
Cyt *a*	31·57±1·57	20·75±1·29	40·55±1·18
Cyt *b*	25·53±2·05	11·11±0·90	17·87±0·75
Cyt $c+c_1$	5·33±0·45	4·1 ±0·28	10·89±0·51
Mitochondrial heme nmol/g protein			
Cyt *a*	15·60±1·30	10·2 ±0·79	17·5 ±1·05
Cyt *b*	6·31±0·52	3·28±0·36	5·16±0·6
Cyt $c+c_1$	3·42±0·41	2·25±0·17	7·82±0·33

Results of three independent experiments.

Discussion

Earlier work in this laboratory has shown that when the freshwater fish *S. mossambica* is exposed to iono-osmotic stress, as an immediate response, there occurs significant dislocation in the bioenergetic functions of mitochondria isolated from the muscle and gill tissues (Indu Bashyam, personal communication; Sulochana *et al.*, 1977). On continued exposure to the iono-osmotic stress (25% sea water), the fish adapts itself to the new environment and the dislocated functions are restored to normalcy. The increased activity of the mitochondrial glutamic dehydrogenase along with an increased [^{3}H] thymidine incorporation into DNA in the epithelial cells of the gill lamella of young salmon in sea water suggested the formation of cells rich in mitochondria (Morris, 1957; Conte and Lin, 1967). In the present studies, changes in the levels of DNA, RNA, protein macromolecules and of heme indicated mitochondrial breakdown in the initial phase and regeneration in the adaptive phase in the gill tissue.

The decrease in the levels of mitochondrial DNA and protein within 24 h of exposure to stress argues in favour of mitochondrial breakdown. The tissue apparently counteracts this by increased biogenesis (figure 1). The rates of breakdown and biogenesis of mitochondria between 1st and 6th day are probably similar resulting in the appreciable change in their levels. The decreased levels of different hemes around the 6th day also strengthen the idea of mitochondrial breakdown. The contrasting trend observed in the RNA levels is somewhat puzzling and a detailed analysis of the RNA species is in progress to understand this.

It is, however, significant that all the parameters measured including microsomal RNA, protein and heme show a steady increase from the 6th day to the 18th day. Restoration of dislocated functions also occur (data not given). These would indicate new synthesis of mitochondria. Such triggering of mitochondriogenesis under stress conditions is not unknown (Rabinowitz *et al.,* 1971; Tata, 1963). There is also a toning up of the general protein synthetic machinery and increase in specific proteins like glutamic dehydrogenase and Na^+–K^+ ATPase have been reported (Epstein *et al.,* 1967; Conte and Lin, 1967).

The experimental system described is being used in this laboratory to understand the interlinking between various bioenergetic functions of mitochondria. The present studies show that the same system is also amenable for investigation of the biogenesis of the organelle in eukaryotic cells and the regulatory mechanisms that govern the process.

Acknowledgement

This research work was made possible from grants from the Indian National Science Academy, New Delhi.

References

Conte, F. P. and Lin, D. H. Y. (1967) *Comp. Biochem. Physiol.,* **23,** 945.

Epstein, F. H., Katz, A. I. and Pickford, G. E. (1967) *Science,* **156,** 1245.

Fleck, A. and Berg, D. (1965) *Biochim. Biophys. Acta,* **108,** 333.

Levin, W., Jacobson, M. and Kuntzman, R. (1972) *Arch. Biochem. Biophys.,* **148,** 262.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.

Morris, R. (1957) *Q.J. Microsc. Sci.,* **98,** 473.

Rabinowitz, M., Aschenbrenner, V. and Albin, R. (1971) in *Cardiac hypertrophy,* ed. N. R. Alpert (New York: Academic Press), p. 283.

Rieske, J. S. (1967) *Methods Enzymol.,* **10,** 492.

Storrie, B. and Attardi, G. (1972) J. *Mol. Biol.,* **71,** 177.

Sulochana, H., Indu Pashyam, Suresh Narayan and Jayaraman, J. (1977) *J. Biomembr. Biomembr.,* **9,** 337.

Tata, J. R. (1963) *Nature* (*London*), **197,** 1167.