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Changes in Expression of Branchial Na+, K± ATPase 1 α- Subunit Isoforms during Acclimation in Different Habitats

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Abstract

In euryhalineteleosts, the biochemical mechanisms for maintenance of constant level of ions in body fluids of fish depend mainly on the activity of gill Na^+, $K^±$ ATPase (NKA). The NKA is a P-type ubiquitous membrane-spanning ATPase that actively transports Na^+ and K^+ out of and into animal cells. The enzyme activities of gill NKA are affected by environmental ion concentrations. The high activity of NKA is mainly located in the tubular system of the MR cells which plays a central role in the process of ion transport in gills of freshwater and seawater-acclimated fishes. The NKA consist of three subunits; α, β and γ.

Keywords: Freshwater; Ions; Fishes; Isoforms

Introduction

The NKA consist of three subunits; α, β and γ [1]. The α-subunit contains binding sites for cations, ATP and ouabain (which is a specific inhibitor of NKA), thus it is responsible for the catalytic and ion regulatory capacity of the NKA, while the β-subunit associated with the protein maturation and anchoring of the enzyme complex in the cell membranes [1]. The γ-subunit is appears to modulate the affinity NKA for Na^+, K^+ and ATP, which has not yet been found in teleosts [2]. Blanco and Mercer [1], reported that, in mammals, four α(α1– α4) and four β(β1–β4) subunit isoforms have been identified, while teleosts display an even wider repertoire of α and β-subunit isoforms [3], many of which are expressed in gills [4]. The molecular weight of the catalytic α-subunit is about 100 kDa, while the smaller glycosylated β-subunit are about 55 kDa, respectively [5].

Function of Na+, K+-ATPase (NKA)

The fish gill Na^+, K^+-ATPase (NKA), involved in ion regulation in both freshwater and seawater. In SW acclimated fishes, the basolateral NKA energizes ion secretion by creating an electrochemical gradient that is used by the $Na^+/K^+/2Cl^-$cotransporter (NKCC) and apical cystic fibrosis transmembrane conductance regulator (CFTR) to provide transcellular secretion of Cl^-, and paracelluar secretion of Na^+, [6]. In FW acclimated fishes, the basolateral NKA is probably also involved in driving

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uptake of NaCl, possibly in conjunction with an apical V-type H^+-ATPase, *via* apical Na^+ channels and $Cl^-/HCO3^-$ exchangers [7]. The NKA is an essential participant in maintaining ionic concentrations and body fluids within appropriate physiological limits for survival in different salinity.

Role of Na+, K+-ATPase (NKA) in Different Habitats

The activity of Na+, K+-ATPase (NKA) is dependent on the environmental ion concentration. In teleost fishes, the gill is a most important organ, plays a principal role in the maintenance of ion homeostasis in both FW and SW acclimated fish. Lin, et al. [8], reported that, when pufferfish (*Tetraodonnigroviridis)* acclimated to FW, BW and SW and resulted in specific activity of gill NKA of fish acclimated to SW was significantly higher than that of fish acclimated to BW and FW (3.3 and 1.8 fold). However, there was no significant difference between BW and SW acclimated fish. When tilapia (*Oreochromismossambicus*) transferred directly from SW to FW, the specific activity of gill NKA dropped significantly within 3 hrs. After 3 hrs, NKA activity reached a stable level at 96 hrs [8]. The Brown trout (*salmotrutta*) acclimated to SW from FW; the specific activity of NKA was significantly higher on day three after SW-transfer and continued to increase on day seven and day 60. When it returns to FW, enzyme activity will be reduced after ten days [9]. The specific activity of NKA land-locked Arctic char (*Salvelinusalpinus*) transfer to SW was no significant difference as compared to control [10].

After SW acclimation of Atlantic salmon parr, the branchial specific activity of NKA was 7 fold higher than the FW acclimation [11]. FW gill NKA activity levels in the Atlantic salmon (*Salmosalar*) anadromous strain increased significantly from April to May, with fivefold higher in May and June than those observed in February. In the Atlantic salmon landlocked strain, FW gill NKA activity levels increased significantly from April to May, with being twofold higher in May and June than those observed in February. Gill NKA activity in FW was significantly lower among fish in the landlocked strain than the anadromous strain in May and June [12]. The three salmonids, Atlantic salmon (*Salmosalar*), rainbow trout (*Oncorhynchusmykiss*) and Arctic char (*Salvelinusalpinus*) acclimated to SW the activity of NKA increased significantly in all three species by days 10 of SW exposure compared with FW controls and continued to rise significantly by days 30 [13]. Atlantic salmon transfer from SW to FW the activity of NKA significantly increased by 43% as compared with the SW acclimated fish. After 30 days of freshwater acclimation, gill NKA activity returned to control levels [14].

In Atlantic salmon, transfer from FW to SW the branchial activity of NKA was significantly elevated at seven days post-transfer [15]. Branchial NKA activity was unaffected first 5 days when rainbow trout (*Oncorhynchusmykiss*) transfer from FW to 80% SW, NKA activity increased 2.4 fold at 15 days post transfer [4]. The branchial activity of gill NKA of milkfish (*Chanoschanos*) acclimated to either FW or BW were significantly higher than that of fish acclimated to SW. Branchial NKA activity in the FW and BW acclimated fishes were approximately 7 and 5 times higher than that of the SW acclimated fishes [16]. The transfer of *Fundulusheteroclitus* from FW to SW salinity ranging from 0.1 to 35 ppt induced a 70% increase in branchial NKA activity 3 hr after transfer. But after 12 hr the activity dropped to initial levels. A second significant increase in activity occurred 3 days after transfer [17].

Yang, et al. [18], studies, when euryhalinesailfin molly (*Poecilialatipinna)* acclimated to FW, BW and SW, branchial NKA activity of SW acclimated fish was significantly higher than that of fish acclimated to BW and FW. There was no significant difference in branchial NKA activity between the BW and FW groups. The density of NKA-rich MRCs and NKA activity was higher in the 4th gill arch in the case of two species of freshwater potamotrygonid stingrays (*Paratrygonaiereba* and *Potamotrygon* sp.). The NKA activity was positively correlated to the NKA-rich MRCs distribution among the gill arches of *P. aiereba* butnot in *Potamotrygon* sp. The levels of NKA activity were not correlated to the gill surface area among the arches for both rays' species, the NKA-rich MRCs is the main site for active ion transport in the gill epithelia and NKA activity plays a crucial role in osmo-ionoregulatory function, resulted that the 4th gill arch is more relevant for osmoregulation and ion balance in these potamotrygonids [19]. When Hawaiian goby (*Stenogobiushawaiiensis*) were acclimated to different salinities ranging from FW to BW (20‰) and SW (30‰) for ten days, resulted in differences in the number, size and staining intensity of NKA immune reactivity in FW and SW acclimated fish. There was a 46% increase in the amount of NKA in gill tissue following SW acclimation. Branchial NKA activity of Hawaiian gobies increased by 24% on transferring to SW, although there was no significant difference between FW fish and SW fish [20]. Similar study was done in Atlantic stingray (*Dasyatis Sabina)*, however yielded opposite results. Stingrays from FW had the highest activity of NKA and the greatest number of NKA-rich cells. When FW stingrays were acclimated to SW for one week, the activity and

abundance of NKA and the number of NKA-rich cells decreased in the gills [21].

Imsland, et al. [22], reported when juvenile turbot (*Scophthalmusmaximus*) was acclimated to different salinities (15‰, 25‰ and 33.5‰), gill NKA activity and plasma chloride were lowest in 15‰ and highest in 33.5‰. Christensen, et al. [23], reported that, the clupeid fish, alewife (*Alosapseudoharengus*) when acclimated to FW to SW environment, the gill NKA activity was up-regulated by 75% and the abundance of the NKA α-subunit were greater in seawater-acclimated individuals by 40%, respectively. Chandrasekar, et al. [24], reported two NKA α-isoforms (NKA α) were expressed in gills of *Etroplussuratensis* during acclimation in FW, BW and SW. Availability of one isoform was controlled in response to marine acclimation, suggesting its role in ion secretion similar to NKA α1b, while expression of another isoform was simultaneously up-regulated in response to both FW and SW acclimation, suggesting the presence of isoforms switching phenomenon during acclimation to different salinities.

Expression of Na+, K+-ATPase 1 α-subunit Isoforms in Different Salinities

When tilapia was transferred directly from SW to FW, relative mRNA abundance of NKA α subunit 1 isoform decreased significantly at 6 hrs post-transfer and relative abundance of NKA α subunit 1 protein decreased gradually from 3 hrs post transfer, was significant at 12 hrs, and became one-fifth of the amount in SW at 24 hrs post transfer [25]. When Land-locked Arctic char (*Salvelinusalpinus*) transferred to SW, the mRNA expression of both the α1a and α1b isoform of gill NKA was detected from both FW acclimated and SW exposed fish. Expression of the α1a isoform was found to be highest in freshwater acclimated char. SW exposure induced a rapid reduction in isoform α1a mRNA. The mRNA levels of the α1b isoform were not different between freshwater and seawater exposed Arctic char [10].

McCormick, et al. [26], reported that, the abundance of gill NKA α1a isoform was high in FW acclimated Atlantic salmon and became nearly undetectable after SW acclimation. However, expression of NKA α1b isoform was present in small amounts in FW acclimated fish, increased 13- fold after SW acclimation. Both NKA α1a andα1b isoforms were detected only in mitochondrial rich chloride cells. In FW environment the mRNA expression of gill NKA α1a isoform of the Atlantic salmon (*Salmosalar*) anadromous strain decreased continuously from February through April, May and June, with expression in June being fourfold lower than those observed in February. In landlocked strain, the mRNA expression of NKA α1a isoform decreased by two folds from February to April and remained stable in May and June, resulting in NKA α1a expression being significantly higher than those of the anadromous strain in May and June. In May and June, the gill NKA α1a mRNA expression were significantly lower in the SW acclimated fish of both strains, when compared to corresponding FW fish. FW gill mRNA expression of NKA α1b isoform in the anadromous strain increased significantly from February through April and May, with relative mRNA expression in May being six-fold greater than those observed in February, followed by a substantial decrease in June. The levels of gill NKA α1c isoform mRNA did not change significantly in either strain in FW from February through June, or following SW exposure. While mRNA expression of NKA α2 was not detected in gills of the fish [12].

Patterns of mRNA expression of gill NKA α1a and α1b isoforms were similar in Arctic char, Atlantic salmon and rainbow trout. The mRNA expression levels of NKA α1a isoform were highest in FW acclimated fish as compare to SW acclimated fish, the levels of α1a decreased rapidly in all three species. In all three SW acclimated species the gills mRNA expression of NKA α1a isoform was significantly different from FW acclimated fish. The mRNA constructionα1b isoform had the opposite reflection form to α1a, being minimum in FW acclimated fishes and increasing significantly upon exposure to SW. All three species had similar mRNA expression patterns for isoform α1b [13]. The mRNA expression of gill NKA α1a isoform increased significantly, by more than 7-fold, during FW acclimation of SW Atlantic salmon, with peak expression was observed after 14 days. Conversely, gill α1b isoform expression decreased significantly in fish within 4 hour of freshwater exposure. However, after 30 days acclimation to freshwater, the gill α1b isoform expression had returned to levels similar to those of control salmon. In contrast to the NKAα1a and α1b isoforms, the mRNA expression of NKA α1c and α3 isoforms were unchanged by FW exposure [14].

Madsen, et al. [27] reported, when Atlantic salmon acclimated in SW environment the mRNA expression of NKA α1a isoform was lowest as compared to FW acclimated fishes at 3 and 7 days after transfer. However, mRNA expression of NKA α1b isoform was highest in SW acclimated than the FW acclimatedat 1, 3 and 7 days after transfer, but the expression of α1c isoform did not affected in SW environment, while the expression of α3isoform was affected, which was generally lower in SW as compared to the FW. Richards, et al. [4] further explained that, the mRNA expression of α1a isoform was

high in FW rainbow trout (*Oncorhynchusmykiss*) and dramatically decreased within 1 day following transfer from FW to 40% and 80% SW. In contrast when the trout was transferred to 80% SW, there was transient increase in α1b mRNA as compared to FW control. Transfer of trout into 40% SW did not affect gill α1b mRNA for the first 5 days post transfer, but significant decreasedα1b mRNA expression at 10 and 15 days post transfer.

Tipsmark, et al. [28] reported that, when FW acclimated *Mozambique tilapia was* transferred to SW environment, resulted in a reduction of gill mRNA expression of NKA α1a isoform within 24 h and a significant increase in mRNA expression of NKA α1b isoform within 7 days after transfer. Khodabandeh and Rajabi [29], reported that, when freshwater acclimated *Salmotruttacaspus* was transferred to brackish water, it resulted in decreased expression of gill NKA α1a isoform and increased expression of NKA α1b isoform. Madsen, et al. [27] reported that, when FW acclimated striped bass (*Moronesaxatilis*) was transferred to SW resulted in increased the gill NKA α mRNA expression. Nilsen, et al. [12], reported that, the FW gill mRNA expression of NKA α1a isoform of juvenile anadromoussalmon (*Salmosalar*) decreased continuously from February through April, May and June, increased the mRNA expression of FW gill NKA α1b significantly from February through April and May and no changes were observed in gill mRNA expression of NKA α1c isoform from February through June. When FW acclimated tilapia (*Oreochromismossambicus*) was transferred to SW, the expression of gill NKA α subunit 5 times increased. Ip, et al. [30], reported that, the climbing perch (*Anabas testudineus*)acclimated to FW, resulted in highest gill mRNA expression of NKA α1c isoform followed by α1a and α1c isoform that is almost undetectable. Further, when it was transferred to SW, it resulted in highest mRNA expression of NKA α1b isoform followed by α1c and α1a.

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