LIPID RESTRUCTURING DOES NOT CONTRIBUTE TO ELEVATED ACTIVITIES OF Na+/K+-ATPase IN BASOLATERAL MEMBRANES FROM THE GILL OF SEAWATER-ACCLIMATED EEL (ANGUILLA ROSTRATA)

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Accepted 25 May; published on WWW 9 August 1999

Summary

In teleost fishes, increases in gill Na+/K+-ATPase activity accompanying the transition from fresh water to sea water may be attributed to changes in either the numbers of enzyme molecules present or to turnover number (kcat). The sensitivity of Na+/K+-ATPase to its chemical/physical environment in the membrane makes it plausible that modulation of enzyme activity may be driven, in part, by changes in membrane properties. In the current study, I test the hypothesis that lipid compositional changes (restructuring) contribute to the modulation of gill Na+/K+-ATPase activity. An enriched preparation of basolateral membranes was prepared from the gills of freshwater- and seawater-acclimated American eel (Anguilla rostrata). Phospholipid class distribution, fatty acyl chain compositions and cholesterol contents were determined. Phosphatidylcholine, the most abundant phospholipid present in gill basolateral membranes, makes up more than 60% of the total phospholipid content in both freshwater- and seawater-acclimated animals. The contents of other phospholipids and major fatty acyl chains are also similar for the two acclimation groups. Cholesterol/phospholipid molar ratios are 0.28 for freshwater and 0.29 for seawater animals. The similarity between lipid compositions in membranes from freshwater- and seawater-acclimated eels indicates that lipid restructuring is not a mechanism for modulation of gill Na+/K+-ATPase activity in Anguilla rostrata, at least during the acclimation time course used in the present study.

Key words: Na+/K+-ATPase, gill, salinity adaptation, plasma membrane, eel, Anguilla rostrata, Na+ pump.

Introduction

Many aquatic organisms exist in fluctuating salinities or spend portions of their life cycle in a mixture of marine, freshwater or estuarine habitats. Despite wide differences in ionic compositions and concentrations of these environments, teleost fishes maintain a relatively constant internal milieu (Evans, 1979). The gill tissue of teleosts is a key interface between an organism and its environment. In addition to its roles in gas exchange, in the elimination of nitrogenous waste and in acid–base balance, the gill plays a central part in ion- and osmoregulatory processes (McDonald et al., 1991).

Variation in environmental salinity invokes a suite of responses in teleosts. These responses involve several different levels of biological organization such as (1) rates of drinking (Hirano, 1974; Karnaky, 1980), (2) cell morphology and composition in the gill (Virabhadrachari, 1961; Karnaky et al., 1976a; Kültz et al., 1995) and (3) the activities of enzymes associated with osmoregulatory processes such as Na+/K+-ATPase (Epstein et al., 1967; Sargent and Thomson, 1974). Modulation of the activity of Na+/K+-ATPase in gill is arguably among the most widespread of these responses. It is well documented that Na+/K+-ATPase activities from the gills of euryhaline organisms are positively correlated with salinity (e.g. Epstein et al., 1967; Sargent and Thomson, 1974; Karnaky et al., 1976a,b; Towle et al., 1977).

Modification of Na+/K+-ATPase activity may involve either changes in gene expression (affecting Na+ pump numbers or subunit isoform expression) or various post-translational adjustments that directly affect catalytic rate (Ewart and Klip, 1995; Lee et al., 1998). Levels of Na+/K+-ATPase α-mRNA rise in the gill of the European eel (Anguilla anguilla) upon transfer from fresh water to sea water (Luke et al., 1993; Cutler et al., 1995). Similarly, a 3 day exposure to 25‰ sea water results in an increase in both Na+/K+-ATPase activities and mRNA levels for the α-subunit of the enzyme in brown trout gill, yet the sixfold elevation in enzyme activity after 50 days is not matched by mRNA levels (Madsen et al., 1995). While an increase in activity of Na+/K+-ATPase during exposure to elevated salinities has been shown to parallel the number of Na+ pumps (measured by ouabain binding) in the gill of...
Fundulus heteroclitus (Karnaky et al., 1976b), this is not always the case. In this same species, a 30 min acute exposure to 30‰ sea water results in an almost twofold increase in pump activity in the gill without a corresponding rise in ouabain binding (Towle et al., 1977). Post-translational modifications affecting turnover number ($k_{cat}$) may include reversible phosphorylation, changes in the subcellular distribution of the enzyme and lipid modulation of enzyme activity (Towle, 1981; Ewart and Klip, 1995). As suggested by Towle (1981), modulation of membrane lipids may be responsible for the rapid rise (after 30 min) of Na$^+$/K$^+$-ATPase activity in the gill of F. heteroclitus exposed to high salinity.

Compositional changes in membrane lipids (restructuring) may, in fact, contribute to variation in Na$^+$/K$^+$-ATPase activity at some point during the animal’s adjustment to varying environmental salinities. Lipid compositional changes have been noted in tissues from fishes acclimated to different salinities (Daikoku et al., 1982; Leray et al., 1984). Furthermore, many membrane-associated proteins are influenced by the chemical and/or physical properties of the membrane (Deuticke and Haest, 1987; Vemuri and Philipson, 1989). Na$^+$/K$^+$-ATPase is among the integral membrane proteins that appear to be particularly sensitive to variation in membrane physical properties (Grisham and Barnett, 1973; Kimelberg and Papahadjopoulos, 1974; Gibbs and Somero, 1990). This sensitivity may reflect the large number of membrane-spanning domains associated with the catalytic ($\alpha$) subunit of the protein. Lingrel and Kuntzweiler (1994) have used a ‘working’ model of 10 membrane-spanning regions accounting for 30% of the protein directly embedded in the lipid matrix of the bilayer.

While phospholipids are required for Na$^+$/K$^+$-ATPase activity (Hokin and Hexum, 1972; Stahl, 1973), it is debatable whether a particular phospholipid class is necessary for maximal activity (Hokin and Hexum, 1972; Stahl, 1973; Harris, 1985). In addition, the neutral lipid cholesterol may also play a role in modulation of enzyme activity. In reconstitution studies using Na$^+$/K$^+$-ATPase from bovine kidney, maximal enzyme activity occurs at physiological (intermediate) levels of cholesterol; a decrease in activity is observed at both low and high levels of membrane cholesterol (Yeagle et al., 1988). Reduced Na$^+$/K$^+$-ATPase activities parallel decreases in membrane fluidity in trout red cell membranes supplemented with cholesterol compared with native membranes (Raynard and Cossins, 1991). Even within the physiological range of cholesterol levels in basolateral membranes (BLMs) from trout intestinal epithelia, Na$^+$/K$^+$-ATPase activity is highest in membranes with the lowest levels of this lipid (Crockett and Hazel, 1997). The fluidity of the bilayer and other physical properties of the membrane seem to be particularly important factors influencing Na$^+$/K$^+$-ATPase activity (Harris, 1985; Yeagle et al., 1988; Gibbs and Somero, 1990).

The purpose of the present study is to test the hypothesis that lipid restructuring plays a part in the modulation of Na$^+$/K$^+$-ATPase activity in the gill of salinity-acclimated euryhaline teleosts. Na$^+$/K$^+$-ATPase activities and lipid compositions (phospholipid class, fatty acyl composition and cholesterol content) were determined in basolateral membranes prepared from the gill of the American eel (Anguilla rostrata) acclimated to either fresh water or sea water.

Materials and methods

Animals

Yellow-phase American eel (Anguilla rostrata LeSueur) were collected in fresh water (Penobscot River, Maine, USA) by commercial fishermen in June 1997. Eels (100–450 g) were acclimated to either fresh water or sea water (32‰) for a minimum of 2 weeks before use. A gradual increase in salinity (5‰ per day) was made before reaching the final salinity in seawater aquaria. Polyvinylchloride tubes were placed in each aquarium for animals to hide in, thus reducing activity levels and stress (Egginton, 1986a). The temperatures in the freshwater aquaria were matched to those of sea water (13°C) using chilling units. Eels were not fed during the acclimation period.

Membrane preparations

Eels were anaesthetized in 0.1% (w/v) MS-222 dissolved in either fresh water or sea water, depending on the acclimation history of the animal, and neutralized with NaHCO$_3$. Eel gills were perfused with 10 ml of 175 mmol l$^{-1}$ NaCl through the ventricle until the gill blanched. Whole gill baskets were removed, and individual arches were cut out, rinsed in homogenization medium (see below) and blotted dry. Gill epithelia were scraped on an ice-cold glass stage. Scrapings were pooled (2–3.5 g wet mass), typically from as many as 10 fish (eight on average), and homogenized in 300 mmol l$^{-1}$ sucrose, 25 mmol l$^{-1}$ Hepes (pH 7.6 at 25 °C) using a motor-driven homogenizer (Biohomogenizer, Biospec Products, Inc.) for 15 s, three times. Homogenates were transferred to, and further homogenized (one pass) in, a 7 ml Ten-Broeck (TB) ground-glass homogenizer.

The starting point for the plasma membrane preparation was from Klaren et al. (1993), although most centrifugation steps and conditions were developed empirically to achieve an enriched basolateral membrane preparation (Fig. 1). Homogenates were centrifuged for 10 min in a fixed-angle rotor (Sorvall SS-34) at 1400g$_{max}$. The supernatant (S1) was set aside, and the pellet (P1) was resuspended (three passes) in 1 ml of homogenization medium in 7 ml TB homogenizers. The resuspended pellet was then brought to 20 ml with homogenization medium and centrifuged again at 1,400g$_{max}$ (Sorvall SS-34), this time for 20 min. The supernatant from this second centrifugation step (S2) was combined with S1. The combined supernatants (S1+S2) were then centrifuged in a fixed-angle rotor (Beckman 70 Ti) at 93,000g$_{max}$ for 90 min. The pellet (P3) from this step was resuspended in 0.5 ml of homogenization medium using three passes in a 2 ml TB ground-glass homogenizer. This suspension was then layered on 18% Percoll (in Hepes-buffered sucrose solution; final
Monitored by assaying both succinate cytochrome c reductase, an enzyme associated with the endoplasmic reticulum (Masters et al., 1967).

Preparation of lipid extracts and analyses of phospholipid class/diacyl chain compositions

Lipids were extracted from plasma membrane fractions (P4) following the method of Bligh and Dyer (1959). Butylated hydroxytoluene (0.01 %) was used in the extraction procedure to prevent autoxidation. Phospholipid class composition for plasma membranes prepared from freshwater- and seawater-acclimated animals was determined using Iatroscan TLC-FID (Mark 5, Bioscan Inc.). The procedure used was similar to that described by Hazel (1985). Chromatards (SIII) were developed overnight prior to use and burned twice immediately before spotting to remove any contamination from the rods. Rods were spotted with 1 μl of standard or sample (10–15 μg standard or sample per microlitre CHCl3) using a blunt-tipped 2 μl syringe (Hamilton 7102). Rods were dried at 60 °C for 5 min before development. Two solvent systems were used for stepwise separation of neutral and polar lipid classes. Each solvent system was allowed to develop completely. The first solvent system (hexane/diethyl ether/formic acid, 80:20:2; development time 34 min) was used to determine whether neutral lipids other than cholesterol (which was analyzed by a different method, see below) could be detected. No significant peak other than that for cholesterol was observed. The second development (to resolve phospholipids) included a polar solvent system (CHCl3/MEOH/H2O, 80:35:3; development time 55 min). Double development with the second solvent system was used to improve the resolution of phospholipid classes. Between and after all developments, rods were dried at 60 °C. Rods were scanned (30 s per rod) with hydrogen at a flow rate of 180 ml min⁻¹ and with air at a flow rate of 21 ml min⁻¹. Standard curves for the phospholipids were generated using four replicates for each of six concentrations. Repeated attempts to separate phosphatidylethanolamine (PE) and phosphatidylinositol (PI) using a variety of different solvent systems were unsuccessful, so PE and PI are reported as a combination of the two phospholipid classes.

Fatty acyl composition analysis was performed by Avanti Polar Lipids, Inc (Alabaster, AL, USA). Briefly, lipid extracts were hydrolyzed and methylated with 1 % sulphuric acid in methanol at 70 °C. After hydrolysis and methylation, hexane was added, and samples were vortexed and centrifuged. The upper phase containing the fatty acid methyl esters (FAMEs) was removed. Samples (2 μl) were injected into a Hewlett Packard 5890 gas chromatograph with flame ionization detection (GC/FID). A J and W column (DB225, 30 m) was used (isothermal at 220 °C, helium as the carrier gas). Samples were analyzed against a FAME calibration standard and control. Peaks were included in the analysis (1) if they constituted more than 1 % of total FAMES, or (2) if less than 1 %, they were identifiable.
Cholesterol, total phospholipid and protein determinations

The cholesterol contents of membrane fractions were analyzed using a fluorometric/enzymatic assay as originally developed and described by Crockett and Hazel (1995). The time courses of the enzymatic reaction (cholesterol oxidation) were examined initially to determine the incubation time necessary for the enzyme (cholesterol oxidase) to react with all membrane cholesterol. A 3 h incubation was determined to be the optimal incubation time for gill plasma membranes. Total phospholipid was quantified as acid-hydrolyzable phosphate (Rouser et al., 1970). The amount of membrane protein present was measured using the bicinchoninic acid method (Smith et al., 1985).

Statistics

Results from freshwater and seawater acclimations were analyzed statistically using Student’s t-test. The level of significance was set at \( P<0.05 \).

Chemicals

Phospholipid class standards were from Avanti Polar Lipids, Inc. (Alabaster, AL, USA) and included saturated fatty acyl chains in the number 1 position on the glycerol backbone and unsaturated fatty acyl chains in the number 2 position on the glycerol backbone. FAME standards were from Nu-Chek Prep, Inc. (Elysian, MN, USA). Pyruvate kinase-lactate dehydrogenase (PK-LDH) used in assays for Na⁺/K⁺-ATPase was from Boehringer-Mannheim (Indianapolis, IN, USA). Bicinchoninic acid (BCA) protein assay reagent was purchased from Pierce Chemical Co. (Rockford, IL, USA). All other biochemicals were from Sigma Chemical Co. (St Louis, MO, USA). Solvents used were HPLC-grade or better.

Results

Enzyme activities from gill homogenates

A comparison of enzyme activities in the gill homogenates from freshwater- and seawater-acclimated eels reveals several trends (Table 1). In keeping with the pattern observed in previous reports, Na⁺/K⁺-ATPase activities are elevated more than twofold in seawater-acclimated fish compared with their freshwater counterparts. In contrast, differences in the activity of the other enzyme associated with the plasma membrane, \( \gamma \)-glutamyltransferase (\( \gamma \)-GT), are not statistically significant between acclimation groups.

Membrane enrichment

Membranes (P4 fractions) prepared from both acclimation groups have eightfold higher protein-specific activities of Na⁺/K⁺-ATPase compared with activities in crude homogenates (Table 1). This result has two implications. First, there is significant enrichment of basolateral membranes in the final membrane fraction and, second, the degree of enrichment is similar for membranes prepared from both freshwater- and seawater-acclimated animals. Enrichment factors for \( \gamma \)-GT are similar to those for Na⁺/K⁺-ATPase. The mitochondrial enzyme (succinate cytochrome c reductase) and the endoplasmic reticulum marker enzyme (NADPH cytochrome c reductase) are enriched threefold or less in the plasma membrane (P4) fraction, indicating some contamination by intracellular membranes. Of the total activity of each of the two plasma membrane markers (Na⁺/K⁺-ATPase and \( \gamma \)-GT) in the crude homogenate, 13 % is recovered in the P4 fraction, while only 4 % of the enzyme activity of intracellular markers is recovered. Importantly, intracellular membranes make up similar portions of the P4 fractions for both freshwater and seawater treatments.

Phospholipid and cholesterol compositions

Phospholipid class compositions are virtually indistinguishable for gill plasma membranes from freshwater- and seawater-acclimated eels (Fig. 2). Phosphatidylcholine (PC) is by far the most abundant phospholipid, making up 61 % of the total phospholipid content of the membrane, while phosphatidylethanolamine (PE) and phosphatidylinositol (PI) combined represent 23 % of the phospholipid component of the membrane. Sphingomyelin (SM) constitutes 12 % and phosphatidylyserine (PS) 4 % of the phospholipid matrix.

Table 1. Marker enzyme activities in homogenates and enrichment factors in final fractions (P4)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Freshwater-acclimated</th>
<th>Seawater-acclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogenate activity</td>
<td>P4</td>
</tr>
<tr>
<td></td>
<td>(( \mu )U mg⁻¹ protein)</td>
<td>Enrichment (fold)</td>
</tr>
<tr>
<td>Na⁺/K⁺-ATPase</td>
<td>39±1.9</td>
<td>8.0±1.0</td>
</tr>
<tr>
<td>( \gamma )-GT</td>
<td>15±0.9</td>
<td>8.3±0.7</td>
</tr>
<tr>
<td>S-CCR</td>
<td>5.3±0.8</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>N-CCR</td>
<td>3.4±0.3</td>
<td>2.9±0.1</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. (N=3).
*** \( P<0.001 \) for freshwater/seawater comparison.
Enrichment factors are calculated as protein specific activity of P4 (membrane) versus protein specific activity of homogenate.
P4, fourth pellet (final membrane fraction); \( \mu \)U, microunits (nmol product formed \( \text{min}^{-1} \)); \( \gamma \)-GT, \( \gamma \)-glutamyltransferase; S-CCR, succinate cytochrome c reductase; N-CCR, NADPH cytochrome c reductase.
Membrane lipids and gill Na+/K+-ATPase in eel

Fatty acyl chain compositions for membranes prepared from freshwater- and seawater-acclimated animals are also similar between acclimation groups (Table 2). An exception to this trend is in the fatty acid 20:2 and an unidentified fatty acid with a retention time relative to 18:0 (RRT) of 3.14. This latter fatty acid is probably a polyunsaturate. Both 20:2 and RRT 3.14 levels are elevated in the seawater-acclimated animals. Three unsaturated fatty acids make up nearly 40% of the total fatty acyl chain composition of membranes from each acclimation group. These include oleic acid (18:1), which makes up 12%, and two polyunsaturated fatty acids (PUFAs), arachidonic acid (20:4), contributing 16–17%, and docosahexaenoic acid (22:6), contributing 10% of the total fatty acid complement of the gill membranes. The two most predominant saturated fatty acids, palmitic (16:0) and stearic (18:0) acids, make up 13–15% and 9–10% of the fatty acid composition, respectively. Unsaturation indices (the mean number of double bonds per fatty acyl chain) are 2.1±0.35 for membranes from both acclimation groups.

Cholesterol contents, normalized to either membrane-associated protein or phospholipid, are not significantly different for the two acclimation groups (Table 3), indicating that cholesterol levels are comparable in the gill plasma membranes of freshwater- and seawater-acclimated eels.

Discussion

Characterization of P4 membranes

A mixture of cell types is present in the gill epithelium of teleost fishes, with the majority of Na+/K+-ATPase occurring in the basolateral membranes of chloride cells (Sargent et al., 1975; Karnaky et al., 1976b; Perry, 1997). It is, therefore, most meaningful to compare lipid compositions in basolateral membranes that originate largely from this cell type to determine whether lipid restructuring contributes to the modulation of Na+/K+-ATPase activity. While the membranes present in P4 (the final membrane fraction) are likely to represent a mixture of cell types, a significant portion of the membranous material in P4 is basolateral membranes of chloride cell origin. The rationale for this is outlined below.

The eightfold enrichment of Na+/K+-ATPase in the final membrane fraction (P4) indicates that the membranes in the P4 fraction are primarily basolateral surfaces. This conclusion is based largely on the fact that the Na+/K+-ATPase is localized to basolateral membranes in gill epithelia of teleosts (e.g. Karnaky et al., 1976b). While the precise localization of a second plasma membrane marker, γ-glutamyltranspeptidase (γ-
GT), in teleost gill is unknown, it is quite plausible that localization of γ-GT in teleost gill may be basolateral given (1) that the enzyme has virtually the same enrichment factor as Na+/K+-ATPase (Table 1) and (2) that γ-GT, in addition to its apical distribution, is also associated with basolateral membranes in other epithelia such as renal proximal tubules (Spater et al., 1982).

A comparison can be made between protein-specific Na+/K+-ATPase activities in the final membrane fraction (P4) obtained in the present study with activities in a chloride cell preparation ('zone 3') reported previously (Sargent et al., 1975). (Zone 3 in the earlier study was prepared from the gill epithelium of a related species, the European eel Anguilla anguilla, using density-gradient centrifugation.) Relative distributions of epithelial cell types were estimated in zone 3. While zone 3 contained only 45% chloride cells by cell number, it contained 90% chloride cells by volume. Because of the larger volume occupied by chloride cells in this preparation, the majority of membranous material is likely to be of chloride cell origin. Preparations fortified with chloride cell membranes should have relatively high protein-specific activities of Na+/K+-ATPase. A comparison of the protein-specific activities of Na+/K+-ATPase in P4 (present study; assayed at 25 °C) and zone 3 (Sargent et al., 1975; assayed at 20 °C) reveals the following: activities from freshwater-acclimated animals are 0.310 μmol product min⁻¹ mg⁻¹ protein for P4 and 0.082 μmol product min⁻¹ mg⁻¹ protein for zone 3, while activities from seawater-acclimated animals are 0.73 μmol product min⁻¹ mg⁻¹ protein for P4 and 0.65 μmol product min⁻¹ mg⁻¹ protein for zone 3. Because protein-specific enzyme activities in P4 are higher than or comparable with those in zone 3, these data indicate that P4 contains a large proportion of basolateral membranes from chloride cells.

Lipid restructuring in salinity-acclimated and -acclimatized animals

To the author’s knowledge this is the first report detailing the lipid composition of enriched plasma membranes from the gills of salinity-acclimatized teleost fishes. While previous studies have characterized gill lipids from freshwater- and seawater-acclimated (or acclimatized) animals, they have done so using whole-gill lipid extracts in which a mixture of plasma and intracellular membrane lipids is present. While the present data reveal the expected rise (upon seawater-acclimation) in Na+/K+-ATPase activity, this increase in activity is not accompanied by any major changes in phospholipid or neutral lipid compositions. The absence of a significant difference in compositions of fatty acyl chains in gill basolateral membranes from freshwater- and seawater-acclimated eels is in keeping with the similarity of total gill fatty acids in the European eel (Anguilla anguilla) acclimated for 3 months to either fresh water or sea water (Thomson et al., 1977). In addition, gill Na+/K+-ATPase activities are higher in 1 °C-acclimated Atlantic cod (Gadus morhua) than in 8 °C-acclimated animals, yet lipid class and fatty acid compositions are the same after a 17 day acclimation period (Staurnes et al., 1994).

The absence of lipid compositional differences noted above is in contrast, however, with the results of other studies that have used either salinity-acclimated or -acclimatized (field-collected) animals. Differences in phospholipid and fatty acyl chain compositions of various tissues (including gill) are found in salinity-acclimated guppies (Daikoku et al., 1982). Variations in fatty acyl chain compositions of the gills have also been noted in the European eel (Thomson et al., 1977) and brown trout (Pagliarani et al., 1991) after the animals have been acclimatized to either fresh water or sea water (eel) or fresh water and brackish water (trout). These differences in fatty acid composition of salinity-acclimatized fishes may largely reflect dietary changes.

Despite the results presented in the present study, the possibility cannot be ruled out that lipid restructuring (when it does occur and regardless of its bases) plays some role in altering the catalytic character of Na+/K+-ATPase during salinity adaptation. Variation in discontinuity temperatures in Arrhenius plots of Na+/K+-ATPase correspond with differences in the relative abundances of certain polyunsaturated fatty acids in salinity-acclimatized teleosts (Thomson et al., 1977). Thomson et al. (1977) reported striking differences in the relative amounts of 20:4 and 22:6 in the gills from freshwater- and seawater-acclimatized Anguilla anguilla. Gill lipids from freshwater-acclimatized animals consist of 22% of 20:4 and only 8% of 22:6, while seawater animals are particularly rich in 22:6 (19%) with relatively low amounts of 20:4 (9%) (Thomson et al., 1977). These data suggest that at least some of the catalytic discrepancies between the enzyme in freshwater and seawater animals may be determined by lipid remodelling.

A role for lipid restructuring during salinity change may be of heightened importance in apical membranes compared with basolateral domains of the gill epithelium. As pointed out by Hazel and Williams (1990), membranes that face varying ionic environments (such as the apical membranes of the gill) may need to undergo compositional changes to ameliorate changes in chemical and physical properties of the membranes that could otherwise occur. Changes in the lipid compositions of apical membranes have been reported for intestinal brush border from rainbow trout after transfer to sea water (Leray et al., 1984). After only 1 day of seawater acclimation, the brush-border membranes have twofold or greater levels of 22:6 in PC and PE (PC representing approximately 45% and PE approximately 22% of the phospholipid class composition of the membrane) than their freshwater counterparts (Leray et al., 1984). Lipid restructuring in this case is not induced by dietary differences. Significant differences are also detected in the relative levels of the saturated fatty acid 18:0, levels of which decline in the brush-border membranes upon seawater acclimation (Leray et al., 1984).

Organisms whose environments span freshwater, marine or brackish environments undergo either (1) a profound metamorphosis (e.g. diadromous fishes such as the American eel) affecting the behaviour, morphology, physiology and
biochemistry of the animal, or (2) adjustments in the absence of metamorphosis (e.g. many intertidal or estuarine organisms). The catadromous adult eel undergoes a ‘silversing’ process that transforms a yellow-phase eel into a silver-phase individual. This metamorphosis entails marked changes in both the locomotory musculature (Egginton, 1986a,b) and the ultrastructural attributes of the gill epithelium (Fontaine et al., 1995). Silvering occurs prior to the downstream migration of the eel through the estuary and is presumably hormonally driven (Fontaine et al., 1995). The time required to migrate through the estuary (i.e. to make the transition between freshwater and seawater environments) is relatively short (4 days; Barbin et al., 1998) and, therefore, the animal has probably acquired most, if not all, of the necessary changes during metamorphosis for life as a marine teleost.

Some of the responses in a yellow-phase eel acclimated to sea water would be expected to be similar to those changes that occur during silverying in the eel. Elevated activities of gill Na+/K+-ATPase, compared with the freshwater condition, appear to be a requisite for life in sea water. Whether different mechanisms are responsible for the increase in enzyme activity in animals acclimated to sea water compared with those undergoing metamorphosis, or even for organisms living in an estuary and experiencing daily salinity fluctuations as a result of tidal cycling, is not known. Seawater-acclimated fishes show changes in Na+/K+-ATPase subunit expression concomitant with altered activities of gill Na+/K+-ATPase (Luke et al., 1993; Cutler et al., 1995; Lee et al., 1998). While altering the rates of subunit gene expression may also be a mechanism for altering the catalytic activity of Na+/K+-ATPase during metamorphosis, it is still plausible that lipid restructuring may also contribute because dramatic changes in fatty acid composition in other tissues have been reported in metamorphic trout (Sheridan et al., 1985).

In summary, the data presented here demonstrate that lipid restructuring in the basolateral membranes of the gill does not contribute to increases in Na+/K+-ATPase activity during seawater-acclimation of yellow-phase eels. This does not, however, exclude the possibility that lipid remodelling may provide a mechanism for influencing enzyme activity in salinity-acclimatized, metamorphic and/or intertidal animals. The underlying mechanism(s) for modulation of Na+/K+-ATPase activity during these different scenarios remain(s) to be elucidated.

Many thanks to Dr Barbara Kent, Gary Gorczyca and the rest of the staff at the Mount Desert Island Biological Laboratory. I especially thank Dr R. Patrick Hassett for all his help with the acclimations and Dr Jeffrey Hazel for comments on the manuscript. This research was supported by NSP 315628952.

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