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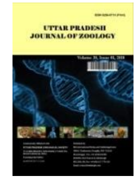

REVIEW ON BIOCHEMICAL ALTERATIONS IN EURYHALINE FISH IN RESPONSE TO CHANGING SALINITIES

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This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

ABSTRACT

Euryhaline organisms are exposed to various kinds of stress during their migration to different salinities. To overcome the stress, they are equipped with different osmoregulatory mechanisms including osmotic pressure changes and morphological alterations. Gills, kidneys and intestine are often subjected to biochemical alterations including changes in the concentration of various enzymes working as ion transporters and the endocrine organs support the osmotic balance through hormonal control. This review gives a brief account on these biochemical changes studied in different species to provide an overall idea about the mechanism used by organism to cope with changing salinity.

Keywords: *Osmoregulation; hormones; ion transporters; Na^+- K^+ - ATPase; CFTR.*

1. INTRODUCTION

Euryhaline organisms are adapted to different salinities and are found in places where regular changes in salinities occur such as estuaries. They are endowed with alterations in morphology and equip them to migrate between freshwater and salt water conditions. There are various extrinsic and intrinsic factors which can trigger the migration in fish as single habitats cannot provide enough food for fish [1]. According to [2], the migratory euryhaline fish face the problem of salinity changes and they compensate through immediate osmoregulatory mechanisms which they attain during their early development or environmental adaptations. For osmoregulation, the euryhaline fish, needs to maintain the pressure of osmotic fluid at a neutral concentration and activate various signalling channels [3]. Various organs like gills, kidneys, intestinal tract, ion and water transport across the gills,

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gastrointestinal tracts and epithelia, regulation of osmolytes and urine are all the osmoregulatory mechanisms in euryhaline organisms and there are various osmosensors like Ion channels, calcium sensing receptors or cytokine receptors which can receive the external environmental changes [4]. To adjust to the changing salinity conditions of the environment, various alterations are observed in fish which include the histological changes, change in the plasma osmolality of the blood, variation in the activity of the osmoregulatory enzymes or transporters in the osmoregulatory organs including gills and kidneys. The key process in analysing any change in the external environmental salinity is known as osmosensing and there are various osmosensors at both cellular and molecular levels which interact together to gather information on the external changes [3]. Changes are also observed in the morphometric characters of many organs. This brief review, summarize the opinion of early researchers about the biochemical changes that takes place in various osmoregulatory organs in the euryhaline fish which helps them better adapt to the changing environmental conditions.

2. HORMONAL ASPECTS OF MIGRATION

[1] Have clearly stated that osmoregulation during the migration in fish is maintained by both extrinsic and intrinsic factors. The extrinsic factors includes temperature, water level, precipitation, photoperiod. According to them, intrinsic factors involved in migration are various hormones and chemicals factors such as prolactin, thyroxin, somatolactin, gonadotropin releasing hormone, growth hormone, follicle stimulating hormone, gonadotropin releasing hormone. In euryhaline fish, the migration to different salinities continues throughout their life cycle which requires the functioning of endocrine system as it helps to regulate homeostasis for maintaining the ion and water balance. When fish encounters sudden changes in the salinity conditions, the endocrine system along with the nervous system plays an important role to acclimate the fish. Various hormones have been categorised as fast- acting hormones which acts suddenly upon exposure to environmental changes and they connects the nervous system and the slow acting hormones [5].

Angiotensin II has been identified to be such a fast acting hormone which can act immediately to the changing environment and start various physiological process at the earliest [4]. In eels, the plasma angiotensin concentration is found to be increased when plasma osmolality increases that is when fish is transferred from freshwater to seawater [6]. The angiotensin levels return to normal levels after the initial increase when fish gets acclimated in the saline environment [4]. Various members of the natriuretic hormone family were identified to be fast acting hormones for saltwater acclimation [1]. When eels were transferred from freshwater to saltwater, the concentration of ANP (Atrial Natriuretic Peptide) was seen to increase in the plasma immediately but returned to earlier levels soon after acclimation and it was identified that increase in the plasma osmolality was the reason for increase or production of ANP [7]. Studies revealed that the saltwater acclimation was achieved by preventing the entry of NaCl by ANP [8]. Guanylins and the neurohypophyseal hormones like the vasotocin and isotocin were also found to affect the osmoregulation in the fish when acclimating to saltwater [9]. Three types of guanylins were present in eel, of which guanylins were produced in intestine and the uroguanylins and renoguanylins were synthesised in digestive tract and kidneys [10,11]. After 24 hours of saltwater acclimation of eels, the mRNA levels of guanylins in the intestine showed a fivefold increase from that of freshwater acclimates ones [11]. The plasma concentration of the vasotocin, a neurohypopyseal hormone showed an increase when the euryhaline *Flounder* were transferred to seawater from freshwater [12] and the concentration decreased when *Flounders* were transferred from to freshwater [13]. Urotensins mainly urotensin II was discovered in a teleost fish where it functioned in maintaining osmoregulation [14]. Its concentration was also found to decrease after the fish was acclimated to the seawater conditions [13]. Various members of the peptide hormone family including adrenomedulins, relaxins, vasoactive intestinal peptides are all identified as rapid acting or fast acting hormones in euryhaline fish [15, 16].

Some of the proteins were classified as slow acting hormones, as they were present in the plasma for long time and regulated many osmoregulatory functions [1]. Prolactin is one such hormone which helps maintain the osmoregulation and they have important role is preventing ion loss and water influx which helps fish to adapt to freshwater environment [17]. When euryhaline fish were transferred to freshwater, the prolactin synthesis, gene expression, secretion and the plasma levels showed an increase [18,17]. Another hormone named growth hormone which like prolactin is included in the pituitary polypeptide hormone is also identified to have the function of osmoregulation other than helping in growth [19]. Growth hormones achieved this by increasing the concentration of chloride cells and the different transporters involved in the salt secretion [20,21]. When *Tilapia mossambica* were transferred to saltwater, the growth hormone levels in the plasma were seen to be increased [22]. Cortisol is a mineralocorticoid which has been found to be involved in increasing the number of protein transporters such as Na^+- K^+ - ATPase (NKA1b), Cystic Fibrosis Trans membrane Conductance Regulator (CFTR), $Na^+/K^+/2Cl^-$ co-transporters (NKCC1) which increase the salt secretion in the gills [23,24,25]. Hormones which help in gonadal maturation were responsible for migration in fish [1]. Two forms of the gonadotropin releasing hormones i.e., sGnRH and cGnRH were found in salmonids [26]. The signals of sGnRH were found in the olfactory nerve and olfactory bulb when fish were away from the spawning ground but these signals were more in the telencephalon and the preoptic area when they were in the spawning ground [27,28,29]. The pituitary is stimulated by the gonadotropin releasing hormone to produce Luteinizing hormone and Follicle stimulating hormone which helps in gamete formation [30].

When euryhaline fish were transferred to freshwater or seawater, the ionic and water balance were maintained at each osmoregulatory organ through the ionic and water exchange and for this, various hormones mentioned above whether fast acting or slow acting, worked together by maintaining absorption through intestine, renal exchange, branchial fluxes and changing the drinking behaviour [8].

3. BIOCHEMICAL CHANGES IN GILLS, KIDNEYS AND INTESTINE

Gills are the organs which fish use for breathing under water and it is the organ which helps in gas exchange. Depending upon the environmental conditions, fish have to either uptake or excrete ions to maintain the osmotic fluid balance and gills are organs which can directly sense these external environmental changes, [31,32]. It is the apical membrane of the epithelial cells in gills which comes in to contact with the external water surface and therefore it is the most severely affected following osmotic stress which is followed by kidneys and intestine [31]. There are numerous cells in gills of which ionocytes are known as chloride cells or Mitochondrion Rich Cells (MRC) and functions in chloride secretion [33]. In freshwater fish, the gill ionocytes function in taking up NaCl from the environment with the help of various transporters like Na^+- Cl^- co-transporter, Na^+/H^+ exchanger or Na^+/K^+- ATPase and in marine environment the ionocytes function in excreting the ions mainly using CFTR, NKCC1 and NKA [34,35,36]. When fish were transferred from freshwater to seawater, the epithelial cells of the gills were able to immediately switch from up taking mode to excretion mode by removing the transporters or channels from freshwater to seawater type [31]. The acid- base regulation in fish in the freshwater and seawater is done through the movement of acidic and basic molecules between the body surface and the environment and the gills are the major organs which helps in this regulation [36,37]. In *Tilapia mossambica*, during acute salinity stress the gill ionocytes were supplied with excess energy by a new type of cells named as Glycogen- Rich Cell (GLR) [38].

Kidney play an important role in osmoregulation in fish. They are very much helpful in secreting divalent ions like Mg^{2+}, Ca^{2+}, and So_4^{2+} [39]. In freshwater fish, the kidneys function in excreting the excess water by producing dilute urine but in saltwater fish, the water is conserved by the kidneys [40]. The euryhaline fish when in freshwater, the filtration rate is high and the reabsorption of the necessary ions is greater and produces dilute urine in order to excrete excess water [41]. When in seawater environment, the filtration rate of the plasma is very low in order to conserve water [42]. NKA activity seems to increase in the gills and was found to be distributed similarly in the renal tubules and collecting duct of freshwater and saltwater acclimated species of *Tilapia mossambica* but was not present in the glomeruli [40]. In the freshwater fish, the glomerular filtration rate is high and the salt absorption takes place in the distal tubules but in saltwater fish, the reabsorption of ions takes place in the proximal tubules [43].

In fish, along with kidneys and gills, intestine is also considered an osmoregulatory organ. In freshwater fish, there is only a minor role played by the intestine but in marine fish, intestine is helpful in absorbing seawater together with Na^+ and Cl^-. In the saltwater fish, the ions like Na^+, K^+, and Cl^- are absorbed by the (NKCCs), which are present in large amount along the mucosal surface of the intestinal epithelium [44]. When fish were transferred to seawater, the outer surface of the digestive tract developed blood vessels and becomes red in colour and the intestinal epithelial cells become thin and transparent which is very much helpful for the functioning of the intestine by actively absorbing ions and water [45]. The lumen and the body surface of the intestine is rich in Cl^- sensors which are helpful in regulating drinking [31]. Water absorption is increased more by secretion of HCO_3^- into the lumen of the intestine and thus decreasing the plasma osmolality [46,47].

4. CHANGES IN GENE ACTIVITIES

The direction of the movement of water and ions and the components of various transporters are the main two system which the teleost use for adapting to different salinities [48]. Various such transporters [35,49,50] were identified in the gills which are the major site of ion transport for osmoregulation. For maintaining osmoregulation, the major strategy used by teleost is regulating the extracellular Na^+ and Cl^- levels [51]. The absorption of Na^+ is powered by the NKA which is present in the basolateral surface [52,53]. The activity of NKA is found to be more in the seawater acclimated fishes than its freshwater counterparts [54,55,56,57,58]. When teleost fish were transferred from freshwater to seawater, the intestinal NKA gene expression seems to increase, which is essential for saltwater osmoregulation [59,60,61]. According to [35], NKA is the major enzyme involved in maintaining homeostasis in fish and function as the major ion transporter in all the transporting organs including gills by taking Na+ out and K+ into the cell. The NKA activity changes according to salinity [36,62]. Fish respond to salinity change by activating the NKA and the MR cells in the gills [35]. In fish like *Tilapia, Eel, Salmon*, which were involved in the hyperosmotic medium, the NKA response was found to be higher along with higher density of MR cells and apical V^- H^+ -ATPase was also found along with NKA which helped in transport of ions [48,62]. In some fish like *Killfish* and *Fundulus* which respond to hyposmotic medium by increasing the NKA activity [63], the V^- H^+-ATPase are found in the basolateral surface in the gill NKA-IR/MR cells Many isoforms of the NKA α- subunit was identified from different species.

Like NKA, the gene expression of (NKCC), also showed an increase in the intestine of euryhaline fish when they were acclimated to seawater from freshwater environment [59]. Their studies also revealed that in saltwater acclimated fish kidneys, the expression of NKCC1 mRNA decreased due to lower rate of urine flow and ion secretion. Out of the two isoforms of NKCC, the NKCC1 was found to be a secreting isoform mainly secreting Cl^-. In fish *like European eel, Killfish and Striped bass*, when transferred to seawater, the NKCC expression was found to be increased as the Cl^- secretion was stimulated [64,65,59]. Studies by [66] also suggested that gills of the saltwater acclimated fish had higher NKA activity and expressed NKCC and CFTR when compared to their freshwater counterpart. The study also revealed that the Cl^-/HCO_3^- exchanger 1 (AE1), and chloride channel 3 (CLC-3) were abundant in the freshwater acclimated group than in the saltwater group. Cl^- channel were found to be abundant in the gills of many saltwater acclimated fishes like *Killfish, Mudskipper, Eel* [67,66,68,62,69]. CFTR appeared in the apical region about 24 hours after transferring to seawater and disappeared within 24 hours when transferred to freshwater in *Killfish* [64,70,62]. In *Tilapia* also apical CFTR appeared at about 12 hours and increased in number after 24 hours [71]. The CFTR mRNA levels increased in both *Killfish* and *Atlantic Salmon* when exposed to saltwater environment for prolonged time and this shows the importance of CFTR in ion regulation [64,25].

5. CONCLUSION

Studies on fish migration and the physiological, biochemical, histological and morphological changes associated with the process of adjusting to the different environments have always been a major concern and research area. From the studies reviewed above, it is clear that the osmoregulation is a complex process and involves many hormones, enzymes, proteins etc. All these factors interact together and enable the fish to cope up with the new environment. The studies suggested that, the major organ for osmoregulation is the gill along with kidneys and intestine which have their own roles. The major transporters which help in ionic transport across the cells to maintain the osmotic balance are all mainly distributed in the gill epithelium which suggest the role and importance of gill in osmoregulation. Many important transporters have been identified of which NKA is given much stress. Various studies have been done to elucidate the role and importance and changing expression of NKA during different salinities. Activities of some of the transporters like CFTR and apical exchangers for chloride transport have to be done further to get a more detailed review of their presence in many fish species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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