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Sulfatide and Na^+-K^+-ATPase: A Salinity-sensitive Relationship in the Gill Basolateral Membrane of Rainbow Trout

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Abstract. We investigated the effect of salinity on the relationship between Na^+-K^+-ATPase and sulfogalactosyl ceramide (SGC) in the basolateral membrane of rainbow trout (*Oncorhynchus mykiss*) gill epithelium. SGC has been implicated as a cofactor in Na^+-K^+-ATPase activity, especially in Na^+-K^+-ATPase rich tissues. However, whole-tissue studies have questioned this role in the fish gill. We re-examined SGC cofactor function from a gill basolateral membrane perspective. Nine SGC fatty acid species were quantified by tandem mass spectrometry (MS/MS) and related to Na^+-K^+-ATPase activity in trout acclimated to freshwater or brackish water (20 ppt). While Na^+-K^+-ATPase activity increased, the total concentration and relative proportion of SGC isoforms remained constant between salinities. However, we noted a negative correlation between SGC concentration and Na^+-K^+-ATPase activity in fish exposed to brackish water, whereas no correlation existed in fish acclimated to freshwater. Differential Na^+-K^+-ATPase/SGC sensitivity is discussed in relation to enzyme isoform switching, the SGC cofactor site model and saltwater adaptation.

Key words: Sulfatide — Na^+-K^+-ATPase — Basolateral membrane — Gill — Rainbow trout — Salinity

Introduction

Sulfatide or sulfogalactosyl ceramide (SGC), is an anionic glycosphingolipid (GSL) present in unusually high quantities in tissues known to contain high levels of Na^+-K^+-ATPase (EC 3.6.1.3). Examples include both osmoregulatory tissues such as avian salt gland (Karlsson, Samuelsson & Steen, 1969, 1973), bovine kidney medulla (Karlsson et al., 1973) dogfish rectal gland (Karlsson, Samuelsson & Steen, 1968, 1974a), and eel gill (Zwingelstein et al., 1980) and bioelectrical tissues such as electric organ of *Torpedo marmorata* (Hansson et al., 1979) and human brain grey matter (Karlsson et al., 1974b).

The high concentration of sulfatide in these Na^+-K^+-ATPase-rich tissues led Karlsson, Samuelsson, and Steen (1968, 1969, 1971, 1973, 1974a, 1974b) to hypothesize that SGC was connected to active Na^+ extrusion via a direct link to Na^+-K^+-ATPase. They showed within these tissues of differing origin and functional status the ratio of Na^+-K^+-ATPase activity to tissue SGC level was conserved (Karlsson et al., 1974b). This ratio was also confirmed in the plasma membranes of human erythrocytes (Hansson, Karlsson & Samuelsson, 1978). Furthermore, Karlsson et al. (1971) demonstrated that when domestic ducks were acclimated to hypertonic saline, both Na^+-K^+-ATPase activity and sulfatide content in the salt gland increased by 200%. Umeda, Egawa, and Nagai (1976) observed a similar relationship in mouse kidney during compensatory renal hypertrophy following hemilateral nephrectomy. Moreover, the accumulation and increased metabolism of SGC has been reported for Madin-Darby canine kidney (MDCK) cell lines cultured in hyperosmotic media (Niimura & Ishizuka, 1989, 1991). Zwingelstein et al. (1980) documented a 31% increase in SGC concentration in the gills of eels exposed to seawater as compared to freshwater control levels. However, these authors considered this level of elevation not significant since the SGC/phospholipid ratio

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remained constant between their freshwater and seawater treatment groups. Nevertheless, concomitant with the increase in Na^+-K^+-ATPase activity associated with seawater acclimation, a heightened rate of sulfate turnover into gill sulfatide was noted.

Karlsson (1977) formulated a cofactor-site model of Na^+-K^+ translocation, in which SGC functioned to donate a K^+ to the enzyme gate site. He postulated that during Na^+-K^+-ATPase activity, SGC was essential for K^+ influx but not for Na^+ efflux. The major evidence for the model was as follows: 1. SGC is located in the outer leaflet of the plasma membrane, which would facilitate the binding of K^+ to its galactose-3-sulfate group; 2. SGC has a higher affinity for K^+ than do other anionic lipids, such as acidic phospholipids (Abramson et al., 1967); 3. For those extraction procedures where SGC is removed, in addition to detergent treatment, a salt extraction step is required to purify Na^+-K^+-ATPase from canine renal medulla (Kyte, 1971) and dogfish salt gland (Ottolenghi, 1975), supporting the notion of a polar interaction between SGC and the enzyme; 4. In such Na^+-K^+-ATPase preparations (i.e., when SGC is absent) the pump appears electrogenic: there is Na^+ efflux with a lessened K^+ influx (Goldin & Tong, 1974). Karlsson's model of coupled function has been subsequently supported by the observation that Na^+-K^+-ATPase activity in microsomes prepared from pig kidney medulla is lost after arylsulfatase-induced SGC hydrolysis and then partially restored following sulfatide addition (Gonzalez & Zambrano, 1983; Jedlicki & Zambrano, 1985).

Direct sulfatide involvement in mammalian kidney Na^+-K^+-ATPase activity was questioned by Zalc et al. (1978) who demonstrated by immunohistochemistry that SGC was present only on the luminal membranes of rabbit renal cells, opposite to the basal site of the Na^+-K^+-ATPase. Based on the lack of a positive correlation between the SGC/phospholipid ratio and Na^+-K^+-ATPase activity upon seawater acclimation Zwingelstein et al. (1980) assumed this to also be the case for eels. However the results of Zalc et al. (1978) must be extrapolated with prudence as SGC has been shown to localize in the basal membrane of ciliary body epithelium of rat eyes (Feeney & Mixon, 1974; Bentley et al., 1976) and assumes a greater basolateral membrane (BLM) concentration in MDCK cells (ver der Bijl, Lopes-Cardozo & van Meer, 1996). It is therefore premature to assume that SGC is absent from gill-cell BLM. In reference to the results of Zwingelstein et al. (1980), a SGC/phospholipid ratio does not indicate SGC tissue concentration and therefore should not be discussed in place of a tissue concentration measurement. Also, Zwingelstein et al. (1980) used an azure A spectrophotometric assay (Kean, 1968) to quantify SGC from a sulfatide purification that contained 10% unknown sulfolipid. The azure A method cannot distinguish between SGC and other sulfolipids, therefore caution is warranted when interpreting their results. In any event, SGC cofactor functioning in gill epithelium Na^+-K^+-ATPase remains a matter of conjecture.

To re-evaluate the teleost model of a sulfatide cofactor function in the Na^+-K^+-ATPase we worked under the premise that SGC was located in gill BLM of *Oncorhynchus mykiss*. We hypothesized that if SGC was present in the BLM and was directly associated with Na^+-K^+-ATPase, its concentration should increase concomitantly with a rise in Na^+-K^+-ATPase activity in saltwater exposed fish. A gill BLM preparation was used to avoid the potential confounding effects of whole-tissue studies. In addition, any ambiguity in SGC measurements was eliminated by quantification by tandem mass spectrometry (MS/MS), which further allowed determination of the SGC fatty acid content and whether it was responsive to salinity change.

Materials and Methods

Fresh and Brackish Water Acclimation and Sampling

One hundred twenty rainbow trout (226.0 ± 12.0 g) were obtained from Alma Research Station (Alma, ON, Canada) and divided into two aerated 1.2 m diameter tanks (750 L) containing either freshwater (FW) or brackish water (BW) (salinity = 20 ppt, Crystal Ocean held at 10°C. Fish exposed to brackish water were brought up to 20 ppt from 0 ppt at a rate of 5 ppt per day. Tanks were static, consequently ½ of the water volume was replaced every day to prevent biofouling; a pH of 8 was maintained throughout. During the acclimation, fish in both tanks were fed a commercial salmon feed daily to satiety. FW acclimation occurred for 29 days, while BW acclimation occurred for 13 (intermediate exposure time to BW (IW)) and 28 (full exposure time to BW) days. At the end of each exposure period fish were sacrificed and gill tissue was obtained.

Basolateral Membrane Isolation Procedure

BLM vesicles were prepared according to Perry & Flik (1988). Gill scrapings were added to 15 mL of hypotonic homogenization buffer (in mM: 25 NaCl, 1 N-2-hydroxyethylpiperazine N′-2 ethanesulfonic acid (HEPES), 1 Tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), and 0.5 ethylenediamine tetraacetic acid (EDTA) (disodium salt), pH 8.0 where they were subsequently homogenized in a dounce homogenizer first with a loosely then tightly fitting pestle (30 strokes each). The volume was then made up to 50 mL and homogenized 10 times with the loosely fitting pestle. Homogenates were divided into two tubes and centrifuged at $550 \times g$ for 15 min (RC5C Centrifuge, Sorvall Instruments, Du Point Canada, Markham, ON, Canada). The subsequent pellets were discarded and the supernatant was centrifuged at $50{,}000 \times g$ for 30 min. The supernatant was then discarded and the light portion of the pellet (plasma membranes) was separated from the dark portion (mitochondria) by gently swirling with 5 ml of sucrose buffer (in mM: 250 sucrose, 5 Mg_2Cl_2, 5 HEPES, and 5 Tris, pH 8.0)

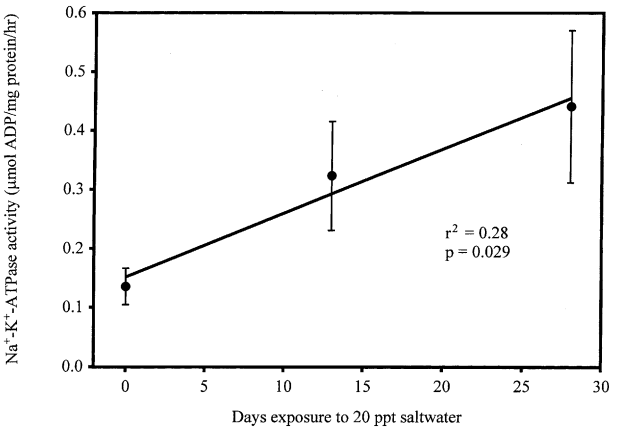

Fig. 1. Basolateral membrane Na^+-K^+-ATPase activity (mean ± SEM) during 29 days of exposure to freshwater (n = 6) and 13 (n = 6) and 28 (n = 5) days exposure to 20 ppt saltwater. ATPase activity increased significantly during the course of saltwater acclimation (P < 0.05).

and then homogenized with 100 strokes of the tight pestle. This homogenate was centrifuged at 1000 × g for 10 min and then immediately at 10,000 × g for 10 min. The pellet (remaining contaminating membranes) was discarded and the supernatant was centrifuged at 30,000 × g for 45 min. The final pellet was resuspended in 1.5 mL of suspension buffer (in mM: Mg_2Cl_2, 150 NaCl, 20 HEPES, 20 Tris-HCl, pH 7.4). Samples were frozen at −80°C for subsequent analysis.

Protein and Na^+-K^+-ATPase Assays

BLM protein was measured with standard Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) at 595 nm (Gary 50 Varian spectrophotometer, Varian, Palo Alto, CA). The Na^+-K^+-ATPase assay was a modification of Gibbs and Somero (1989) and McCormick (1993). NADH oxidation-dependent ATP hydrolysis at 25°C was measured spectrophotometrically at 340 nm in the presence and absence of 10 mM ouabain within an ATPase mix (in mM: 100 NaCl, 20 KCl, 5 Mg_2Cl_2, 50 imidazole, 3 ATP, 2 phospho(enol)pyruvate, 0.2 NADH, excess pyruvate kinase and lactate dehydrogenase, pH 7.5). Activity was expressed as µmol ADP/mg BLM protein/h.

Sulfatide Extraction

BLM GSLs were extracted in 7.5 mL of 2:1 (chloroform:methanol, v/v) and mixed in a wrist action shaker (Burrell model 75, Pittsburgh, PA) overnight. Volumes were then adjusted to form a Folch partition (2:1:0.6, chloroform:methanol:PBS, $v/v/v$, where PBS was (in mM) 140 NaCl, 30 KCl, 80 Na_2HPO_4 (anhydrous), 10 KH_2PO_4, pH 7.4). The lower phase was extracted and dried under a gentle stream of nitrogen. The GSLs were then re-dissolved in 100 µL of 2:1 (chlorofor:methanol, v/v).

Thin Layer Chromatography (tlc) and Antibody Binding Overlay

Together with 4 µg of sulfogalactosylacylalkylglycerol (SGG, also termed seminolipid; Ishizuka, Suzuki & Yamakawa, 1973) standard and 5 µg, 4 µg, 3 µg, 2 µg, 1 µg, and 0.5 µg of SGC standards (cerebroside sulfate, Sigma, St. Louis, MO), 10 µL of each glycolipid extract was separated on 7 mm lanes on a silica gel plate (Macherey-Nagel, Polygram SIL G/UV_{254}, Easton, PA) with a 50 mL mobile phase of 65:25:4 (chloroform:methanol:water, v/v/v). SGC and SGG were detected using a modification of the TLC antibody-binding overlay procedure of Kushi et al. (1996). Plates were initially blocked with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS) (50 mM Tris, 154 mM NaCl, pH 7.4) and then incubated with Sulph 1 monoclonal antibody (Fredman et al., 1988) (1/1000 in 1% BSA in 50 mM TBS) overnight at room temperature. Plates were then washed 3 × with 50 mL of 50 mM TBS and incubated with horseradish-peroxidase-conjugated goat anti-mouse IgG (secondary antibody, Sigma, 1/2000 in 1% BSA in 50 mM TBS) for 2 h at room temperature. Plates were washed again (3 × with 50 mL of 50 mM TBS) prior to development, with a combination of 1 volume of 4-chloro-1-naphthol solution (Sigma; 3 mg/mL in methanol) with 5 volumes of TBS and 1 µL of 30% H_2O_2 per 10 mL of TBS.

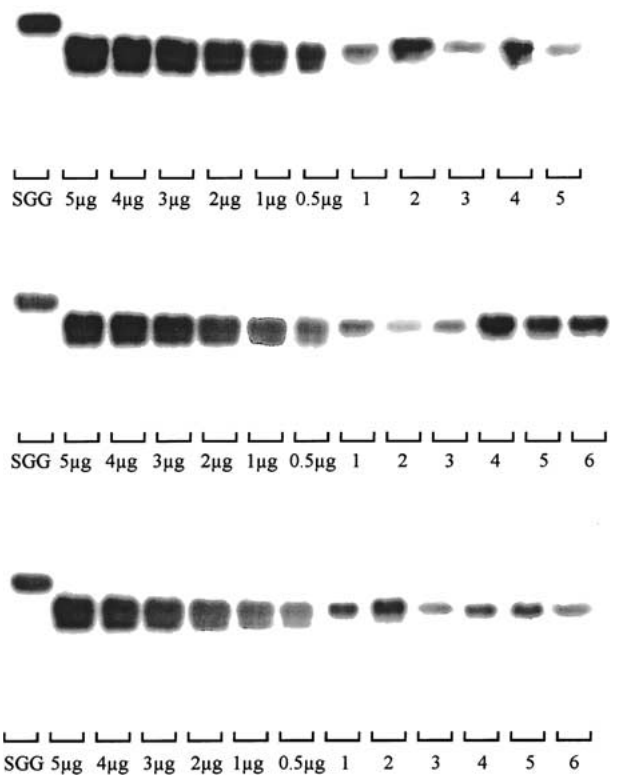

Fig. 2. SGC in rainbow trout gill cell basolateral membranes as visualized by TLC antibody overlay. Standards included 4 µg of SGG and, as denoted by the lane numbers, 5 µg, 4 µg, 3 µg, 2 µg, 1 µg, and 0.5 µg of SGC. The numbered lanes on the right correspond to 10 µl of glycolipid extracted from basolateral membranes of gill epithelium from rainbow trout exposed to: freshwater for 29 days (n = 6; *bottom panel*); 20 ppt saltwater for 13 days (n = 6; *middle panel*); and 20 ppt saltwater for 28 days (n = 5; *top panel*).

Mass Spectrometry and Sulfatide Quantification

SGC content in the BLM was measured by tandem mass spectrometry (high performance triple quadrapole mass spectrometer, Micromass Quattro Ultima, Waters Corporation, Milford, MA) using electrospray ionization in the negative mode. SGC was quantified by MS/MS analysis by multiple reaction monitoring (MRM) according to a modification of Whitfield et al. (2001).

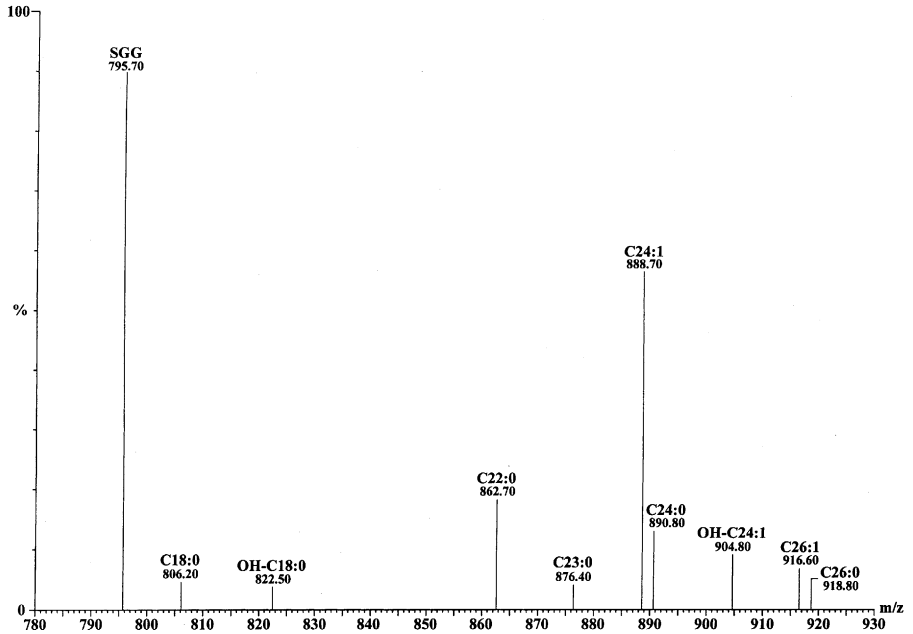

Fig. 3. Representative mass spectrum for C18:0; OH-C18:0; C22:0; C23:0; C24:1; C24:0; OH-C24:1; C26:1; C26:0 SGC isoforms in the basolateral membrane (BLM) of rainbow trout gill epithelium. The 795 amu peak denotes the internal standard, sulfogalactosylacylalkylglycerol (SGG). Note that peaks in this figure are not directly comparable, as they have not been quantified and standardized to BLM protein concentration.

GSL extracts or SGC standards (in ng/mL: 10, 25, 250, 1000 and 3000) were added to 25 μL of 10 μM SGG (in methanol). They were then dried down under a gentle stream of nitrogen and redissolved in 200 μL of methanol. Aliquots of 10 μL of sample/standard with SGG were then injected into the mass spectrometer; 10 μL of 100% methanol was injected between sample/standard runs to minimize carry over. The concentration of individual fatty acid SGC species was calculated from 9 standard curves (one for each isoform), each based on the ratio of the signal intensity of an individual SGC isoform standard relative to the signal intensity of the SGG internal standard at each SGC concentration. SGC isoform concentration was expressed as nmol SGC/mg BLM protein. Total BLM SGC content was calculated from the sum of each SGC isoform concentration.

Data Analysis

Na^+-K^+-ATPase activity and SGC concentration in the BLM were regressed against the BW exposure time. SGC isoform concentrations were analyzed under the factorial ANOVA model: SGC concentration $\sim\mu$ + salinity + isoform + isoform × salinity + error. SGC isoform concentrations within and between salinity treatment groups were compared with Tukey's test. Variances were assessed with Levene's test. For each salinity treatment group, Na^+-K^+-ATPase activity was plotted against total SGC and concentration and non-linear regression statistics were used to evaluate the extent of correlation. A 95% confidence level was employed throughout.

Results

BLM Na^+-K^+-ATPase activity significantly increased upon increasing exposure time to BW ($P < 0.05$, $n = 17$; Fig. 1). TLC overlay showed that SGG was absent from the BLM (Fig. 2), therefore this sulfogalactolipid could serve as a valid internal standard for subsequent mass spectrometry analysis. Sulfatide was present in the BLM (Fig. 2); we measured 9 SGC fatty acid species prominent in mammals, N-acyl chain: C18:0; OH-C18:0; C22:0; C23:0; C24:1; C24:0; OH-C24:1; C26:l; C26:0 (Fig. 3). Under our ANOVA model, there was no significant isoform by salinity interaction ($P > 0.05$, $n = 17$) and the variances between treatment groups were equal ($P > 0.05$, $n = 17$). Total SGC concentration and the relative proportion of SGC isoforms were conserved between each salinity treatment group ($P > 0.05$, $n = 17$; Fig. 4). Within each salinity treatment group the concentration of the C22:0 SGC isoform was significantly greater than the other SGC species ($P < 0.05$, $n = 17$), and the OH-C16:0 isoform concentration was significantly greater than that of the C26:0 isoform ($P < 0.05$, $n = 17$). The other SGC species did not differ significantly from each other ($P > 0.05$, $n = 17$).

In the BLM from FW and IW fish there was no correlation between Na^+-K^+-ATPase activity and total SGC concentration (Fig. 5). However, a strong exponential correlation was observed between Na^+-K^+-ATPase activity and total SGC concentration of BLM from BW fish ($P < 0.05$, $r^2 = 0.94$, $n = 5$). We noted the same statistically significant negative relationship between Na^+-K^+-ATPase activity and SGC concentration for each individual SGC isoform from BW fish ($P < 0.05$, $n = 5$), whereas no such correlation was found for individual SGC isoforms from fish acclimated to FW ($P > 0.05$, $n = 6$) or IW ($P > 0.05$, $n = 6$) (*not shown*).

Discussion

In rainbow trout, sulfogalactolipids have only been reported in testis (Levine et al., 1975) and have subsequently been implicated in mammalian spermato-

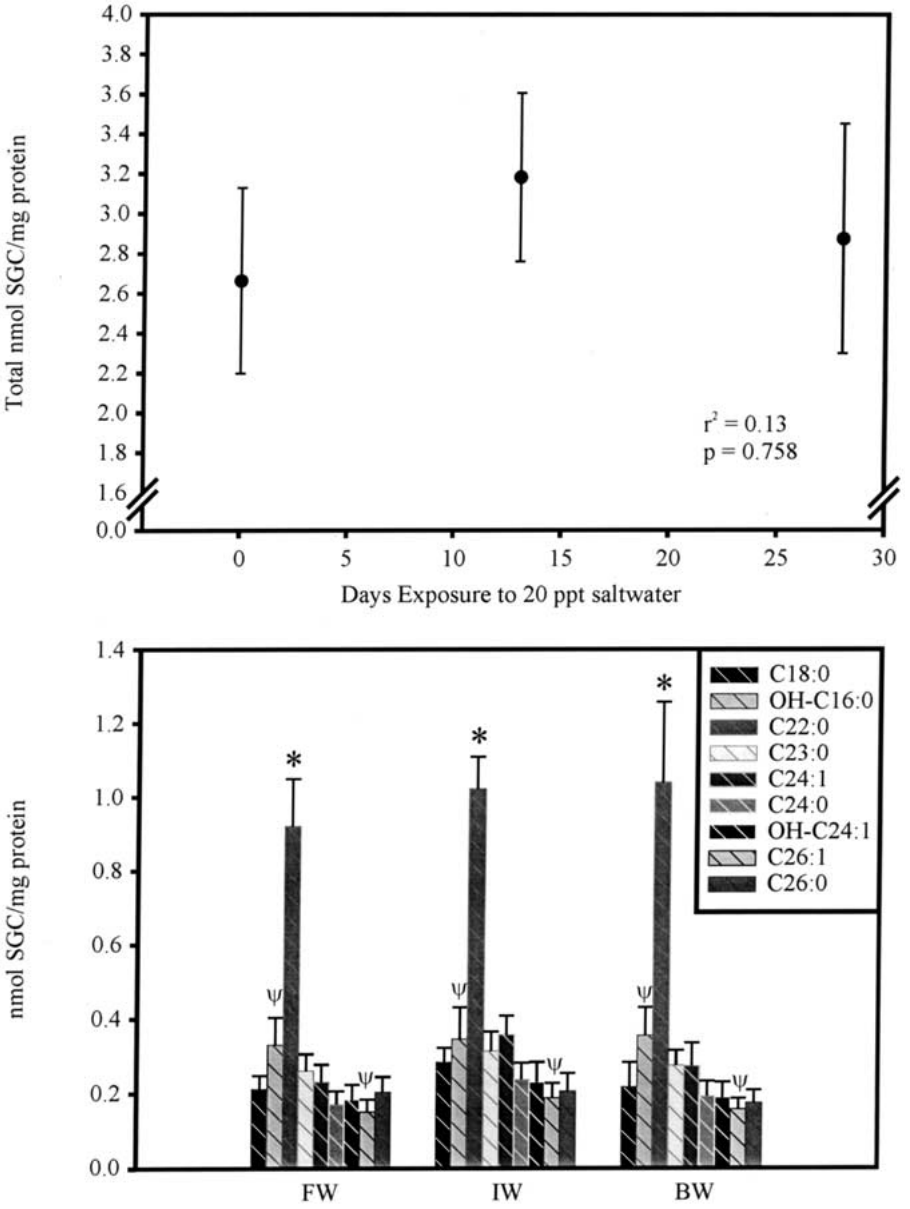

Fig. 4. Rainbow trout gill cell basolateral membrane SGC content as measured by tandem mass spectrometry. Fish were exposed to freshwater for 29 days (*FW*; $n = 6$); 20 ppt saltwater for 13 days (*IW*; $n = 6$); and 20 ppt saltwater for 28 days (*BW*; $n = 5$). Upper panel shows total SGC concentration (mean ± SEM) during these treatments. Lower panel shows the concentration of individual N-acyl chain SGC isoforms (mean ± SEM) in each salinity group. Within each salinity treatment group significant differences are denoted by * and 'ψ' ($P < 0.05$, $n = 17$). SGC concentration profiles were conserved between salinity treatment groups ($P > 0.05$, $n = 17$)

genesis (Lingwood, 1986; Fujimoto et al., 2000) and sperm-egg interactions (White et al., 2000). The presence of SGC in the BLM of rainbow trout gill tissues was expected, given its specificity for Na^+-K^+-ATPase-rich organs and basal membranes. Our results contradict the assumption of Zwingelstein et al. (1980) who, based on the mammalian immunohistological data of Zalc et al. (1978), proposed that SGC was absent from the BLM of eel gills and therefore not involved in Na^+-K^+-ATPase activity. Furthermore the results of Zalc et al. (1978) must be interpreted with caution, as GSLs are often cryptic and not readily accessible as epitopes (Shayman & Radin, 1991). In any event, it appears that gill-cell BLM can provide a location in which an SGC co-factor site model may operate.

The rainbow trout gill BLM SGC fatty acid isoform proportions were constant across salinity groups. Similar constancies in lipid class distribution, phospholipid fatty acid chain composition and cholesterol content have been reported in the BLM of fresh- and saltwater-acclimated eels (Crockett, 1999). However, the SGC pattern in rainbow trout BLM does not consistently compare with SGC profiles reported for Na^+-K^+-ATPase-rich tissues including dogfish rectal gland (Karlsson et al., 1974b), the salt glands of eider ducks or herring gulls (Karlsson et al., 1974b), the electric organ of *Torpedo marmorata* (Hansson et al., 1979), or mouse kidney (Sandhoff et al., 2002). However, a quantification of all SGC species in rainbow trout BLM is required before any definitive conclusions can be drawn.

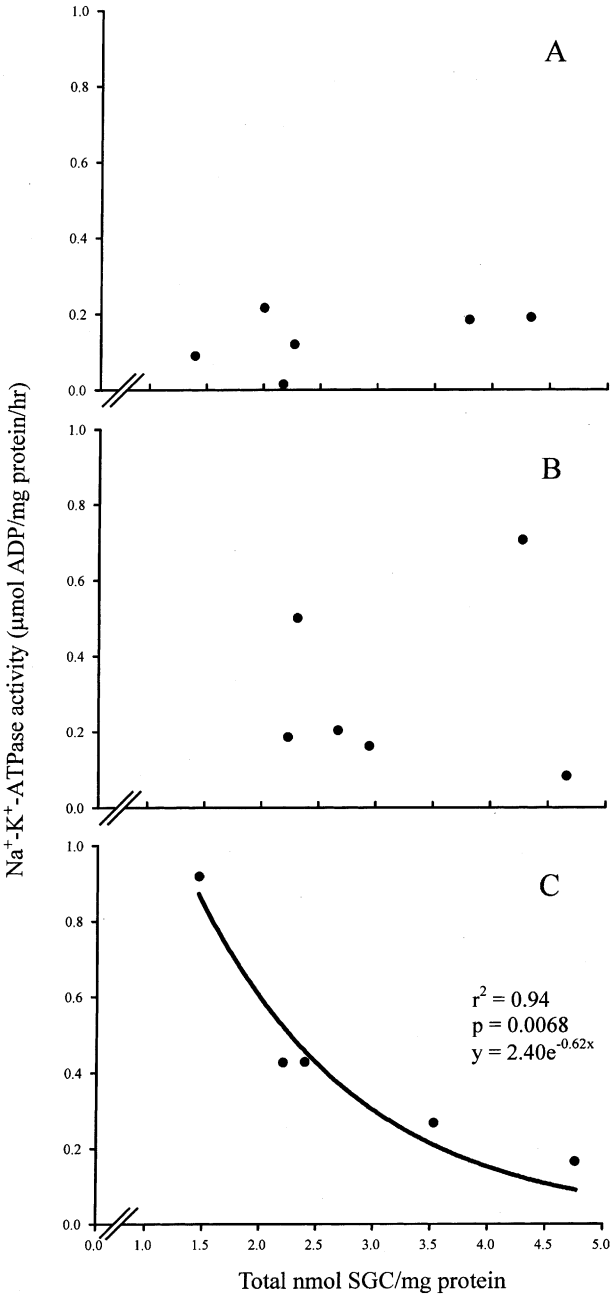

Fig. 5. Total SGC concentration versus Na^+-K^+-ATPase activity in the basolateral membrane of rainbow trout gill cells (*A*) Fish ($n = 6$) acclimated to freshwater for 29 days. (*B*) Fish ($n = 6$) acclimated to 20 ppt saltwater for 13 days. (*C*) Fish ($n = 5$) acclimated to 20 ppt saltwater for 28 days; note the significant exponential correlation ($P < 0.05$).

The increase in BLM Na^+-K^+-ATPase activity observed in response to BW acclimation is supported extensively throughout the literature. Gill Na^+-K^+-ATPase activity is accepted as a measure of seawater adaptability (Borgatti, Paliarani & Ventrella, 1992) and usually correlates positively with saltwater exposure (Epstein, Katz & Pickford, 1967; Karnaky, Ernst & Philpott, 1976; Karnaky et al., 1976). In contrast to our initial hypothesis, average SGC concentration remained constant throughout the acclimation to BW (Fig. 4). However, within the BW group we observed a negative correlation between Na^+-K^+-ATPase activity and BLM SGC content (Fig. 5). Zwingelstein et al. (1980) also found a negative relationship between SGC concentration and Na^+-K^+-ATPase activity in eel gill Na^+-K^+-ATPase 'concentrates' (crude membrane preparations designed to concentrate Na^+-K^+-ATPase), although the trend was conserved for both FW- and seawater-acclimated fish. However, these authors presented SGC content as nmol SGC/μmol phospholipid; if their units are changed to ng SGC/mg protein (the units used in our study), their data matches ours: the inverse relationship between Na^+-K^+-ATPase activity and SGC concentration remains in seawater-adapted eels but disappears in freshwater-adapted eels.

Our results may be explained by differential sensitivity to SGC between salinities. Richards et al. (2003) showed that Na^+-K^+-ATPase α1a and α1b isoforms are differentially expressed in rainbow trout gills following seawater transfer. It may be that the activity of the α1b Na^+-K^+-ATPase isoform predominantly expressed in seawater is more effectively modulated by SGC; indeed, the enzyme's K_m for both the Na^+ and K^+ greatly increased in the gills of rainbow trout exposed to BW (Pagliarani et al., 1991). In addition, the constancy of SGC isoform proportions during BW acclimation may be indicative of a novel situation where activity is regulated by changing the amount of SGC-sensitive enzyme itself rather than changing its lipid environment. The negative correlation with activity is not intuitive given the supportive role of SGC in the cofactor model, however, a similar salinity-sensitivity profile (FW insensitive and saltwater sensitive) has been observed between Na^+-K^+-ATPase activity and cholesterol content in the gill BLM of arctic char (Bystriansky & Ballantyne, unpublished data). Therefore, there may be a link to cholesterol. Strong binding of choline phospholipids to SGC has been shown by titration changes for phosphate (Abramson & Katzman, 1968) and was hypothesized to interfere with SGC-assisted K^+ transport (Karlsson, 1977). This binding was inhibited by cholesterol, which in theory could perhaps serve to track and assist SGC cofactor function. Whether this system operates in the gill BLM of rainbow trout awaits further investigation.

The level of SGC in the BLM may also be governed by ionic parameters. The accumulation of sulfated groups on extracellular proteoglycans is thought to be a mechanism through which some molluscs and crustaceans adapt to increased salinity (Nader et al., 1983; Grimm-Jorgensen, Ducor & Piscatelli, 1986). The result is an infiltration of water

and the formation of a gel that contributes osmotically but obstructs the transport of ions. Comper & Laurent (1978) estimated that NaCl migration across membranes of densely charged tissues is reduced to approximately 8/10 of its value in water. Surface mucus can therefore modulate cellular osmoregulation by changing the ion gradients at the cell surface. Ishizuka and Yamakawa (1985) suggested that SGC may act as one such ion barrier or ion trap. Given the prevalence of SGC in Na^+-K^+-ATPase-rich tissues it is possible that this ion barrier exists in rainbow trout gill BLM. If so then perhaps the total BLM SGC content in the FW and BW condition reflects a level that meets the SGC concentration requirement to form a support annulus according to Karlsson (1977), yet at the same time minimizes the barrier-associated disruption to overall ion gradients.

In the context of an SGC ion barrier, an explanation for the negative relationship between Na^+-K^+-ATPase activity and BLM SGC concentration in BW fish is not exclusive to an isoform-switching hypothesis. Saltwater adaptation requires gill tissue to transform from a salt-absorbing epithelium to a salt-secreting epithelium (reviewed by Jüress & Bastrop, 1995). Salt secretion in the gill is accomplished by an upregulation of the chloride cell system where Cl^- extrusion against a concentration gradient (driven by BLM Na^+-K^+-ATPase in conjunction with the Na^+-$2Cl^-$-K^+ cotransporter and apical cystic fibrosis transmembrane conductance regulator) is matched to the passive paracellular exit of Na^+. An ion barrier created by SGC in the BLM would have the potential to disrupt the ion gradients necessary to create the transepithelial voltage required for salt excretion. This may therefore account for the BW results where fish that were better or poorer osmoregulators (as indicated by Na^+-K^+-ATPase activity) had lower and higher BLM SGC concentrations, respectively. It is also possible that an SGC-induced ion barrier would have the potential to disrupt effectors of individual cell hyperosmotic adaptation, including Na^+-$2Cl^-$-K^+ cotransport with associated Na^+-H^+ antiport and HCO_3^--Cl^- antiport, and the accumulation of compatible solutes and organic osmolytes. In any event, the overall ability of SGC to disrupt BLM ion gradients requires confirmation before a decisive argument can be made.

In conclusion, SGC was found in the BLM of rainbow trout gill cells. Both the proportions of SGC isoforms and its total concentration did not change upon acclimation to BW, however, fish exposed to saltwater exhibited a negative relationship between Na^+-K^+-ATPase activity and SGC BLM content. Perhaps this represents a mechanism where enzyme activity is regulated by changing the relative amounts of lipid-sensitive isoforms rather than altering the lipid environment. SGC in rainbow trout gill BLM could still assist Na^+-K^+-ATPase operation, but only perhaps at a level that does not comprise ion gradients necessary for salt secretion and cellular osmo-adaptation. How SGC relates to Na^+-K^+-ATPase isoform switching in response to salinity transfer warrants further study.

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