VARIABILITY OF Na+, K+-ATPASE ACTIVITY AND LOCATION OF CHLORIDE CELLS IN THE GILLS OF TENUALOSA ILISHA IN RESPONSE TO DIFFERENT SALINITY ZONES OF BHAGIRATHI-HOOGHLY RIVER SYSTEM, WEST BENGAL, INDIA

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ABSTRACT

The size and location of chloride cells in gill of Hilsa, Tenualosa ilisha, from marine (Digha) and fresh water (Farakka) environments were studied in this paper. Changes in gill and kidney histopathology in terms of Na+, K+-ATPase activity were also investigated. Fish samples were collected from fresh water (FW) (salinity ~ 0.1psu) and marine water (MW) (salinity ~ 29 psu) environment. In FW, the chloride cells were observed on the epithelium of the filaments (mainly in inter-lamellar regions) and on the lamellae of Hilsa gill. On the contrary, in the MW samples, the abundance of Na+, K+-ATPase increased and few chloride cells were observed on the lamellae. MW samples showed a high density of chloride cells on the epithelium of the filaments, and a few cells on the lamellae. Na+, K+-ATPase intensity was significantly differed in two unlike environment (sustainability higher in MW samples compared to FW samples) where kidney samples showed the opposite trend. The capability of T.ilisha to change the number and size of gill chloride cells, as well as their activities indicated that the high degree of adaptability of T.ilisha to a wide range of salinity.

INTRODUCTION

Diadromy is a term used to describe migrations of fishes between fresh waters and the sea; these migrations are regular, physiologically mediated movements which occur at predictable life history phases, typically for breeding and reproduction, in each diadromous species (McDowall, 1997). Tenualosa ilisha is such type of anadromous fish, commonly known as Hilsa (in eastern part of India and Bangladesh). Moreover, Tenualosa ilisha belongs to the clupeidae family and it is one of the most commercially important fish species in a number of countries bordering the Bay of Bengal, Indian Ocean, Persian Gulf and Arabian Sea. It is a large anadromous shad that occurs in the large river systems of southern Asia from the Arabian Gulf to Myanmar and northern Sumatra (McDowall RM 1997).

During migration anadromous fishes developed some unique strategies regarding their physiology with the evolution of the ion regulating organ like gill, kidney, intestinal tract etc, for regulating the ion concentration and osmolality of their body fluid at a level that is different from their external environments (Khodabandeh et al.2009). Among them gill plays the most important role as the ion regulating organ. Previous researches indicate that active extrusion of NaCl across the gill of sea water teleost’s is carried out by specialized mitochondria or ionocytes rich cells, called chloride cells (Evans et al. 1984). These cells are characterized by an elaborate system of intracellular canals (Masoni and Payan, 1974). Chloride cells can individually transform between ion absorption and ion secretion states in response to salinity changes (Uchida et al., 2000). Osmoregulatory activities enable aquatic animals to adapt to external medium salinity fluctuations (Evans 1984). In freshwater, the organism is subjected to water uptake and ion loss, as they are hypertonic to the medium and their blood has lower water concentration than the surrounding medium. Thus hyper-osmoregulatory mechanisms compensate with a low water intake, active absorption of ions by the gills and production of hypotonic urine by kidney (Evans 1984). In seawater, hypo-osmoregulatory mechanisms compensate for water loss and ionic invasion. To avoid the dehydration Tenualosa ilisha makes up the extra water loss through high rate of drinking sea water. The water is absorbed by intestine and gills reject the excess ions come inside through water (Jensen et al., 1998; Hawkings et al., 2004; Evans 1984). Ion transport by gill is facilitated by chloride cells present in the gill’s epithelium that possess a plasma membrane associated Na+, K+-ATPase enzyme. The driving force for the active transport is Na+, K+-ATPase, which maintains intracellular Na+...
MATERIALS AND METHODS

The kidney of fish is usually located in a retroperitoneal position up against the ventral aspect of the vertebral column. The ventral portion of the fishes were dissected, and slowly all the organs were removed, cleaned and kidney was dissected carefully without losing blood. After dissecting, kidney was immediately transferred to 4% para-formaldehyde for 24 hours at room temperature for histopathological analysis. The samples were then sectioned to a thickness of 5µm with a microtome (Leitz, Germany), and stained with haematoxylin and eosin, and the histology analysis was performed same as histology analysis.

Gill Na+,K+-ATPase activity was measured on crude gill homogenates using the methods outlined by McCormick (1993). Gills are covered by a bony operculum and four hollow branches are present in each operculum, of which one side was selected for the study. These sections are cut from each arch on ice, immediately homogenized in ice cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and immediately stored in -80°C until assay. During assay, samples were rapidly thawed, again homogenized and centrifuged at 2,000 rpm at 40°C for 30 seconds. Samples (10-µL) were run in two sets of duplicates, one set containing the assay mixture and the other containing the assay mixture and 0.5 mM ouabain. The resulting ouabain-sensitive ATPase activity is expressed as µg PI g protein⁻¹ hour⁻¹. Protein concentrations were determined with a BSA protein assay kit (Pierce). Both assays were run on a THERMOMax microplate reader using SoftMax software (Molecular Devices) at 405 nm and 750 nm respectively. Therefore, kidney Na,K ATPase activity was measured on crude kidney homogenates using the methods outlined by McCormick (1993) as well as gill sample.

RESULTS

Gill & kidney histopathology studies

Sagittal sections (stained) of the gills of T. ilisha collected from different saline zones showed an oval to elongated shape with an epical positioned nucleus (Figure 1). The pink colour stained the chloride cells with nucleus in the middle. In fresh water, lamellar chloride cells appeared at the base close to the filament and expanded proximal to distal part of the lamellae (Figure 1 and Figure 2). Moreover, lamellar chloride cells increased significantly and predominated over filament cells. Chloride cells in the filament have decreased significantly in the freshwater samples (Figure 2). In gill filament of T. ilisha from both marine and freshwater sources, chloride cells occupied 10 to 15 % out of the total space (Figure 2).

The sagittal section of kidney tissue of T. ilisha from different saline zone was done using hematoxylin and eosin staining procedure. Kidney of fresh water juvenile and adult fish is composed of numerous Bowman capsules with well-developed glomeruli and a system of renal tubules like proximal tubules. (Figure 3), whereas in marine water there are few Bowman capsules and no proximal tubule shows in the juvenile T. ilisha (Figure 3) sample but in case of adult some kidney tubules are followed in the tissue.
samples (Farakka barrage, Bhagirathi-Hooghly river) showed significantly higher intensities in the gills of different size group of *T. ilisha*. Na⁺, K⁺-ATPase intensity was significantly higher (Figure 4) in freshwater compared to marine water. In case of kidney showed an opposite trend compared with the results for gill tissues, kidney Na⁺, K⁺-ATPase protein of fresh water fish proved to be higher than the sea water acclimated fish (Figure 4).

### DISCUSSION

Evans (1984) estimated at about 95% of the teleosts are stenohaline, while only 5% are euryhaline, of which *T. ilisha* can tolerate a wide variation in salinity during its life history, and thus provides a very good model for osmo-regulation research. The present study also confirms the same for *T. ilisha*, , the histopathology of gill, kidney changes, as well as, the Na⁺, K⁺-ATPase activity with increasing salinity. The gills and kidney are the main osmoregulatory organs and are sensitive to environmental factors (i.e salinity) (Uchida et al.,2000). The most radical change observed in the present
study is that, the appearance of chloride cells in the lamellae and its abundance in the epithelium of gills during migration of T. ilisha from sea water to fresh water. Previous study showed that most branichial chloride cells were detected in the filament and inter lamellar region of T. ilisha from marine environment, whereas lamellar chloride cells become evident in fish from fresh water environment. Chloride cells in T. ilisha were abundant on gill filament, but rarely appeared on lamellae (Varsamos et al., 2002). The occurrence of lamellar chloride cells is thought to satisfy the physiological demand of ion uptake in some euryhaline teleost’s (Uchida et al., 2000; Sasai et al., 1998) but not in others (Laurent and Perry, 1991). The enzyme that is important in the process of ion exchange, according to previous studies in some fishes like Milk fish (Chanos chanos), Tilapia (Oreochromis mossambicus) was identified as Na+, K+-ATPase. Na+, K+-ATPase is a plasma-membrane-associated enzyme which catalyses ubiquitous ATP-driven Na+/K+ transport. This enzyme is crucial to ion and water regulation in both freshwater and seawater fish (Evans 1984). In T. ilisha the activity of gill Na+, K+-ATPase is higher in sea water, while decreased in fresh water, but up to that extent. In seawater conditions, Na+, K+-ATPase still pumps Na+ from the intracellular compartment of chloride cells into the extracellular space across the basolateral surface. The strong Na+ gradient drives a secondary active co-transport of Cl- into the cell through a Na+, K+, 2Cl- co-transporter. This creates an electrochemical gradient that favours diffusion of Cl- through a Cl- channel on the apical side out to the seawater. It enables the Hilsa to secrete excess salts efficiently and thus acclimate adaptation of ilisha (Jensen et al., 1998). In contrast it has been seen that euryhaline fishes such as the Mozambique tilapia (Oreochromis mossambicus): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zoological Science, 17(2):149-160.

CONCLUSION

Comparing the values obtained for Na+, K+-ATPase intensity of Hilsa from the Digha and Farakka, we can conclude that Hilsa have high degree of adaptability in the wide range of salinity during the different phases of its life cycle. This species uses specific osmoregulation mechanisms during its migration from marine to freshwater including changes in the size and number of gill chloride cells.

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