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Sodium or Potassium Ions Activate Different Kinetics of Gill Na, K-ATPase in Three Seawater- and Freshwater-Acclimated Euryhaline Teleosts

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ABSTRACT The effects of $[Na^+]$ or $[K^+]$ on Na, K-ATPase activity of FW-acclimated and SW-acclimated tilapia, puffer and milkfish were examined in gill homogenates. $[Na^+]$ or $[K^+]$ stimulated Na, K-ATPase hydrolyzing ATP in all experimental groups. ATP hydrolysis stimulated by $[Na^+]$ or $[K^+]$ followed Michaelian-Menten kinetics. Km values for $[K^+]$ (i.e., Km_K), were lower in SW- than FW-acclimated tilapia and puffer fishes (tilapia: 8.69 ± 0.22 vs. 11.93 ± 1.17 mM; puffer: 13.51 ± 1.39 vs. 30.52 ± 2.66 mM). Km values for $[Na^+]$ (i.e., Km_{Na}), were lower in FW- than SW-acclimated milkfish (3.76 ± 0.54 vs. 7.55 ± 1.08 mM). These data suggest that $[K^+]$ stimulates ATP hydrolysis to rates higher in SW- than FW-acclimated tilapia and puffer fishes, while $[Na^+]$ stimulated ATP hydrolysis at rates higher in FW- than SW-acclimated milkfish. This is the first demonstration that Na, K-ATPase activity of euryhaline tilapia, puffer, and milkfish modulated by $[Na^+]$ or $[K^+]$ have different effects between FW- and SW-acclimated groups. Such responses as changes in properties of branchial Na, K-ATPase may contribute to improve the osmoregulatory capacity of tilapia, puffer and milkfish to acclimate in seawater and fresh water. *J. Exp. Zool. 303A:57–65, 2005.*

INTRODUCTION

Euryhaline teleosts are able to survive in environments with a broad spectrum of salinities. Effective mechanisms of ion-regulation enable teleosts to retain an osmotic and ionic constancy in their internal milieus and survive in hypertonic or hypotonic environments. The gill is the major organ responsible for ion-regulation in euryhaline teleosts (Perry et al., 2003).

Na^+- and K^+-activated ATP hydrolysis is a widely distributed, phylogenetically conserved key mechanism, hence there are extensive investigation on Na, K-ATPase (NKA) structure-function relationships (Scheiner-Bobis, 2002). Fish gill epithelium contains NKA, which provides the driving force for many transporting system and thus maintain intracellular homeostasis (Marshall, 2002; Hirose et al., 2003). Immunocytochemical studies demonstrated that NKA is located mainly in mitochondria-rich (MR) cells of gill epithelia in euryhaline teleosts (Wilson and Laurent, 2002). The energy of ATP hydrolysis drives the coupled extrusion of Na^+ and uptake of K^+ ions by the membrane-spanning enzyme NKA in most animal cells. This heterodimeric integral membrane protein is a P-type ATPase consisting of an $(\alpha\beta)_2$ protein complex and a catalytic α-subunit with molecular weight of about 100 kDa, while the smaller glycosylated β-subunit exists with a molecular weight of approximately 60 kDa. Four α (α1–4) and three β (β1–3) isoforms as well as a small γ subunit have been found in mammals and birds. It is suggested that tissue specific expression of different isoforms are associated with various physiological functions (Blanco and Mercer, '98; Scheiner-Bobis, 2002).

Most euryhaline teleosts exhibit adaptive changes in NKA activity following salinity changes (Marshall, 2002). Previous works classify euryhaline teleosts, which respond with higher NKA activity in SW as belonging to the diadromous paradigm (Lin et al., 2003). Some species studied, which agree with the paradigm, include killifish (*Fundulus heteroclitus*, Epstein et al., '67), tilapia (*Oreochromis mossambicus*, Hwang et al., '89, '98; Morgan et al., '97; Uchida et al., 2000; Lee et al.,

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2003), and puffer (*Tetraodon nigroviridis*, Lin et al., 2004b). Alternatively, studies of marine species outline a remaining group of euryhaline fish which respond with higher NKA activity in FW include flounder (*Platichthys flesus*, Stagg and Shuttleworth, '82), mudskipper (*Periophthalmodon schlosseri*, Ip et al., '93), mullet (*Mugil cephalus*, Ciccotti et al., '94), European sea bass (*Dicentrarchus labrax*, Jensen et al., '98), sea bream (*Mylio macrocephalus*, Kelly et al., '99), and milkfish (*Chanos chanos*, Lin et al., 2003). Among them, tilapia and puffer resided naturally in FW and estuary, and milkfish in SW habitats. Sets of salinity-challenging experiments were conducted in our laboratory to reveal the NKA expression in these three euryhaline species (Lee et al., 2000, 2003; Lin et al., 2003, 2004a, b).

In vitro activity assays demonstrated that gill NKA of euryhaline or anadromous teleosts, e.g., eel, trout, tilapia, and sea bass, is sensitive to ionic strength (Johnson et al., '77; Ho and Chan, '80; Trigari et al., '85; Hwang et al., '88; Pagliarani et al., '88). Hwang et al. ('88) and Pagliarani et al. ('88) reported that NKA activities of gill homogenates in FW tilapia and SW sea bass elevated with increasing sodium and potassium concentrations of the reaction media. These studies however, focused on gill NKA kinetics for $[Na^+]$ and $[K^+]$ of studied species only in their natural habitats (i.e., FW or SW). Since the affinities of gill NKA in euryhaline teleosts to $[Na^+]$ or $[K^+]$ in environments of varied salinities may be different and lead to changes of NKA activity, the kinetic characteristics of this enzyme in euryhaline fish from either FW or SW, instead of merely their natural habitats, should be evaluated to illustrate different patterns of changing NKA activities among euryhaline species. In this study, direct effects of sodium and potassium ions on hydrolytic activity of the Na, K-ATPase in microsomal fractions from gill tissues of euryhaline tilapia, puffer, and milkfish acclimated to hypertonic SW or hypotonic FW were examined. We demonstrated that varied patterns of NKA activities found in gills of euryhaline teleosts upon salinity challenges were modulated by sodium and potassium ions. This kinetic analysis of NKA provides a better understanding of mechanisms of salinity-adaptation in different euryhaline teleosts.

MATERIAL AND METHODS

Fish and experimental environments

Tilapia (*Oreochromis mossambicus*), puffer (*Tetraodon nigroviridis*) and milkfish (*Chanos chanos*) obtained from laboratory populations were 8.5 ± 0.2, 6.2 ± 0.5 and 12.9 ± 1.0 cm respectively in total length. Seawater (35‰; SW) mixed from local tap water with appropriate amounts of synthetic sea salt, Instant Ocean (Aquarium Systems Co., Mentor, OH). Fishes were reared in SW ($[Na^+]$, 582.86 mM; $[K^+]$, 10.74 mM; $[Ca^{2+}]$, 15.75 mM; $[Mg^{2+}]$, 32.92 mM; $[Cl^-]$, 520.84 mM) or fresh water (FW; $[Na^+]$, 2.6 mM; $[K^+]$, 0.04 mM; $[Ca^{2+}]$, 0.58 mM; $[Mg^{2+}]$, 0.16 mM; $[Cl^-]$, 0.18 mM) at $27 \pm 1^\circ$C with a daily 12 hr photoperiod for at least two weeks before experimental analyses. The water was continuously circulated through fabric-floss filters. Fish were fed a daily diet of commercial frozen sludgeworm (for puffer) or pellet (for tilapia and milkfish).

Preparation of gill homogenates

Gill arches of the fish from FW and SW were excised and blotted dry. The gill epithelia were immediately scraped off the underlying cartilage with a scalpel. All subsequent operations were carried out in ice. The gill scrapings (0.9–1.2 g wet wt.) were suspended in 3 ml of homogenization solution according to Hwang et al. ('89) (100 mM imidazole-HCl buffer, pH 7.6; 5 mM Na_2EDTA; 200 mM sucrose; 0.1% sodium deoxychlolate). Homogenization was performed in a glass Potter-Elvehjem homogenizer with a motorized Teflon pestle at 600 rpm for 20 strokes. The homogenate was then centrifuged at 13000g 4°C for 20 min. Protein concentrations of the supernatant were determined with the reagents of Bio-Rad Protein Assay Kit using bovine serum albumin as a standard.

Enzyme specific activity (ESA) of Na, K-ATPase

ESA of Na, K-ATPase in fish gills was determined as described by Hwang et al. ('89). Aliquots of the suspension of prepared gill homogenates, were used for determination of protein and enzyme activities. ESA was assayed by adding the supernatant to the reaction medium (100 mM imidazole-HCl buffer, pH 7.6, 125 mM NaCl, 75 mM KCl, 7.5 mM $MgCl_2$, 5 mM Na_2ATP). The reaction was run at 37°C for 30 min and stopped by addition of 200 μl of ice-cold 30% trichloroacetic acid. Inorganic phosphate concentration was measured according to Peterson's method ('78). The ESA of Na, K-ATPase was defined as the difference between the inorganic phosphate liberated in

the presence and absence of 3.75 mM ouabain in the reaction medium.

Estimation of kinetic parameters

To analyze the effects of different sodium or potassium concentrations on ESA of Na, K-ATPase, choline chloride ($C_5H_{14}NOCl$) was used to replace KCl or NaCl in the reaction medium. Different concentrations of sodium or potassium used in reaction medium of the experiments were 2.5mM, 5mM, 10mM, 20mM, 40mM, 80mM, 160mM, and 210mM, respectively. ESA is normalized to compare the effects of sodium or potassium on ESA of Na, K-ATPase. Normalized ESA is defined as: ESA of each concentration point divided by the maximum ESA in the same group (SW or FW group). A rank of one in each group represents the highest activity. The Michaelis-Menten constant (Km) for $[Na^+]$ and $[K^+]$ (i.e., Km_{Na} and Km_K) were calculated as described by Trigari et al. ('85). Km given in the Tables or Figures were calculated by results of gill homogenates from different individuals (N=4).

Statistical analysis

Values were compared using a one-way analysis of variance (ANOVA) (Fisher's pair-wise method). Values were expressed as the means $\pm$ SEM.

RESULTS

Effect of choline chloride on enzyme specific activity (ESA) of gill Na,K-ATPase

ESA of branchial Na,K-ATPase of experimental fishes are not affected by increasing concentrations of choline chloride in the reaction medium (Table 1). Thus, choline chloride was used in substitution of NaCl or KCl in determination of ESA of Na, K-ATPase in the following experiments.

Effects of $[Na^+]$ or $[K^+]$ on ESA of gill Na,K-ATPase

The effects of increasing $[Na^+]$ or $[K^+]$ on ATP hydrolysis by the Na, K-ATPase in gills of tilapia, puffer and milkfish are shown in Figs. 1–3. The ESA of Na, K-ATPase were stimulated by modifying $[Na^+]$ and $[K^+]$ in all experimental groups. ESA values for $[Na^+]$ and $[K^+]$ are higher in SW- than FW-acclimated tilapia (Fig. 1) and puffer (Fig. 2) fishes, while milkfish (Fig. 3) showed opposed results. Although ESA values for $[Na^+]$ and $[K^+]$ were different between FW- and SW-aclimated groups, ESA increased 3–5 folds on average with increasing $[Na^+]$ and $[K^+]$ (from the lowest to the highest concentrations, Figs. 1–3). The Michaelis-Menten constant (Km) of Na, K-ATPase were estimated in gills of SW- and FW-acclimated fishes. In SW- and FW-acclimated tilapia and puffer, Km_{Na} values were similar (Table 2; Figs. 1A and 2A), while Km_K values revealed significant differences in SW- and FW-acclimated individuals (Table 2; Figs. 1B and 2B). On the contrary, in SW- and FW-acclimated milkfish, Km_{Na} values showed significant differences (Table 2; Fig. 3A), while Km_K values were similar (Table 2; Fig. 3B).

DISCUSSION

Three euryhaline species with varied natural habitats were studied. The Mozambique tilapia (*Oreochromis mossambicus*) is a euryhaline FW cichlid that tolerates salinities of up to 120‰ (Stickney, '86), making it a good model organism for studies on ionic and osmotic adaptations in teleost fishes. The green spotted

TABLE 1. Effects of different concentrations of choline chloride on ESA of Na,K-ATPase in gills of tilapia, puffer and milkfish

Euryhaline species	Milieu	choline chloride (mM) 0	50	100	200
tilapia	FW	7.37 $\pm$ 0.55	7.43 $\pm$ 0.60	7.00 $\pm$ 0.60	7.60 $\pm$ 0.50
	SW	14.70 $\pm$ 1.44	13.60 $\pm$ 1.43	14.00 $\pm$ 1.54	13.90 $\pm$ 1.34
puffer	FW	3.00 $\pm$ 0.50	3.20 $\pm$ 0.40	3.40 $\pm$ 0.30	3.20 $\pm$ 0.25
	SW	6.77 $\pm$ 0.44	6.60 $\pm$ 0.40	6.89 $\pm$ 0.33	6.64 $\pm$ 0.28
milkfish	FW	4.77 $\pm$ 0.44	4.60 $\pm$ 0.43	4.89 $\pm$ 0.54	4.64 $\pm$ 0.43
	SW	1.37 $\pm$ 0.15	1.43 $\pm$ 0.23	1.40 $\pm$ 0.17	1.60 $\pm$ 0.15

ESA of Na,K-ATPase is expressed in μmole Pi/mg protein/hr

All reaction media contained 5mM Na_2ATP, 125mM NaCl, 75mM KCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. Data are expressed as mean $\pm$ SEM. (N=3)

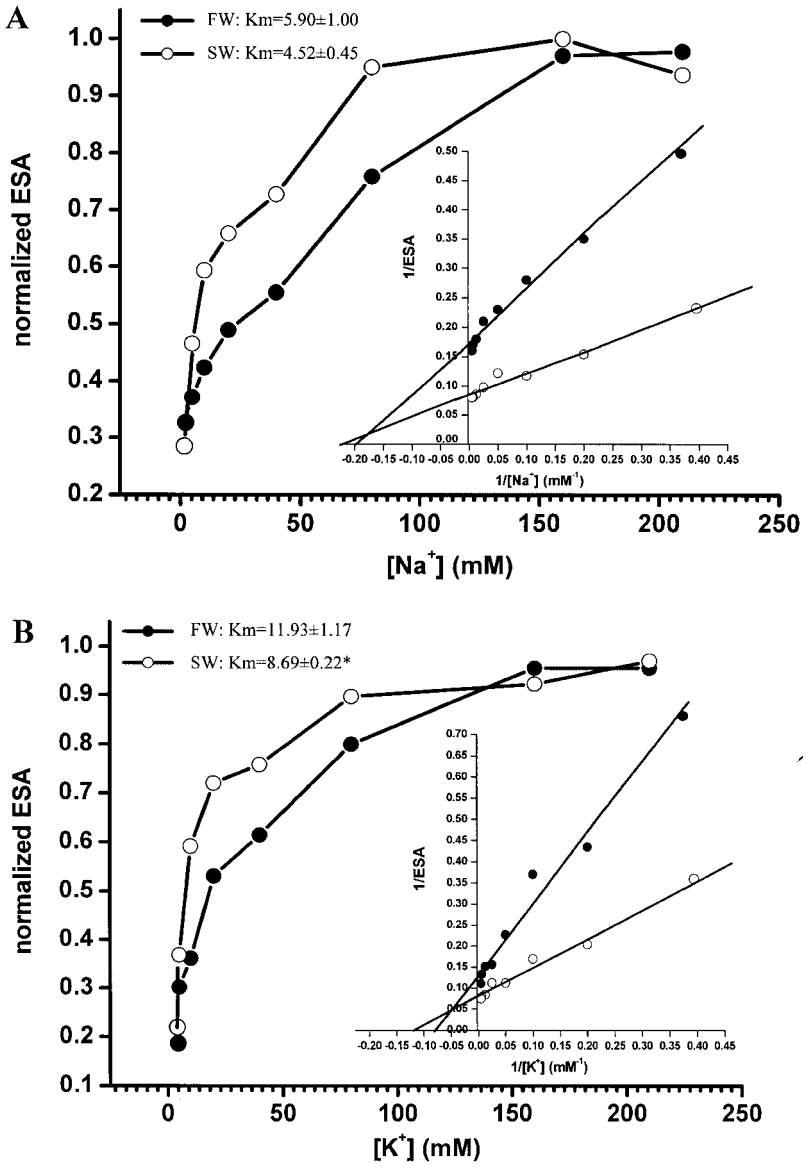

Fig. 1. Characteristics of gill Na, K-ATPase in tilapia. (A) The effects of Na^+ concentrations on the enzyme specific activity (ESA). All reaction media contained 5mM Na_2ATP, 75mM KCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (B) The effects of K^+ concentrations on the ESA. All reaction media contained 5mM Na_2ATP, 125mM NaCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (●) FW-acclimated tilapia; (○) SW-acclimated tilapia. Each point is an average of four fishes. ESA is expressed in μmole Pi/mg protein/hr. Km values are means ± SEM (N=4). The asterisk (*) indicates significantly different Km values ($P<0.05$).

pufferfish (*Tetraodon nigroviridis*) is a tetraodontid teleost whose native range covers the rivers and estuaries of SouthEast Asia (Rainboth, '96). As a peripheral FW inhabitant (Helfman et al., '97), this puffer is an efficient osmoregulator in experimental conditions, tolerating direct transfer from FW to SW or vice versa. The milkfish, a marine inhabitant, is widely distributed throughout the tropical and subtropical Indo-Pacific (Bagrinao, '94). It displays extremely euryhaline behavior throughout its life history, although, it does not appear that a freshwater environment is essential for any part of its life cycle (Bagrinao, '94). Compared to the FW-acclimated individuals among these three species, SW-acclimated tilapia or puffer revealed higher NKA activity similar to the typical ''anadromous paradigm'' (i.e., eel: Thomson and Sargent, '77; salmon: D'Cotta et al., 2000; killifish: Epstein et al., '67; tilapia: Lee et al., 2003; puffer: Lin et al., 2004b), while SW-accli-

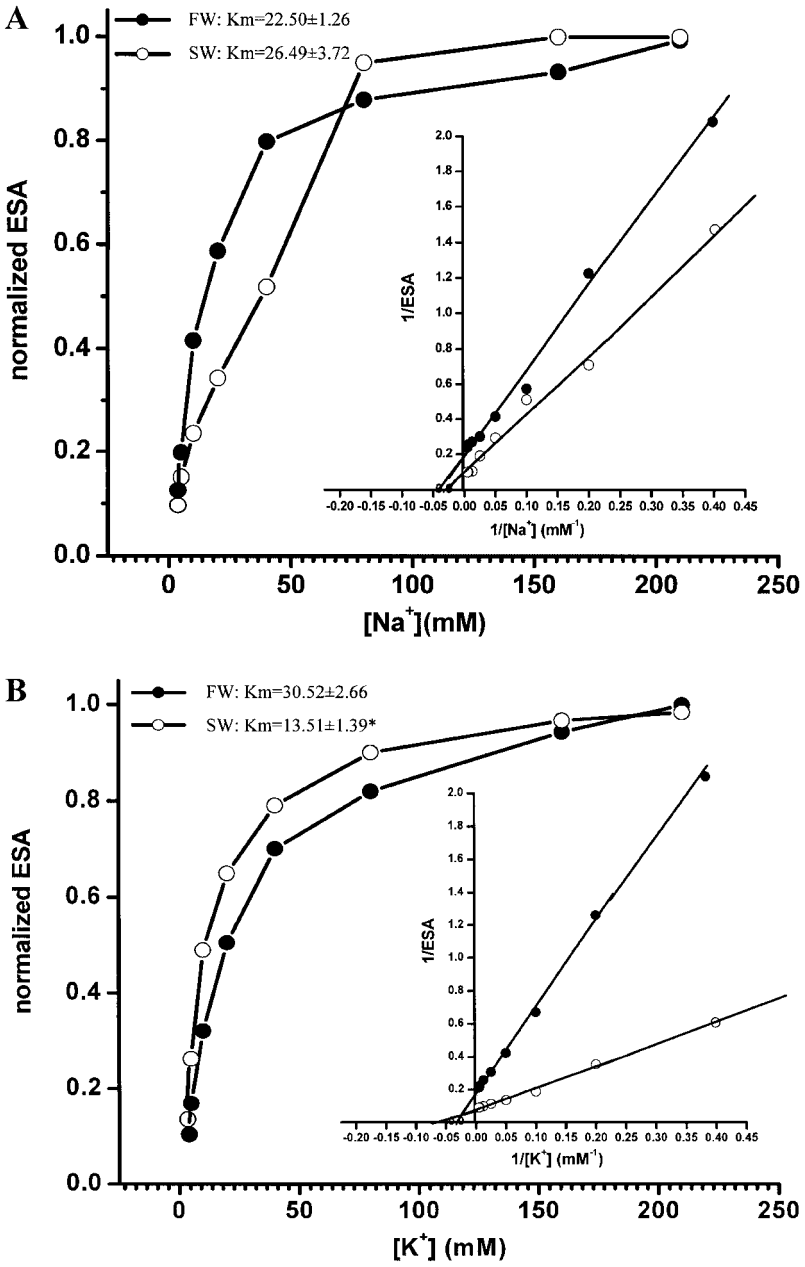

Fig. 2. Characteristics of gill Na, K-ATPase in puffer. (A) The effects of Na^+ concentrations on the enzyme specific activity (ESA). All reaction media contained 5mM Na_2ATP, 75mM KCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (B) The effects of K^+ concentrations on the ESA. All reaction media contained 5mM Na_2ATP, 125mM NaCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (●) FW-acclimated puffer; (○) SW-acclimated puffer. Each point is an average of four fishes. ESA is expressed in μmole Pi/mg protein/hr. Km values are means ± SEM (N=4). The asterisk (*) indicates significant different Km values ($P<0.05$).

mated milkfish showed lower NKA activity as that in other marine species (i.e., flounder: Stagg and Shuttleworth, '82; mudskipper: Ip et al., '93; mullet: Ciccotti et al., '94; European sea bass: Jensen et al., '98; sea bream: Kelly et al., '99; milkfish: Lin et al., 2003). This present study demonstrated that varied patterns of NKA activities found in gills of euryhaline teleosts upon salinity challenges were modulated by sodium or potassium ions.

Apart from enzyme activity differences, the two habitats (FW or SW) impose a different enzyme

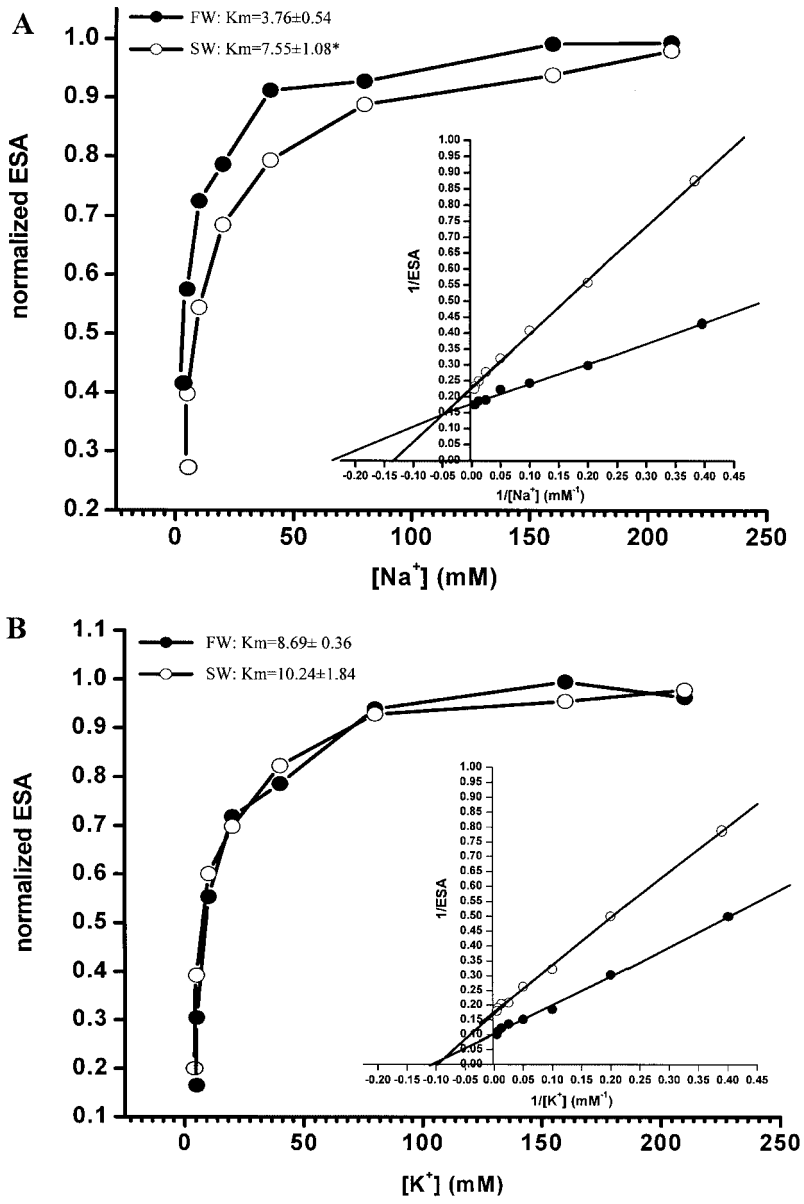

Fig. 3. Characteristics of gill Na, K-ATPase in milkfish. (A) The effects of Na^+ concentrations on the enzyme specific activity (ESA). All reaction media contained 5mM Na_2ATP, 75mM KCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (B) The effects of K^+ concentrations on the ESA. All reaction media contained 5mM Na_2ATP, 125mM NaCl, 7.5mM $MgCl_2$, 100mM imidazole-HCl buffer (pH=7.6), and the presence or absence of 3.75mM ouabain. (●) FW-acclimated milkfish; (○) SW-acclimated milkfish. Each point is an average of four fishes. ESA is expressed in μmole Pi/mg protein/hr. Km values are means ± SEM (N=4). The asterisk (*) indicates significantly different Km values ($P<0.05$).

response to various effectors. The membrane-spanning enzyme NKA is responsible for the active transport of Na^+ out of and K^+ into animal cells. The function of the enzyme in absorbing or secreting Na^+ and K^+ and, secondarily, other solutes, requires tight regulation of the enzyme to maintain normal levels of Na^+ and K^+ as external salinity is altered (Marshall, 2002). Upon salinity challenge, if ions traffic over a pathway catalyzed by a rate-limiting enzyme, e.g., NKA, increases, the specific activity of the enzyme must enhance. When the traffic decreases, however, simultaneous diminish of high specific enzyme activity may not be necessary. To maintain homeostasis, NKA activity can be directly modulated by ligands, including monovalent cations, ATP and

TABLE 2. Properties of branchial Na,K-ATPase in tilapia, puffer and milkfish adapted to FW and SW

Fish	Milieu	$K_{m,Na}$(mM)	$K_{m,\ K}$(mM)	ESA of Na,K-ATPase (μmole Pi/mg protein/hr)	Relative protein abundance of Na,K-ATPase α-subunit	
Tilapia	FW	5.90 ± 1.00	11.93 ± 1.17	7.37 ± 0.55	177.35 ± 41.00	(Lee et al., 2003)
	SW	4.52 ± 1.45	8.69 ± 0.22*	14.77 ± 1.44*	389.27 ± 55.00*	
Puffer	FW	22.50 ± 1.26	30.52 ± 2.66	3.00 ± 0.50	867.50 ± 55.00	(Lin et al., 2004b)
	SW	26.49 ± 3.72	13.51 ± 1.39*	6.77 ± 0.34*	529.00 ± 18.00*	
Milkfish	FW	3.76 ± 0.54	8.69 ± 0.36	4.77 ± 0.44	120.00 ± 10.00	(Lin et al., 2003)
	SW	7.55 ± 1.08*	10.24 ± 1.84	1.37 ± 0.15*	18.00 ± 5.00*	

The asterisks (*) indicated significant differences ($p < 0.05$) between FW and SW group.
Data are expressed as mean ± SEM.

its analogs, or cardiac glycosides and their putative physiological counterparts. The simplest and most straightforward determinants of NKA activity are the concentrations of substrates (Therien and Blostein, 2000). Previous in vitro studies reported that gill NKA activity in euryhaline teleosts was affected by different concentrations of Na^+ or K^+ in media (i.e., Japanese eel: Kamiya and Utida, '68; Ho and Chan, '80; Chinook salmon: Johnson et al., '77; European sea bass: Trigari et al., '85; Jensen et al., '98; Mozambique tilapia: Hwang et al., '88; rainbow trout: Pagliarani et al., '91). Normally NKA works below its maximal capacity (Vmax) (Rossier et al., '87). Similar to previous data of other species, SW- or FW-acclimated tilapia, puffer, and milkfish studied here displayed elevated NKA activity with higher sodium or potassium concentrations in reaction media (Figs. 1–3).

Although NKA activities among SW- or FW-tilapia, puffer, and milkfish were elevated with increasing salt concentrations in the reaction media, disparity between the two habitats lies in the activation kinetics by Na^+ and K^+ (Table 2). The Km values for potassium in gill NKA of SW-acclimated tilapia and puffer were significantly lower than for FW groups, while the Km values for sodium in gill NKA of SW-acclimated milkfish were significantly higher than for FW individuals (Table 2). Thus, gill NKA had a high affinity for K^+ in SW-acclimated tilapia and puffer but high affinity for Na^+ in FW-acclimated milkfish. NKA is a P-type ATPase in which each cycle of work includes (i) binding of 3 Na^+, (ii) hydrolysis of ATP, (iii) shipping the Na^+ out of the cell, and (iv) carrying 2 K^+ into the cell (Lingrel and Kuntzweiler, '94). Every NKA cycle may create membrane potential or a chemical gradient necessary for transporting different ions (Marshall, 2002; Hirose et al., 2003). Improvement of the efficiency at any step of the NKA work-cycle, e.g., higher affinities to Na^+ or K^+, will lead to increased enzyme effectiveness. SW acclimation induced higher efficiency (higher K^+ affinity; Table 2) and greater protein abundance of gill NKA in FW tilapia and puffer (Lee et al., 2003; Lin et al., 2004b) to meet the physiological demands for survival in hypertonic environments. On the contrary, FW acclimation stimulated higher efficiency (higher Na^+ affinity; Table 2) and more protein abundance of gill NKA in SW-dwelling milkfish (Lin et al., 2003) to compensate for the hypotonic milieu.

NKA is reported to consist of an $(\alpha\beta)_2$ protein complex with four α (α1–4) and three β (β1–3) isoforms as well as a small γ subunit (reviewed by Scheiner-Bobis, 2002). High levels of homology prevail among all the α-subunit sequences identified in animal species ranging from *Drosophila*, *Artemia* to vertebrates (Vasilets and Schwarz, '93), including teleosts (Schönrock et al., '91; Culter et al., '95; Seidelin et al., 2001; Feng et al., 2002; Semple et al., 2002). Moreover, different isoforms of the α-subunit were found in gills of anadromous eel (*Anguilla anguilla*; Cutler et al., '95) and salmon (*Salmo salar*; D'Cotta et al., '96; Seidelin et al., 2001), as well as euryhaline killifish (*Fundulus heteroclitus*; Semple et al., 2002) and tilapia (*Oreochromis mossambicus*; Hwang et al., '98; Feng et al., 2002). Isoforms of the β-subunit are also reported in gills of eel (*Anguilla anguilla*; Cutler et al., '95, 2000) and salmon (*Salmo salar*; Seidelin et al., 2001). Responsible for catalytic and transport work of NKA, α-subunit isoforms revealed distinct differences in their affinities to Na^+ and K^+ in rat brain and kidney (Urayam and Nakao, '79), and in brine shrimp (Cortas et al., '89). From the adipocytes of rat, Lytton ('85) found the α1-isozyme has a Km for Na^+ that is threefold lower than that of α2-isozyme. Other studies have

shown that the central isoform-specific region of α-subunit was involved in PKC regulation and resulted in different potassium sensitivity of the enzyme (Lingrel and Kuntzweiler, '94; Mercer, '93; Pierre et al., 2002). On the other hand, the NKA β-subunit maintained basis of normal enzyme activity and regulated affinity of NKA for sodium and potassium ions (Blanco et al., '95; Chow and Fortel, '95; Eakle et al., '94, '95; Jaisser, '92). In euryhaline teleosts, SW-adapted tilapia revealed different levels of increase of α1- and α3-mRNA (about 5 and 2 folds) as well as α1- and α3-protein in the gill (about 3 and 1 folds) from that of FW-adapted fish (Lee et al., '98; Feng et al., 2002). Expression of various levels of NKA isoforms may lead to different Na^+ or K^+ affinities and fulfill some of the requirements for altered enzyme behavior in gills of SW- or FW-acclimated euryhaline teleosts studied here.

In conclusion, different affinities of NKA for sodium and potassium may represent adaptive physiological mechanisms that operate to enhance the osmoregulatory capability in FW by euryhaline tilapia and puffer acclimated to SW, or in marine environments, for euryhaline milkfish acclimated to FW.

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LITERATURE CITED

Bagrinao, T. 1994. Systematics, distribution, genetics and life history of milkfish, *Chanos chanos*. Environ Biol Fish 39: 23–41.

Blanco G, Koster JC, Sanchez G, Mercer RW. 1995. Kinetic properties of the α2β1 and α2β2 isozymes of the Na, K-ATPase. Biochem 34:319–325.

Blanco G, Mercer RW. 1998. Isozymes of the Na, K-ATPase: heterogeneity in structure, diversity in function. Am J Physiol 275:F633–F650.

Chow DC, Forte JG. 1995. Functional significance of the β subunit for heterodimeric P-type ATPases. J Exp Biol 198:1–17.

Ciccotti E, Marino G, Pucci P, Cataldi E, Cataudella S. 1994. Acclimation trial of *Mugil cephalus* juveniles to freshwater: morphological and biochemical aspects. Environ Biol Fish 43:163–170.

Cortas N, Arnaout M, Salon J, Edelman IS. 1989. Isoforms of Na, K-ATPase in *Artemia salina*: II. Tissue distribution and kinetic characterization. J Membr Biol 108:187–195.

Culter CP, Sanders IL, Hazon N, Cramb G. 1995. Primary sequence, tissue specificity, and expression of Na, K-ATPase α1 subunit in the European eel (*Anguilla anguilla*). Comp Biochem Physiol 111B:567–573.

Culter CP, Brezillon S, Bekir S, Sanders IL, Hazon N, Cramb G. 2000. Expression of a duplicate Na, K-ATPase β_1-isoform in the European eel (*Anguilla anguilla*). Am J Physiol 279:R222–R229.

D'Cotta HC, Gallais C, Saulier B, Prunet P. 1996. Comparison between parr and smolt Atlantic salmon (*Salmo salar*) α subunit gene expression of Na^+, K^+- ATPase in gill tissue. Fish Physiol Biochem 15:29–39.

D'Cotta HC, Valotaire C, Gac FL, Prunet P. 2000. Synthesis of gill Na^+-K^+-ATPase in Atlantic salmon smolts: differences in α-mRNA and α-protein levels. Am J Physiol 278: R101–110.

Eakle KA, Kabalin MA, Wang SG, Farley RA. 1994. The influence of β subunit structure on the stability of Na^+/K^+-ATPase complexes and interaction with K^+. J Biol Chem 269:6550–6557.

Epstein FH, Katz AI, Pickford GE. 1967. Sodium-potassium-activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. Sci 156: 1245–1247.

Feng SH, Leu JH, Yang CH, Fang MJ, Huang CJ, Hwang PP. 2002. Gene expression of Na, K-ATPase α1 and α3 subunits in gills of the teleosts *Oreochromis mossambicus*, adapted to different environmental salinities. Mar Biotechnol 4: 379–391.

Helfman GS, Collette BB, Facey DE. 1997. The Diversity of Fishes. Malden, MA: Blackwell Science.

Hirose S, Kaneko T, Naito N, Takei Y. 2003. Molecular Biology of major components of chloride cells. Comp Biochem Physiol Pt. B 136:593–620.

Ho SM, Chan DKO. 1980. Branchial ATPase and ionic transport in the eel, *Anguilla japonica* I. Na^+, K^+-ATPase. Comp Biochem Physiol 66B:255–260.

Hwang PP, Sun CM, Wu SM. 1988. Characterization of gill Na^+, K^+-activated adenosine triphosphatase from tilapia *Oreochromis mossambicus*. Bull Inst Zool Academia Sinica 27:49–56.

Hwang PP, Sun CM, Wu SM. 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia *Oreochromis mossambicus* during seawater acclimation. Mar Biol 100: 295–299.

Hwang PP, Fang MJ, Tsai JC, Huang CJ, Chen ST. 1998. Expression of mRNA and protein of Na,K-ATPase α subunit in gills of tilapia (*Oreochromis mossambicus*). Fish Physiol Biochem 18:363–373.

Ip YK, Lee CY, Chew SF, Low WP, Peng KW. 1993. Differences in the responses of two mudskippers to terrestrial exposure. Zool Sci 10:511–519.

Jaisser F, Horisberger JD, Rossier BC. 1992. The β subunit modulates potassium activation of the Na-K pump. Ann NY Acad Sci 671:113–119.

Jensen MK, Madsen SS, Kristiansen K.1998. Osmoregulation and salinity effects on the expression and activity of Na^+,K^+-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). J Exp Zool 282:290–300.

Johnson SL, Ewing RD, Lichatowich JA. 1977. Characterization of gill (Na^+/K^+)-activated adenosine triphosphatase from Chinook salmon, *Oncorhynchus tshawytscha*. J Exp Zool 199:345–354.

Kamiya M, Utida S. 1968. Changes in activity of sodium-potassium-activated adenosine triphosphatase in gills during adaptation of the Japanese eel to sea water. Comp Biochem Physiol 26:675–685.

Kelly SP, Chow INK, Woo NYS.1999. Haloplasticity of black seabream (*Mylio macrocephalus*): hypersaline to freshwater acclimation. J Exp Zool 283:226–241.

Lee TH, Tsai JC, Fang MJ, Yu MJ, Hwang PP. 1998. Isoform expression of Na^+-K^+-ATPase α subunit in gills of the teleost, *Oreochromis mossamibcus*. Am J Physiol 275: R926–R932.

Lee TH, Hwang PP, Shieh YE, Lin CH. 2000. The relationship between 'deep-hole' mitochondria-rich cells and salinity adaptation in the euryhaline teleost, *Oreochromis mossambicus*. Fish Physiol Biochem 23:133–140.

Lee TH, Feng SH, Lin CH, Hwang YH, Huang CL, Hwang PP. 2003. Ambient salinity modulates the expression of sodium pumps in branchial mitochondria-rich cells of Mozambique tilapia, *Oreochromis mossambicus*. Zool Sci 20:29–36.

Lin YM, Chen CN, Lee TH. 2003. The expression of gill Na, K-ATPase in milkfish, *Chanos chanos*, acclimated to seawater, brackish water and fresh water. Comp Biochem Physiol Pt.A 135:489–497.

Lin CH, Hwang CL, Yang CH, Lee TH, Hwang PP. 2004a. Time course changes in the expression of Na, K-ATPase and morphometry of mitochondrion-rich cells in gills of euryhaline tilapia (*Oreochromis mossambicus*) during freshwater acclimation. J Exp Zool 301A:85–96.

Lin CH, Tsai RS, Lee TH. 2004b. Expression and distribution of Na, K-ATPase in gill and kidney of the green spotted pufferfish, *Tetraodon nigroviridis*, in response to salinity challenge. Comp Biochem Physiol Pt.A 138:287–295.

Lingrel JB, Kuntzweiler T. 1994. Na, K-ATPase. J Biol Chem 269:19659–19662.

Lytton. J, Lin JC, Guidotti G. 1985**.** Identification of two molecular forms of Na,K-ATPase in rat adipocytes. Relation to insulin stimulation of the enzyme. J Biol Chem 260: 1177–1184.

Marshall WS. 2002. Na^+, Cl^-, Ca^{2+} and Zn^{2+} transport by fish gills: retrospective review and prospective synthesis. J Exp Zool 293:264–283.

Mercer RW. 1993. Structure of the Na, K-ATPase. Inter Review Cytol 137C:139–168.

Morgan JD, Sakamoto T, Grau EG, Iwana GK. 1997. Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp Biochem Physiol 117A:391–398.

Pagliarani A, Ventrella V, Trombetti F, Trigari G, Borgatti AR. 1988. (Na^+/K^+)- and Na^+-stimulated Mg^{2+}-dependent ATPase activities in kidney of sea bass (*Dicentrarchus labrax* L.). Comp Biochem Physiol 90B:41–52.

Pagliarani A, Ventrella V, Ballestrazzi R, Trombetti F, Pirini M, Trigari G. 1991. Salinity- dependence of the properties of gill Na^+, K^+-ATPase in rainbow trout (*Oncorhynchus mykis*). Comp Biochem Physiol 100B:229–236.

Pierre SV, Duran MJ, Carr DL, Pressley TA. 2002. Structure/function analysis of Na^+-K^+- ATPase central isoform-specific region: involvement in protein kinase C regulation. Am J Physiol 283:F1066–F1074.

Perry SF, Shahsavarani A, Georgalis T, Bayaa M, Furimsky M, Thomas SLY. 2003. Channels, pumps, and exchangers in the gill and kidney of freshwater fishes: their role in ionic and acid-base regulation. J Exp Zool 300A:53–62.

Peterson GL.1978. A simplified method for analysis of inorganic phosphate in the presence of interfering substances. Anal Biochem 84:164–172.

Rainboth WJ. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome, p. 265.

Rossier BC, Geering K, Kraehenbuhl JP. 1987. Regulation of the sodium pump: How and why? Trends Biomed Sci 12:483–487.

Schönrock C, Morley SD, Okawara Y, Lederis K, Richter D. 1991. Sodium and potassium ATPase of the teleosts fish, *Catostomus commersoni*. Sequence, protein structure, and evolutionary conservation of the α-subunit. Biol Chem Hopper-Seyler 372:279–286.

Scheiner-Bobis G. 2002. The sodium pump. Eur J Biochem 269:2424–2433.

Seidelin M, Madsen SS, Cutler CP, Cramb G. 2001. Expression of gill vacuolar-type H^+-ATPase B subunit, and Na, K-ATPase α1 and β1 subunit messenger RNAs in smolting *Salmo salar*. Zool Sci 18:315–324.

Semple JW, Howard JG, Schulte PM. 2002. Molecular cloning and characterization of two Na^+, K^+-ATPase isoforms in *Fundulus heteroclitus*. Mar Biotechnol 4:512–519.

Stagg RM, Shuttleworth TJ.1982. The effect of copper on ionic regulation by the gills of the seawater-adapted flounder (*Platichthys flesus* L.) J Comp Physiol 149:83–90.

Stickney RR. 1986. Tilapia tolerance of saline waters: A review. Prog Fish-Cult 48:161–167.

Therien AG, Blostein R. 2000. Mechanism of sodium pump regulation. Am J Physiol 279:C541–C566.

Thomson AJ, Sargent JR. 1977. Changes in the levels of chloride cells and Na^+/K^+ dependent ATPase in the gills of yellow and silver eels adapting to sea water. J Exp Zool 200:33–40.

Trigari G, Borgatti AR, Pagliarani A, Ventrella V.1985. Characterization of gill (Na^+/K^+)- ATPase in the sea bass (*Dicentrarchus labrax* L.). Comp Biochem Physiol 80B: 23–33.

Uchida K, Kaneko T, Miyazaki H, Hasegawa S, Hirano T. 2000. Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool Sci 17:149–160.

Urayama O, Nakao M. 1979. Organ specificity of rat sodium- and potassium-activated adenosine triphosphatase. J Biochem (Tokyo) 86:1371–1381.

Vasilets LA, Schwarz W. 1993. Structure-function relationships of cation binding in the Na,K ATPase. Biochim Biophys. Acta 1154:201–222.

Wilson JM, Laurent P. 2002. Fish gill morphology: inside out. J Exp Zool 293:192–213.