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Comparative Biochemistry and Physiology, Part A 144 (2006) 451–457
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Decoupling the Na^+–K^+–ATPase in vivo: A possible new role in the gills of freshwater fishes

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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

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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