Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 144 (2006) 451–457
CBP
www.elsevier.com/locate/cbpa

Decoupling the Na^+–K^+–ATPase in vivo: A possible new role in the gills of freshwater fishes

D. Lingwood [a,*], G. Harauz [b], J.S. Ballantyne [a]

[a] Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1
[b] Department of Molecular and Cellular Biology, University of Guelph, 50 Stone Road East Guelph, Ontario, Canada N1G 2W1

Received 5 October 2005; received in revised form 28 March 2006; accepted 28 March 2006
Available online 24 May 2006

Abstract

The literature suggests that when Na^+–K^+–ATPase has reduced access to its glycosphingolipid cofactor sulfogalactosyl ceramide (SGC), it is converted to a Na^+ uniporter. We recently showed that such segregation can occur within a single membrane when Na^+–K^+–ATPase is excluded from membrane microdomains or 'lipid rafts' enriched in SGC (D. Lingwood, G. Harauz, J.S. Ballantyne, J. Biol. Chem. 280, 36545–36550). Specifically we demonstrated that Na^+–K^+–ATPase localizes to SGC-enriched rafts in the gill basolateral membrane (BLM) of rainbow trout exposed to seawater (SW) but not freshwater (FW). We therefore proposed that since the freshwater gill Na^+–K^+–ATPase was separated from BLM SGC it should also transport Na^+ only, suggesting a new role for the pump in this epithelium. In this paper we discuss the biochemical evidence for SGC-based modulation of transport stoichiometry and highlight how a unique asparagine–lysine substitution in the FW pump isoform and FW gill transport energetics gear the Na^+–K^+–ATPase to perform Na^+ uniport.

Keywords: Sulfatide; Raft; Na^+–K^+–ATPase; Na^+–ATPase; Gill membrane; Asparagine–lysine substitution

1. Introduction

Na^+–K^+–ATPase (EC 3.6.1.37) is a membrane bound enzyme that uses the energy from the hydrolysis of one molecule of ATP to transport two K^+ into and three Na^+ out of most animal cells. As with all lipoprotein enzymes, a membrane environment is necessary for this activity (Roelofsen and Deenen, 1973). Na^+–K^+–ATPase can, however, exhibit specificity in its lipid requirement, in particular for the glycosphingolipid sulfogalactosyl ceramide (SGC). Karlsson (1977, 1982) proposed that SGC is a cofactor for Na^+–K^+–ATPase-catalyzed K^+ translocation. His cofactor model, which has subsequently been supported by extensive biochemical evidence (Gonzalez et al., 1979; Zambrano et al., 1981; Gonzalez and Zambrano, 1983; Jedlicki and Zambrano, 1985), stated that SGC functions to donate a K^+ (SGC exhibits a $K^+ > Na^+$ selective charge interaction via its sulfate group (Abramson et al., 1967)) to the enzyme gate site (i.e., SGC is responsible for K^+ import). Furthermore, Karlsson (1982) proposed that when SGC and Na^+–K^+–ATPase are localized to different cellular compartments, the pump performs Na^+uniport without reciprocal K^+ transport. This was based on the observation that in Na^+–K^+–ATPase preparations where SGC is absent there is Na^+ efflux with no K^+ influx (Goldin and Tong, 1974).

In a recent study we showed that this enzyme and cofactor can be functionally segregated within a single membrane: Na^+–K^+–ATPase localizes to SGC-enriched microdomains or 'lipid rafts' (see Lai (2003) for a recent lipid raft review) in the gill basolateral membrane (BLM) of rainbow trout exposed to seawater (SW) (electrolyte secreting gill epithelia) but not freshwater (FW) (electrolyte absorbing epithelia) (Lingwood et al., 2005). Additionally, we found that arylsulfatase-induced desulfation of BLM SGC reduced Na^+–K^+–ATPase activity in SW but not FW trout; suggesting that partitioning between SGC-enriched rafts results in a functional difference with respect to enzyme catalysis. We proposed that the raft-mediated, co-localization of enzyme and cofactor of the SW gill was

* Corresponding author. Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany.
E-mail address: lingwood@mpi-cbg.de (D. Lingwood).

1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.03.016

adaptive in the sense that it helped facilitate the up-regulation of Na^+–K^+–ATPase activity necessary for trout seawater adaptation. We also suggested that the raft-mediated, segregation of enzyme and cofactor in the FW gill, decoupled the Na^+–K^+–ATPase to a Na^+–ATPase, defining a new role for this pump in this tissue. This paper is an illustration of how SGC/membrane raft-based modulation of Na^+–K^+–ATPase transport stoichiometry can work in concert with the general thermodynamic and physiological gill transport factors and specific adaptations in pump amino acid residues to facilitate transformation to a Na^+–ATPase in FW.

2. Decoupling the Na^+–K^+–ATPase

2.1. Modulation of transport stoichiometry by SGC

Through the manipulation of ionic medium Na^+–K^+–ATPase can be induced to perform Na^+ uniport without reciprocal K^+ transport:

1. Uncoupled Na^+ efflux: in the absence of extracellular Na^+ and K^+ the Na^+–K^+–ATPase catalyzes an ouabain-sensitive ATP supported Na^+extrusion (Garrahan and Glynn, 1967a,b; Glynn et al., 1974; Karlish and Glynn, 1974; Glynn and Karlish, 1975; Lew et al., 1973; Glynn, 1988); the widely accepted Albers–Post scheme (Fahn et al., 1966; Post et al., 1969) predicts a transport stoichiometry of $3Na^+_{cyt}$/1ATP hydrolyzed (no compensating ions are returned) which has been measured in shark rectal gland (Cornelius, 1989).
2. Na^+/K^+-congener exchange: in the absence of extracellular K^+ the Na^+–K^+–ATPase catalyzes an ouabain-sensitive ATP supported Na^+/Na^+ exchange in which Na^+ substitutes for extracellular K^+ (Lee and Blostein, 1980; Blostein, 1983); transport stoichiometry is $3Na^+_{cyt}$: $2Na^+_{ext}$/ 1 ATP hydrolyzed (Cornelius and Skou, 1987; Yoda and Yoda, 1987).

Although such decoupling events have not been thought to occur in vivo (De Weer et al., 1988), we propose that differential partitioning of Na^+–K^+–ATPase to SGC membrane subdomains or lipid rafts represents a novel physiological mechanism for similar departures from $3Na^+_{cyt}$:$2K^+_{ext}$ transport stoichiometry. Several earlier studies are consistent with this concept. An ouabain-sensitive Na^+ efflux rate equivalent to that obtained in a medium lacking K^+ is produced from erythrocytes treated with arylsulfatase (Zambrano et al., 1981); native ouabain-sensitive Na^+ efflux is only restored by SGC repletion and not K^+ addition. Additionally, arylsulfatase treatment of microsomes from pig kidney medulla inhibits both ouabain-sensitive, K^+-sensitive activity (Gonzalez and Zambrano, 1983) and K^+ inducible dephosphorylation of the Na^+–K^+–ATPase phospho-intermediate (Jedlicki and Zambrano, 1985); restoration in both cases only occurs following SGC repletion and not K^+ addition. These data indicate that when Na^+–K^+–ATPase has limited access to SGC, it transports Na^+ only. Since arylsulfatase treatment mimics the transport dynamics of systems lacking extracellular K^+, we contend that non-SGC-enriched raft Na^+–K^+–ATPase also pumps Na^+ only. The resultant transport stoichiometry is not clear and ADP stimulated Na^+/Na^+ exchange (Garrahan and Glynn, 1967a,b; De Weer, 1970; Glynn and Hoffman, 1971; Cavieres and Glynn, 1979; Kennedy et al., 1986) (in absence of K^+ the enzyme can catalyze a one for one Na^+ exchange; it is electroneutral and net ATP hydrolysis is zero) cannot be ruled out. Nevertheless, we predict that the $3Na^+_{cyt}$:$2K^+_{ext}$ coupling ratio is predominantly conserved by SGC/raft associated enzyme.

2.2. Na^+–K^+–ATPase vs Na^+–ATPase

K^+ independent, ouabain insensitive Na^+–ATPase activity has been detected in a number of different tissues of different organisms (Proverbio et al., 1991). These systems have been generally distinguished from Na^+–K^+–ATPase by preferential inhibition by ethacrynic acid and furosemide (Proverbio et al., 1989). However, ethacyrnic acid is not an ideal inhibitor because it penetrates the cell and progressively inhibits other cell functions (Epstein, 1972a,b; Gaudemer and Foucher, 1967; Gordon, 1968). Furthermore, specificity according to furosemide sensitivity should be assessed with caution as furosemide is only a general inhibitor of Cl^-/cation co-transport (Engström et al., 1991). Na^+–ATPase and Na^+–K^+–ATPase do exhibit different responses to adenosine (Caruso-Neves et al., 1997), angiotensin II (Rangel et al., 1999) and bradykinin (Caruso-Neves et al., 1999). However, Na^+–K^+–ATPase isozymes also possess considerably different kinetic properties and modes of regulation (Mobasheri et al., 2000). Moreover, differentiation according to glycoside sensitivity is questionable since Na^+–K^+–ATPase isozymes differ in ouabain sensitivity (Blanco and Mercer, 1998). Finally, K^+ independence as a criterion for defining Na^+–ATPase is suspect since in the absence of K^+, the Na^+–K^+–ATPase will perform ATP-consuming Na^+ transport (see Na^+/K^+-congener exchange described above). The question of whether the functionally different Na^+–ATPase and Na^+–K^+–ATPase possess distinct structures or whether they represent two different isoform expressions of the same enzyme remains unanswered (Ventrella et al., 2001).

2.3. The solvent capacity of water

Compared with Na^+, K^+ is relatively large and therefore has a lower charge density. This means that Na^+ packs more densely with water than does K^+ (Hochachka and Somero, 1984). Several workers (Hazelwood, 1979; Ling, 1979; Wiggins, 1971, 1979) have proposed that as intracellular water is highly structured due to the presence of proteins, membranes, nucleic acids and thousands of organic metabolites, the different demands of K^+ and Na^+ on the solvent capacity of water can govern the ease with which these two ions enter the cell. For an inorganic cation to be accommodated in such a structured solvent, it requires a relatively low demand on solvent capacity. Selective accumulation of K^+ instead of Na^+ may, therefore, be a physical–chemical consequence of incorporating ions into already densely packed solutions. Ling (1979) suggested that specific ATPase ion pumps may not even be needed to maintain high intracellular K^+ and low Na^+. We are not advocating this extreme view of ion transport, nevertheless the different

influences of K^+ and Na^+ on water structure strengthen the notion of Na^+–K^+–ATPase- catalyzed Na^+ uniport without reciprocal transport in vivo.

3. Decoupling the pump in the freshwater gill

3.1. SW and FW Na^+–K^+–ATPase isoforms have different transport stoichiometric capacities

Richards et al. (2003) discovered that two gill Na^+–K^+–ATPase catalytic (α) subunit isoforms are differentially expressed during rainbow trout salinity transfer. We suggested that in contrast to the FW isoform (α1a), the isoform upregulated in SW (α1b) had an ability to partition into SGC-enriched membrane microdomains (Lingwood et al., 2005). There are also differences in the sequences of these isoforms that contribute to a resultant decoupled Na^+–K^+–ATPase in FW.

The efficacy of ion-binding is a function of the amino acids residues that make up a transporter's binding site. With respect to Na^+–K^+–ATPase, the residues Asn^{324} and Asn^{776} on the α-subunit have been found to act as cation coordination centres (Jorgensen and Pedersen, 2001). Specifically, ion occlusion studies on modified α-subunit from the kidney of *Sus scrofa* show that single substitution of Asn^{776} to Lys completely eliminates high affinity binding of Tl^+ (a K^+ analogue); replacement of Asn^{324} to Lys also causes a large depression in high affinity Tl^+ occlusion. Substitution of both residues for Lys, however, increases Tl^+ binding to one Tl^+ ion occluded per ouabain binding site. Energy analysis shows that the single substitution of Asn^{324} or Asn^{776} to Lys reduces the free energy of binding with 5.7 or 15.8 kJ/mol, respectively, while the interaction between the two Lys residues after double Lys substitution favours Tl^+ binding by −18.3 kJ/mol (Jorgensen and Pedersen, 2001).

Such a double Lys substitution has been found to occur in an α-subunit isoform expressed in the chloride cells of the salt gland of the brine shrimp *Artemia salina* (Baxter-Lowe et al., 1989; Escalante et al., 1995; Fig. 1); additionally, this isoform is up-regulated upon increasing salt exposure (Jorgensen and Domenech, 2005). Jorgensen and Pedersen (2001) suggested that since the electrochemical work required to transport Na^+ from hemolymph to the brine shrimp's high salt environment (200 to 300 ppt) is greater than the free energy if ATP hydrolysis, the double Lys substitutions facilitated a compensatory alteration in Na^+–K^+–ATPase transport stoichiometry. Specifically the pump (activity is 10–20% with double Lys substitution) would transport one K^+ and two Na^+ per ATP hydrolyzed. Upon examination of the rainbow trout FW (α1a) and SW (α1b) isoforms it becomes apparent that in FW there is a similar Asn to Lys substitution, but only at Asn^{776} (Fig. 1); the SW form exhibits no Lys substitutions. The replacement therefore differs from that of the shrimp in that it is in the direction of FW adaptation and is only a single substitution. Given the fact that Lys replacement at Asn^{776} eliminates K^+ binding (interestingly much more than a single substitution at Asn^{324}, see above), and that in this case there is no recovery of K^+ occlusion by an additional Lys substitution at Asn^{324}, we can propose that the FW isoform should transport Na^+ only. The nature of that stoichiometry, given the costs of Na^+ across gill epithelium will be discussed later.

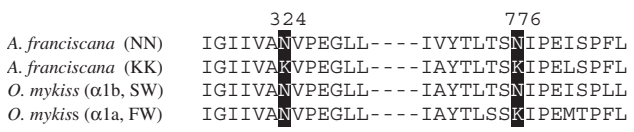

Fig. 1. Asn–Lys substitutions in Na^+–K^+–ATPase catalytic subunit isoforms from *Artema franciscana*, and *Oncorhynchus mykiss* (exposed to seawater (SW) or freshwater (FW)). Brine shrimp exhibit an isoform where Asn is doubly replaced by Lys at Asn^{324} and Asn^{776} upon increasing salinity exposure (KK) (Jorgensen and Domenech, 2005). With respect to the trout isoforms that are differentially expressed according to salinity exposure (Richards et al. (2003)), there is a Lys substitution but only at Asn^{776} of the FW isoform (α1a). Sequences were obtained from the SwissProt database.

3.2. Physiological considerations

In order to maintain ionic homeostasis, the fish gill absorbs salt in freshwater. Although the mechanism is controversial, gill chloride cells (also termed mitochondria rich cells) house much of the machinery thought to perform this function (Perry, 1997). In the current model of salt uptake (Fig. 2A; reviewed by Marshall, 2002) an apical vacuolar type H^+–ATPase generates an electrochemical gradient favourable for the passive uptake of Na^+ through an epithelial Na^+ channel and acidifies the boundary layer. In the apical membrane, a Cl^-–HCO_3^- antiport, possibly driven by low HCO_3^- in the boundary layer, drives Cl^- into the cell. Intracellular H^+ and HCO_3^- are thought to be derived from the hydration of CO_2. Cl^- probably exits the cell via basolateral Cl^- channels, while Na^+ uptake is completed by the Na^+–K^+–ATPase.

Although freshwater chloride cells exhibit lower Na^+–K^+–ATPase levels than their seawater counterparts (Sakamoto et al., 2001), these levels are far greater than those possessed by their surrounding non-chloride gill cell types (Perry, 1997). Current models of salt uptake do not require Na^+–K^+–ATPase activity beyond a generic housekeeping level. The reason for heightened Na^+–K^+–ATPase in FW chloride cells therefore remains unclear (Perry, 1997; Evans et al., 2005).

Na^+–ATPase is generally found in higher levels in the basal membranes of electrolyte-adsorbing epithelia (Proverbio et al., 1991). It is suggested to produce a vectorial flux of Na^+ into the interstitium that is thought to facilitate salt absorption in kidney, small intestine and gill epithelium (Proverbio et al., 1991). If non-SGC/raft associated Na^+–K^+–ATPase acting as a Na^+–ATPase performs a similar function, it explains why no Na^+–K^+–ATPase was raft associated in the FW trout (Lingwood et al., 2005); rainbow trout gill Na^+–ATPase activity is higher in fish acclimated to FW than those adapted to SW (Ventrella et al., 1992). This addresses the unclear role of Na^+–K^+–ATPase in salt uptake by the gill epithelium of freshwater fishes. Based on their existence in SW adapted gill epithelia (Evans et al., 2005), current models of salt uptake in freshwater fish gill assume putative, unidentified basolateral K^+ channels to account for K^+ recycling (Fig. 2A). Passive K^+

leak constitutes an enormous energetic loss for absorptive epithelia (Karlsson, 1982). Under our scheme, the majority of Na^+–K^+–ATPase pumps Na^+ only (Fig. 2B). Therefore, K^+ leak at a lower level that matches housekeeping levels of Na^+–K^+–ATPase catalyzed K^+ influx could be assumed. This does not necessarily predict that intracellular K^+ is lower in FW branchial epithelium, but rather, is recycled at a lower rate.

Transforming Na^+–K^+–ATPase to a Na^+–ATPase may also help facilitate Cl-uptake in the FW gill. Experimental evidence indicates that both uncoupled Na^+ efflux and Na^+/K^+-congener exchange are electrogenic (Cornelius and Skou, 1987; Yoda and Yoda, 1987; Cornelius, 1989). However, uncoupled Na^+ efflux from red blood cell ghosts is accompanied by an ouabain-sensitive efflux of the anions present in solution (SO_4^{2-}) (Cornelius, 1991). Additionally, an electroneutral uncoupled Na^+ efflux has been observed in proteoliposomes at pH 7.0 (Goldshleger et al., 1990); the authors suggest co-transport of Cl^- or counter-transport of H^+ in order to explain this. Finally, in Na^+–K^+–ATPase preparations where SGC is absent

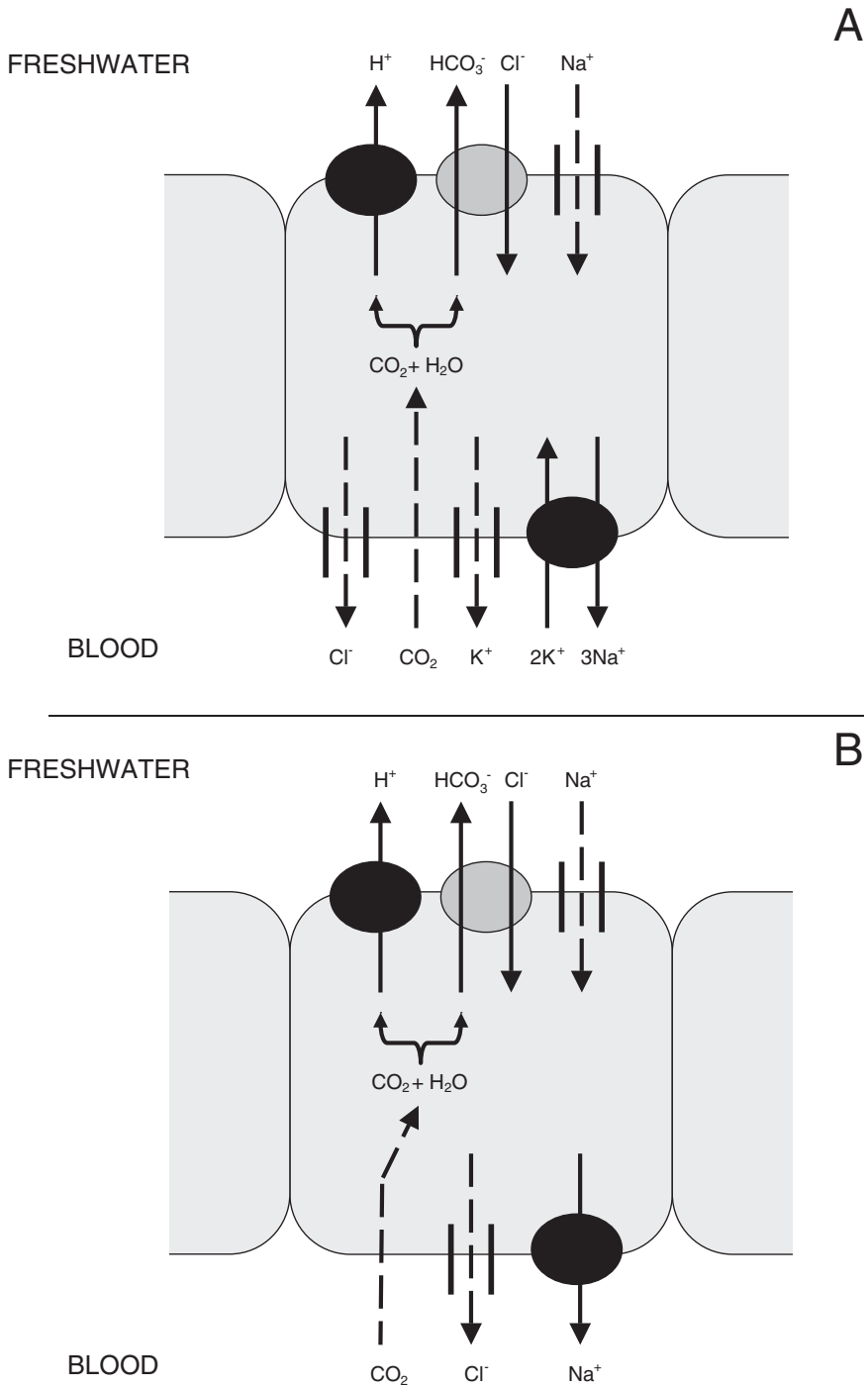

Fig. 2. Current model for freshwater gill chloride cell salt uptake (A) (Redrawn from Marshall, 2002) and proposed model with decoupled Na^+–K^+–ATPase (B).

Table 1
Energetic cost of Na^+ transport across the gills of FW and SW adapted fish

	$[Na^+]_1$	$[Na^+]_2$	$\Delta\psi$	Energy cost per Na^+	Meaning
FW					
Apical membrane ($AM_{FW\ Na^+}$)	$[Na^+]_{cell}$=66 mM (a)	$[Na^+]_{freshwater}$=1 mM (b)	−0.047 V (cell side negative) (b)	5.3 kJ/mol	Freshwater→cell active step (Na^+ in coupled to apical H^+–ATPase)
BLM ($BLM_{FW\ Na^+}$)	$[Na^+]_{blood}$=150 mM (b)	$[Na^+]_{cell}$=66 mM (a)	−0.042 V (cell side negative)	5.8 kJ/mol	Cell→blood active step (Na^+ out by BLM Na^+–K^+–ATPase)
Epithelium ($EPITH_{FW\ Na^+}$)	$[Na^+]_{blood}$=150 mM (b)	$[Na^+]_{freshwater}$=1 mM (b)	−0.005 V (water reference) (b)	11.4 kJ/mol	Freshwater→blood ~ $AM_{FW\ Na^+}$+$BLM_{FW\ Na^+}$)
SW					
Apical membrane ($AM_{SW\ Na^+}$)	N/A	N/A	N/A	N/A	No movement of Na^+ across AM
BLM ($BLM_{SW\ Na^+}$)	$[Na^+]_{blood}$=170 mM (b)	$[Na^+]_{cell}$=37 mM (b)	−0.08 V (cell side negative)	11.2 kJ/mol	Cell→blood active step (Na^+ out by BLM Na^+–K^+–ATPase)
Epithelium ($EPITH_{SW\ Na^+}$)	$[Na+]_{saltwater}$=450 mM (b)	$[Na^+]_{blood}$=170 mM (b)	−0.025 V (water reference) (b)	−0.1 kJ/mol	Blood→saltwater passive para cellular Na^+ exit

Literature values used to calculate cost of Na^+ transport according to $\Delta G = RT\ln([Na^+]_1/[Na^+]_2)+zF\Delta\psi$ where R=8.31J/°K/mol, T=283°K, z=+1, F=96.5 kJ/mol, $\Delta\psi$=electrical difference across barrier BLM_{FW} $\Delta\psi$ calculated from difference between the $\Delta\psi$ of $AM_{FW\ Na^+}$ and $\Delta\psi$ of $EPITH_{FW\ Na^+}$$BLM_{SW}$ $\Delta\psi$ calculated from the Goldman Equation $\{\Delta\psi=(RT/F)\ln(([K^+]_{in}+0.01[Na^+]_{in})/([K^+]_{out}+0.01[Na^+]_{out}))\}$ a = values obtained from Morgan et al. (1994); b = values obtained from Wright (1991).

(Karlsson, 1977), Na^+ translocation (with no reciprocal K^+ transport) is accompanied by a co-efflux of Cl^-, presumably by a Cl^- transporter (Goldin and Tong, 1974). Current models of salt uptake by the FW gill predict that Cl^- absorption into the blood is completed by a Cl^- channel in the gill basolateral membrane (Fig. 2A); a translocation that would therefore be favourable in the presence of decoupled Na^+ transport (Fig. 2B).

3.3. Decoupled Na^+ transport energetics

Decoupled Na^+ transport itself appears more thermodynamically plausible in the FW gill. Using available literature values (Wright, 1991; Morgan et al., 1994) we calculated the energy barriers for Na^+ transport across whole epithelium, apical membrane and BLM of FW acclimated (Na^+ absorbing) and SW adapted (Na^+ secreting) gill epithelia (Table 1). Assuming no junctional potentials these values are reliable because energy is conserved in FW ($EPITH_{FW\ Na^+}$ ~ $BLM_{FW\ Na^+}$$AM_{FW\ Na^+}$) and $EPITH_{Na^+}$ is less than zero in SW (a negative value is indicative of the passive paracellular Na^+ extrusion driven by the transepithelial potential associated with apically concentrated Cl^- (Evans et al., 2005)). BLM_{Na^+} represents the cost of Na^+ transport by the basolaterally restricted Na^+–K^+–ATPase in SW and FW, whereas the cost associated with $AM_{FW\ Na^+}$ is consistent with the energy consumption by the apical H^+–ATPase modeled for salt uptake in FW (Evans et al., 2005). Although total energetic costs to Na^+ movement appear similar in FW and SW ($EPITH_{FW\ Na^+}$ ~ $BLM_{FW\ Na^+}$+ $AM_{FW\ Na^+}$ ~ $BLM_{SW\ Na^+}$+ $EPITH_{SW\ Na^+}$ ~ 11 kJ/mol), the FW gill divides this energy into two active steps (Table 1) making it energetically cheaper to transport Na^+across its BLM ($BLM_{FW\ Na^+}$ < $BLM_{SW\ Na^+}$). Conservatively, if we assume Na^+–K^+–ATPase operates at <60% efficiency (Kirschner, 2004), the energy released by ATP hydrolysis in a cycle (~ −63 kJ (Civan et al., 1983)) could support a novel 6–7Na^+_{cyt}/1ATP stoichiometry (5× $BLM_{FW\ Na^+}$) in FW but only the traditional 3Na^+_{cyt}/1ATP stoichiometry in SW (3× $BLM_{SW\ Na^+}$). We would therefore contend that decoupling Na^+–K^+–ATPase to a Na^+–ATPase is more favourable in the Na^+ absorbing system.

If such decoupling is conserved across the animal kingdom, we would predict variability in the transport stoichiometry of other absorptive tissues: a 5Na^+_{cyt}:1K^+_{ext} antiport ratio has been measured in dog kidney medulla (Anner et al., 1977); a 3Na^+_{cyt}:1K^+_{ext} stoichiometry associated with greater Na^+ reabsorption has been observed in renal proximal tubules of Dahl salt-sensitive rats (Orosz and Hopfer, 1996); and the Na^+ to K^+ coupling ratio can exceed the value of 3Na^+_{cyt}:2K^+_{ext} during periods of enhanced pump activity in amphibian renal proximal tubules (Sackin and Boulpaep, 1983). The extent to which coupling plasticity is linked to Na^+–K^+–ATPase SGC/raft partitioning and/or adaptations in amino acid signature warrants future investigation.

4. Future directions

To summarize, we have proposed a thermodynamically and physiologically viable mechanism whereby Na^+–K^+–ATPase may be transformed to a Na^+–ATPase via its separation from SGC due to partitioning into different membrane subdomains. This concept is of wide interest since it relates to the functioning of the main ion transporter in most cells with applications in understanding the regulation of all epithelial electrolyte absorbing/transport processes; particularly those of the FW gill where it is complemented by adaptations in amino acid residues and gill transport energetics. The challenge now is to consistently account for altered Na^+–K^+–ATPase transport stoichiometry both as a function of cation binding residues and pump position relative to SGC microdomains within the membrane.

Acknowledgements

The authors thank Jason Bystriansky and Ben Speers-Roesch for the helpful discussion. This work was supported by a NSERC PGS scholarship to D. Lingwood, a NSERC operating grant to J.S. Ballantyne and an operating grant from the Multiple Sclerosis Society of Canada to G. Harauz.

References

Anner, B.M., Lane, L.K., Schwartz, A., Pitts, B.J.R., 1977. A reconstituted Na^++K^+ pump in liposomes containing purified (Na^++K^+)–ATPase from kidney medulla. Biochim. Biophys. Acta 467, 340–345.

Abramson, M.B., Katzman, R., Curci, R., Wilson, C.E., 1967. The reactions of sulfatide with metallic cations in aqueous systems. Biochemistry 6, 295–304.

Baxter-Lowe, L.A., Guo, J.Z., Bergstrom, E.E., Hokin, L.E., 1989. Molecular cloning of the Na,K–ATPase alpha-subunit in developing brine shrimp and sequence comparison with higher organisms. FEBS Lett. 257, 181–187.

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

Blostein, R., 1983. Sodium pump-catalyzed sodium–sodium exchange associated with ATP hydrolysis. J. Biol. Chem. 258, 7948–7953.

Caruso-Neves, C., Francisco-Pedro, L.G., Souza, L.P., Chagas, C., Lopes, A.G., 1997. Effect of adenosine on the ouabain-insensitive Na^+–ATPase activity from basolateral membrane of the proximal tubule. Biochim. Biophys. Acta 1329, 336–344.

Caruso-Neves, C., Siqueira, A.S.E., Iso-Cohen, G., Lopes, A.G., 1999. Bradykinin modulates the ouabain-insensitive Na^+–ATPase activity from the basolateral membrane of the proximal tubule. Biochim. Biophys. Acta 1431, 485–491.

Cavieres, J.D., Glynn, I.M., 1979. Sodium–sodium exchange through the sodium pump: the roles of ATP and ADP. J. Physiol. 297, 637–645.

Civan, M.M., Peterson-Yatorno, K., DiBona, D.R., Wilson, D.F., Erecinska, M., 1983. Bioenergetics of Na transport across frog skin, chemical and electrical measurements. Am. J. Physiol. 245, F691–F700.

Cornelius, F., 1989. Uncoupled Na^+-efflux on reconstituted shark Na, K–ATPase is electrogenic. Biochem. Biophys. Res. Commun. 160, 801–807.

Cornelius, F., 1991. Functional reconstitution of the sodium pump. Kinetics of exhange reactions performed by reconstituted Na/K–ATPase. Biochim. Biophys. Acta 1071, 19–66.

Cornelius, F., Skou, J.C., 1987. The sided action of Na^+ and of K^+ on reconstituted shark (Na^+ K^+)–ATPase engaged in Na^+–Na^+ exchange accompanied by ATP hydrolysis. I. The ATP activation curve. Biochim. Biophys. Acta 904, 353–364.

De Weer, P., 1970. Effects of intracellular adenosine-5′-diphosphate and orthophosphate on the sensitivity of sodium efflux from squid axon to external sodium and potassium. J. Gen. Physiol. 56, 583–620.

De Weer, P., Gadsby, D.C., Rakowski, R.F., 1988. Overview: stoichiometry and voltage dependence of the Na/K pump. In: Skou, J.C., Norby, J.G., Maunsbach, A.B., Esmann, M. (Eds.), Progress in Clinical and Biological Research, vol. 268A. Alan R. Liss Inc., New York, pp. 421–434.

Engström, K.G., Sandström, P.-E., Sehlin, J., 1991. Volume regulation in mouse pancreatic β-cells is mediated by a furosemide-sensitive mechanism. Biochim. Biophys. Acta 1091, 145–150.

Epstein, R.W., 1972a. The binding of ethacrynic acid to rabbit kidney cortex. Biochim. Biophys. Acta 274, 119–127.

Epstein, R.W., 1972b. The effects of ethacrynic acid on active transport of sugars and ions and on other metabolic processes in rabbit kidney cortex. Biochim. Biophys. Acta 274, 128–139.

Escalante, R., Garcia-Saez, A., Sastre, L., 1995. In situ hybridization analyses of Na, K–ATPase alpha-subunit expression during early larval development of Artemia franciscana. J. Histochem. Cytochem. 43, 391–399.

Evans, D.H., Piermarini, P.M., Choe, K., 2005. The multifunctional fish gill: dominant site of gas exchange, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.

Fahn, S., Koval, G.J., Albers, R.W., 1966. Sodium–potassium-activated adenosine triphosphatase of electrophorus electric organ. I. An associated sodium-activated transphosphorylation. J. Biol. Chem. 241, 1882–1889.

Garrahan, P.J., Glynn, I.M., 1967a. The behaviour of the sodium pump in red cells in the absence of external potassium. J. Physiol. 192, 159–174.

Garrahan, P.J., Glynn, I.M., 1967b. The sensitivity of the sodium pump to external sodium. J. Physiol. 192, 175–188.

Gaudemer, Y., Foucher, B., 1967. Influence of sodium ethacrynate on some reactions connected with the mechanism of oxidative phosphorylation. Biochim. Biophys. Acta 131, 255–264.

Glynn, I.M., 1988. The coupling of enzymatic steps to the translocation of sodium and potassium. In: Skou, J.C., Norby, J.G., Maunsbach, A.B., Esmann, M. (Eds.), Progress in Clinical and Biological Research, vol. 268A. Alan R. Liss Inc., New York, pp. 435–460.

Glynn, I.M., Hoffman, J.F., 1971. Nucleotide requirements for sodium–sodium exchange catalysed by the sodium pump in human red cells. J. Physiol. 218, 239–256.

Glynn, I.M., Karlish, S.J., 1975. ATP hydrolysis associated with an uncoupled efflux of Na through the Na pump. J. Physiol. 250, 33–34.

Glynn, I.M., Karlish, S.J., Cavieres, J.D., Ellory, J.C., Lew, V.L., Jorgensen, P.L., 1974. The effects of an antiserum to Na^+, K^+–ATPase on the ion-transporting and hydrolytic activities of the enzyme. Ann. N. Y. Acad. Sci. 242, 357–371.

Goldin, S.M., Tong, S.W., 1974. Reconstitution of active transport catalyzed by the purified sodium and potassium ion-stimulated adenosine triphosphatase from canine renal medulla. J. Biol. Chem. 249, 5907–5915.

Goldshleger, R., Shahak, Y., Karlish, S.J.D., 1990. Electrogenic and electro-neutral transport modes of renal Na/K ATPase reconstituted into proteoliposomes. J. Membr. Biol. 113, 139–154.

Gonzalez, E., Zambrano, F., 1983. Possible role of sulphatide in the K^+-activated phosphatase activity. Biochim. Biophys. Acta 728, 66–72.

Gonzalez, M., Morales, M., Zambrano, F., 1979. Sulfatide content and (Na^+K^+)–ATPase activity of skin and gill during larval development of the Chilean frog, *Calyptocephalella caudiverbera*. J. Membr. Biol. 21, 347–359.

Gordon, E.E., 1968. Site of ethacrynic acid action on Ehrlich ascites tumor cells. Biochem. Pharmacol. 17, 1237–1242.

Hazelwood, C.F., 1979. A view of the significance and understanding of the physical properties of cell-associated water. In: Drost-Hansen, W., Clegg, J. (Eds.), Cell-Associated Water. Academic Press, New York, pp. 165–259.

Hochachka, P.W., Somero, G.N., 1984. Biochemical Adaptation. Princeton University Press, Princeton, NJ.

Jedlicki, A., Zambrano, F., 1985. Role of sulfatide on phosphoenzyme formation and ouabain binding of the (Na^++K^+) ATPase. Arch. Biochem. Biophys. 238, 558–564.

Jorgensen, P.L., Pedersen, P.A., 2001. Structure function relationships of Na^+, K^+, ATP, or Mg^{2+} binding and energy transduction in Na, K–ATPase. Biochim. Biophys. Acta 1505, 57–74.

Jorgensen, P.L., Domenech, F.A., 2005. Salt induction of doubly lysine substituted α-subunit isoform of the Na–K pump of *Artemia franciscana*. Na,K–ATPase and Related Pumps: Structures, Mechanisms, and Diseases, 11th International ATPase Conference and 59th Annual Meeting and Symposium of the Society of General Physiologists. Woods Hole, MA, USA.

Karlish, S.J., Glynn, I.M., 1974. An uncoupled efflux of sodium ions from human red cells, probably associated with Na-dependent ATPase activity. Ann. N. Y. Acad. Sci. 242, 461–470.

Karlsson, K.-A., 1977. Aspects on structure and function of sphingolipids in cell surface membranes. In: Abrahamsson, S., Pascher, I. (Eds.), Structure of Biological Membranes. Plenum Press, New York, pp. 245–274.

Karlsson, K.-A., 1982. Glycosphingolipids and surface membranes. In: Chapman, D. (Ed.), Biological Membranes, vol. 4. Academic Press, London, pp. 1–74.

Kennedy, B.G., Lunn, G., Hoffman, J.F., 1986. Effects of altering the ATP/ADP ratio on pump-mediated Na/K and Na/Na exchanges in resealed human red blood cell ghosts. J. Gen. Physiol. 87, 47–72.

Kirschner, L.B., 2004. The mechanism of sodium chloride uptake in hyperregulating aquatic animals. J. Exp. Biol. 207, 1439–1451.

Lai, E., 2003. Lipid rafts make for slippery platforms. J. Cell. Biol. 162, 365–370.

Lee, K.H., Blostein, R., 1980. Red cell sodium fluxes catalysed by the sodium pump in the absence of K^+ and ADP. Nature 285, 338–339.

Lew, V.L., Hardy, M.A., Ellory, J.C., 1973. The uncoupled extrusion of Na^+ through the Na^+ pump. Biochim. Biophys. Acta. 323, 251–266.

Ling, G.N., 1979. The polarized multilayer theory of cell water according to the association–induction hypothesis. In: Drost-Hansen, W., Clegg, J. (Eds.), Cell-Associated Water. Academic Press, New York, pp. 261–269.

Lingwood, D., Harauz, G., Ballantyne, J.S., 2005. Regulation of fish gill Na^+–K^+–ATPase by selective sulfatide-enriched raft partitioning during seawater adaptation. J. Biol.Chem. 280, 36545–36550.

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

Mobasheri, A., Avila, J., Cozar-Castellano, I., Brownleader, M.D., Trevan, M., Francis, M.J.O., Lamb, J.F., Martin-Vasallo, P., 2000. Na^+–K^+–ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. Biosci. Rep. 20, 51–91.

Morgan, I.J., Potts, W.T.W., Oates, K., 1994. Intracellular ion concentrations in branchial epithelial cells of brown trout (*Salmo trutta* L.) determined by X-ray microanalysis. J. Exp. Biol. 194, 139–151.

Orosz, D.E., Hopfer, U., 1996. Pathphysiological consequences of changes in the coupling ratio of Na,K–ATPase for renal sodium reabsorption and its implications for hypertension. Hypertension 27, 219–227.

Perry, S.F., 1997. The chloride cell: structure and function in the gills of freshwater fishes. Annu. Rev. Physiol. 59, 325–347.

Post, R.L., Kume, S., Tobin, T., Orcutt, B., Sen, A.K., 1969. Flexibility of an active center in sodium-plus-potassium adenosine triphosphatase. J. Gen. Physiol. 54, 306–326.

Proverbio, F., Marin, R., Proverbio, T., 1989. The "second" sodium pump and cell volume. Curr. Membr. Transp. 34, 105–119.

Proverbio, F., Marin, R., Proverbio, T., 1991. The ouabain-insensitive sodium pump. Comp. Biochem. Physiol., A 99, 279–283.

Rangel, L.B.A., Caruso-Neves, C., Lara, L.S., Brazil, F.L., Lopes, A.G., 1999. Angiotensin II activates the ouabain-insensitive Na^+–ATPase from renal proximal tubules through a G-protein. Biochim. Biophys. Acta 1416, 309–319.

Richards, J.G., Semple, J.W., Bystriansky, J.S., Schulte, P.M., 2003. Na^+-K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J. Exp. Biol. 206, 4475–4486.

Roelofsen, B., Deenen, L.L.M., 1973. Lipid requirement of membrane bound ATPase. Eur. J. Biochem. 40, 245–257.

Sackin, H., Boulpaep, E.L., 1983. Rheogenic transport in the renal proximal tubule. J. Gen. Physiol. 82, 519–551.

Sakamoto, T., Uchida, K., Yokota, S., 2001. Regulation of the ion-transporting mitochondrion-rich cell during adaptation of teleost fishes to different salinities. Zool. Sci. 18, 1163–1174.

Ventrella, V., Trombetti, F., Pagliarani, A., Trigari, G., Pirini, M., Borgatti, A.R., 1992. Salinity dependence of the ouabain-insensitive Mg^{2+}-dependent Na^+–ATPase in gills of rainbow trout (*Oncorhynchus mykiss* Walbaum) adapted to fresh and brackish water. Comp. Biochem. Physiol., B 101, 1–7.

Ventrella, V., Pagliarani, A., Trombetti, F., Pirini, M., Trigari, G., Borgatti, A.R., 2001. Response of rainbow trout to gill Na^+-ATPase to T_3 and NaCl administration. Physiol. Biochem. Zool. 74, 694–702.

Wiggins, P.M., 1971. Water structure as a determinant of ion distribution in living tissue. J. Theor. Biol. 32, 131–146.

Wiggins, P.M., 1979. Metabolic control of the properties of intracellular water as a universal driving force for active transport. In: Drost-Hansen, W., Clegg, J. (Eds.), Cell-Associated Water. Academic Press, New York, pp. 69–114.

Wright, S.H., 1991. The interface of animal and aqueous environment: strategies and constraints on the maintenance of solute balance. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and molecular biology of fishes. Phylogenetic and biochemical perspectives. Elsevier, Amsterdam, pp. 165–180.

Yoda, A., Yoda, S., 1987. Two different phosphorylation–dephosphorylation cycles of Na,K–ATPase proteoliposomes accompanying Na^+ transport in the absence of K^+. J. Biol. Chem. 262, 110–115.

Zambrano, F., Morales, M., Fuentes, N., Rojas, M., 1981. Sulfatide role in the sodium pump. J. Membr. Biol. 63, 71–75.