Hypotonicity-induced Exocytosis of the Skate Anion Exchanger skAE1

ROLE OF LIPID RAFT REGIONS*

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Upon hypotonic volume expansion, skate erythrocytes lose solutes via a pathway that requires participation of anion exchangers (AEs). Three skate AE isoforms (skAEs) are expressed, and at least skAE1 has been shown to mediate this effect when expressed in oocytes. Under isoosmotic conditions, only a small fraction of skAE1 is expressed on the external plasma membrane. Under these conditions, a portion of skAE1 may be found in non-ionic detergent-insoluble regions. However, the detergent-insoluble material is found intracellularly. Cellular volume expansion by hypoosmotic volume expansion but not volume expansion by isoosmotic medium by permeant solutes (ethylene glycol, diethyl urea, or ammonium chloride) stimulates the appearance of skAE1 in the external plasma membrane, and a significant portion of this is found in detergent-insoluble regions. Upon hypoosmotic volume expansion nearly half of the skAE1 is found as oligomers. SkAE1 in these detergent-insoluble fractions is highly tyrosine phosphorylated. These data suggest that volume expansion by hypoosmotic medium stimulates movement of skAE1 from an intracellular pool contained in detergent-insoluble lipid rafts to the plasma membrane. This skAE1 associates to form oligomers that could be involved in the solute efflux that occurs upon volume expansion.

Regulation of cell volume is a requirement for nearly all cells. Upon volume expansion, cells will swell and must lose water. To decrease volume, cells will undergo a regulatory volume decrease that involves the loss of a number of solutes and, therefore, water, that follows these solutes. A large number of pathways exist in various cells to accomplish this regulatory volume decrease. Electrolytes, notably K⁺ and Cl⁻, may exit through KCl cotransport in a variety of cell types (1–6). Many cells utilize the efflux of non-metabolized non-electrolytes such as betaine, sorbitol, and the α-amino acid taurine (for reviews, see Refs. 7 and 8). The nature and regulation of pathways for solute efflux that occurs upon volume expansion.

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††MATERIALS AND METHODS

Isolation and Taurine Uptake of Skate Erythrocytes—Little skates (Raja erinacea) were caught off Frenchman’s Bay, ME, or Woods Hole, members of the anion exchanger (AE) family. Although best known for their role in Cl⁻/HCO₃⁻ exchange in red cells, AEs appear to participate in the loss of taurine in red blood cells of certain species, including trout (9, 10), eel (11), and skate (12–14). AEs have been cloned from trout and skate, and while many similarities with human and murine AEs exist, significant differences are present as well (15, 16). Indeed expression of trout or skate red cell AEs, but not human or murine red cell AEs, in oocytes results in increased transport of taurine (15, 17). These data support past work using pharmacologic inhibition of red cell AEs, which block volume-expanded stimulated taurine efflux, as well as a phylogenetic approach demonstrating that red cells from species that have little or no AE activity (e.g. lamprey and hagfish) do not elicit volume-expanded stimulation of taurine efflux.

Regulation of transporter activities is complex and may involve multiple mechanisms of regulation. Transporters exist in the external or plasma membrane, and their activity in this location may be regulated directly by biochemical events such as phosphorylation or association with adaptor/accessory proteins. Transport activity may also be regulated by the number of transport proteins inserted into the external membrane through exocytosis. A previous study on skate red blood cells demonstrated increased binding of a ligand for the AEs, e.g. the stilbene DIDS (18), during hypotonic volume expansion. Using proteolytic digests, no new binding sites could be identified. It appeared that additional transporters may have been introduced into the plasma membrane. The goals of the present study were to determine whether additional skAE1 could be identified in the plasma membrane and to determine the membrane domain(s) in which the exchangers reside.

Non-ionic detergents have been used to define regions of the plasma membrane that are rich in cholesterol and glyco-/ sphingolipids with high saturated fatty acids. Due to the lipid interactions, these regions are insoluble in many mild, non-ionic detergents and have been called lipid rafts (19, 20). Recent work supports a large diversity of these lipid raft regions with respect to protein as well as lipid composition. Therefore, we aimed to determine the distribution of skAE1 in these lipid raft regions upon volume expansion.

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Hypotonicity-induced Exocytosis of skAE1

**Fig. 1.** Hypotonicity-induced volume expansion stimulates appearance of skAE1 on the surface of skate erythrocytes. Cells were kept in isoosmotic medium (940 EIM), exposed to hypotonic medium (460 EIM) for 10 min, or volume expanded in isoosmotic medium with the inclusion of the permeant solutes ethylene glycol, diethyl urea, or ammonium chloride (specific concentrations are listed in text, all for 30 min). Cells were surface-labeled with sulfo-NHS-biotin for 30 min at 4 °C. Ghosts were isolated, and subsequently membrane fractions of the ghosts were isolated. Western blotting of the fractions was determined using the procedure in “Materials and Methods.” Images shown are representative of images from four separate experiments. Densitometry was performed using NIH Image version 1.54 software; data are means ± S.E. *p < 0.05 compared with the value of the same group in isoosmotic cells by analysis of variance with a Bonferroni correction. Surf, surface.

MA and kept in running seawater. Blood was removed from a tail vessel into a heparinized syringe. Cells were pelleted (400 × g for 2 min at room temperature), and the plasma and buffy coat were removed by aspiration. Erythrocytes were resuspended in 5 volumes of isotonic (940 mosmol/liter) elasmobranch incubation medium (940 EIM) (300 mmol/liter NaCl, 5.2 mmol/liter KCl, 2.7 mmol/liter MgSO4, 5 mmol/liter CaCl2, 370 mmol/liter urea, 15 mmol/liter Tris, pH 7.4), washed twice, and resuspended at 50% hematocrit in 940 EIM. To volume expand the cells, erythrocytes were diluted 1:10 into 460 EIM (NaCl was reduced to 100 mm, and urea was reduced to 250 mm). To each incubation, [H]taurine (PerkinElmer Life Sciences) was added (1 μCi/ml), and at varying times thereafter, aliquots were removed, added to appropriate osmolarity EIM with 10 μm unlabeled taurine, and pelleted (600 × g for 2 min at room temperature). Pellets were resuspended in 5 ml of appropriate osmolarity EIM with 0.1 μm taurine, pelleted, and washed a second time in these media. Pellets were extracted with 10% (v/v) perchloric acid, allowed to sit on ice for at least 15 min, and protein-precipitated (2000 × g for 5 min at room temperature); the supernatant was removed; and the pH was quantitated by liquid scintillation spectrometry.

Separation of skAE1 in Varying Membrane Domains—Red blood cell membranes were isolated by protocols published previously (21–24). Briefly red cell ghosts were made by lysing cells in 20 volumes of 10 mm Tris, pH 7.2, 5 mM EDTA with protease inhibitors (Complete inhibitor mixture, Roche Applied Science) and phosphatase inhibitors (0.1 mM NaF, 5 mM EDTA with protease and phosphatase inhibitors (Complete inhibitor mixture, Roche Applied Science). To surface biotinylate, cells were exposed to sulfo-NHS-biotin (Pierce) for 60 min. Biotin reacts with free amine groups (as the biotin does not react with the biotin, as the biotin reacts with free amine groups (as the biotin). skAE1 was characterized previously (27). After primary antibody, blots were washed five times with T-TBS, incubated with peroxidase-conjugated secondary antibody for 60 min, washed four times with T-TBS, washed once with TBS, and then developed using an enhanced chemiluminescence system (SuperSignal, Pierce). When blots were analyzed for phosphotyrosine, bovine serum albumin (3%, w/v) replaced the milk in the blocking solution. The 4G10 monoclonal anti-phosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY) was used. Other antibodies used were rabbit polyclonal anti-flicillin-2 (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal anti-caveolin-1 (Upstate Biotechnology, Charlotteville, VA). Densitometry of all blots was performed using NIH Image version 1.54 software.

Surface Biotinylation—To label proteins that may expose portions to the cell exterior, the cell-impermeant labeling agent sulfo-NHS-biotin (Pierce) was used. Since biotin reacts with free amine groups (as the e-amino groups of lysine residues in proteins), all amine groups, including Tris and urea, were removed from the medium and replaced with HEPES and mannitol. Since mannitol is not generally used in our elasmobranch buffers, its effect on hypotonic stimulated taurine efflux was determined. Cells exposed to isosmotic buffer with mannitol did not demonstrate increased basal taurine efflux, and hypotonic medium with mannitol did not alter the stimulated efflux (data not shown). The osmolarity of all buffers was confirmed using a vapor point osmometer (Wescor, Logan, UT). To surface biotinylate, cells were exposed to medium compositions, pelleted, and chilled to 4 °C to minimize endocytosis and exocytosis. Because certain buffers contained reagents that might react with the biotin, e.g. ammonium chloride or diethyl urea, analyzed in all fractions by the procedure of Hilderson (25) and was found to correlate with an increased absorbance at 600 nm. Therefore, in subsequent experiments, fractions were analyzed by absorbance, and appropriate fractions were pooled and concentrated using a 10-kDa molecular mass cut-off filter (Amicon, Milford, MA) to approximately one-fifth volume. Protein concentrations, whether in membranes or in concentrated raft fractions, were determined using the bicinchoninic acid procedure (26). Samples were mixed with 3× Laemmli stop solution, heated to 65 °C, and analyzed by Western blotting within 2 days.

**Western Blot Analysis**—Proteins were resolved by 7.5% SDS-PAGE except for oligomerization experiments in which a 5% acrylamide concentration was used. Completed gels were immediately transferred to polyvinylidene difluoride membranes (Polyscreen, PerkinElmer Life Sciences) using 10× transfer buffer (25 mM Tris, 192 mM glycine, pH 8.8, with 10% (v/v) methanol). Blots were blocked in 5% (v/v) nonfat dry milk in Tris-buffered saline containing Tween 20 (T-TBS; 140 mmol/liter NaCl, 5 mmol/liter KCl, 10 mmol/liter Tris, pH 7.4, with 0.05% (v/v) Tween 20) for 60 min. Blots were incubated overnight at 4 °C with a polyclonal antiserum directed against 17 amino acids of the amino terminus of isoform 1 of the skate anion exchangers (skAE1) characterized previously (27). After primary antibody, blots were washed five times with T-TBS, incubated with peroxidase-conjugated secondary antibody for 60 min, washed four times with T-TBS, washed once with TBS, and then developed using an enhanced chemiluminescence system (SuperSignal, Pierce). When blots were analyzed for phosphotyrosine, bovine serum albumin (3%, w/v) replaced the milk in the blocking solution. The 4G10 monoclonal anti-phosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY) was used. Other antibodies used were rabbit polyclonal anti-flicillin-2 (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal anti-caveolin-1 (Upstate Biotechnology, Charlotteville, VA). Densitometry of all blots was performed using NIH Image version 1.54 software.

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RESULTS

Distribution of skAE1 upon Volume Expansion—The amount of skAE1 expressed on the surface of the cells as well as in the non-ionic detergent-resistant membrane (DRM) fractions was first determined. To determine the amount of skAE1 in the external plasma membrane, cells were incubated in isoosmotic or hypotonic conditions in buffers modified so that no free amines were available for the surface biotinylation (replacing Tris with HEPES and urea with mannitol). Under isoosmotic conditions, a small percentage of cell skAE1 was labeled with the cell-impermeant biotin. However, the labeling increased significantly 10 min after hypotonic volume expansion (Fig. 1, top panels, second lanes, Surf). There was no change in the total cell skAE1 expression over this time (Fig. 1, top panels, first lanes, Total). Replacement of NaCl and mannitol with permeant solutes such as ethylene glycol, diethyliurea, or ammonium chloride also causes volume expansion. However, the kinetics of the response differs from hypotonic medium-stimulated volume expansion. Whereas hypotonic medium-stimulated volume expansion occurs within 5–15 min, volume expansion using permeant solutes in isoosmotic medium requires 30 min. Functionally little or no increase in efflux of the amino acid taurine occurs in permeant solute-stimulated volume expansion in contrast to hypotonic-stimulated volume expansion that stimulates taurine efflux in 5–15 min (14). Volume expansion using isoosmotic media with permeant solutes did not result in the appearance of skAE1 on the surface (Fig. 1, bottom panels, second lanes). Therefore, the appearance of skAE1 on the surface was an effect unique to hypotonic medium-stimulated volume expansion.

To determine how much of each pool, total and surface-expressed, was in detergent-insoluble regions, skAE1 was analyzed in membranes solubilized with the non-ionic detergent Triton X-100. This fraction was analyzed as total Triton-insoluble material or DRM. Under isoosmotic conditions, a significant percentage could be found in DRM (Fig. 1, top left panels, third lanes, DRM), and this increased slightly when isolated from hypotonic volume-expanded cells (Fig. 1, top right panel, third lane, DRM). No changes were noted in DRM from cells after 30 min in isoosmotic permeant solute volume-expanding media (Fig. 1, bottom panels, third lanes, DRM). To determine how much skAE1 in the DRM was surface-expressed, DRMs were isolated from cells that had been surface-labeled with biotin, and biotinylated DRM material was isolated with streptavidin. Although skAE1 was present in the

![Fig. 2. Hypotonicity-induced Exocytosis of skAE1](image-url)
from four separate experiments. *, isoosmotic condition, only data from membranes from cells in hypoosmotic condition are presented. Images shown are representative of images conditions, and C provides data from both buoyant (raft) fractions as well as denser material at the bottom. As tetramers are not present under isoosmotic conditions, only data from membranes from cells in hypoosmotic conditions are presented. Images shown are representative of images from four separate experiments. *, p < 0.05; +, p < 0.01 compared with isoosmotic by paired Student's t test. Iso, isoosmotic; Hypo, hypoosmotic; Sol, soluble; Insol, insoluble; IP, immunoprecipitation; PY, phosphotyrosine.

To more completely analyze the nature of skAE1 in DRM, skAE1 in DRM was separated on continuous sucrose density gradients. SkAE1 in DRM may exist in a number of domains. Some DRM material may be in lipid raft regions that are buoyant due to their lipid nature. As shown in Fig. 2, skAE1 is found at both the bottom and the surface of sucrose gradient DRM from cells under hypoosmotic and hypotonic conditions. The fractions were tested for cholesterol, and those fractions that contained the buoyant skAE1 contained cholesterol as opposed to neighboring fractions that contained little or no cholesterol. These fractions also had a higher cholesterol/protein ratio than the material at the bottom of the sucrose gradient (data not shown). As confirmation that these buoyant fractions of the sucrose density gradients represented membrane lipid raft regions, fractions were also analyzed for two markers of lipid raft regions. Caveolin-1 has been demonstrated to be highly enriched in certain lipid rafts and may coordinate their structure. Additionally, proteins of the band 7.2 family, such as flotillin and stomatin, have been noted to be enriched in these buoyant membrane lipid raft regions (28). As shown in Fig. 2, top panel, those fractions that contained skAE1 and possessed cholesterol also had both caveolin-1 and flotillin-2, further confirming that these fractions represented the membrane raft domains. The panels presented for caveolin-1 and flotillin-2 are from a sample of cells in hypoosmotic medium; however, similar patterns were observed when DRMs from cells in isoosmotic medium were analyzed (data not shown).

To determine whether the material in the fractions was surface-expressed, skAE1 was isolated from the sucrose gradient DRM fractions after hypotonic stimulated volume expansion (22). Under isoosmotic conditions, skAE1 is found nearly in an equivalent distribution as monomers and dimers as compared with hypoosmotic conditions, which resulted in the formation of tetramers (Fig. 3A). No skAE1 tetramers could be found under isoosmotic conditions as expected, and the tetramer form could be detected in both Triton-soluble and -insoluble forms (Fig. 3B, lanes 3 and 4). To determine whether the tetramers were in the buoyant raft fractions of the detergent-insoluble membranes, this material was separated on sucrose gradients, and membranes from the bottom as well as the buoyant fractions were analyzed. SkAE1 tetramers could be detected in both buoyant raft fractions as well as in the membranes at the bottom of the gradient (Fig. 3C).

Phosphotyrosine Levels of skAE1—Upon volume expansion, tyrosine phosphorylation of skAEs increases. We first analyzed phosphotyrosine of non-modified skAE1. In this case, skAE1 runs as a 97-kDa protein. SkAE1 was immunoprecipitated from isoosmotic and hypoosmotic cells as presented in Fig. 4, far left panel. While skAE1 is tyrosine phosphorylated under basal conditions, this increases ~90% upon volume expansion.

To determine the state of tyrosine phosphorylation of the lipid raft skAE1 and whether this increase was associated with the appearance in the plasma membrane, skAE1 was immunoprecipitated from both Triton-soluble and -insoluble fractions after cross-linking. Under basal conditions, skAE1 in both the monomer and dimer is tyrosine phosphorylated, and this is...
true for skAE1 in both Triton-soluble and -insoluble fractions (Fig. 4, A and B). Tyrosine phosphorylation of skAE1 tetramer increases under hypoosmotic conditions (Fig. 4A), and this may be found in both Triton-soluble and -insoluble fractions, although the greater increase occurs in the Triton-insoluble fraction. In A, to determine that increased phosphotyrosine was observed when samples from BS3 cross-linked samples were used, blots were scanned, and the densitometric units of the monomer, dimer, and tetramer were summed. As shown Fig. 4A, below the right panel, increased phosphotyrosine levels were detected.

The levels of tyrosine phosphorylation of the tetramers in the buoyant raft fraction as well as the denser material at the bottom fractions of the sucrose gradient were determined. In both fractions, skAE1 phosphorylated tetramers were detected (Fig. 4C).

**Effect of Cholesterol Depletion on Volume-expanded Stimulated Taurine Uptake**—Volume expansion of skate erythrocytes results in a rapid loss of taurine. This increased permeability may also be measured as increased taurine uptake as the permeability is bidirectional (11, 12). To determine whether cholesterol-rich lipid regions of the membrane were involved, erythrocytes were treated with the cholesterol-depleting agent methyl-β-cyclodextrin (MBCD) at 2.5 mM for 30 min in isoosmotic (940) EIM prior to taurine uptake measurement. MBCD was removed by washing cells in isoosmotic EIM once prior to initiation of uptake. Disruption of lipid raft regions was confirmed by analyzing samples of cells after 30 min in isoosmotic, hypoosmotic, or hypoosmotic conditions after treatment with MBCD by measuring the cholesterol content of all fractions of the detergent-resistant material placed on sucrose gradients. Protein concentrations of the fractions were also determined. Within 30 min, the appearance of fractions with cholesterol was decreased nearly 35%, and by 60 min, over half of the cholesterol of what would be predicted in the “peak” fractions (fractions 8 and 9) was decreased (2.61 ± 0.44 to 1.33 ± 0.27 μg/10⁸ cells, n = 3). This decrease was also observed at the level of protein concentrations in these fractions (decreasing from 74.8 ± 12.2 to 41.1 ± 14.6 μg/10⁸ cells, n = 3) using 2.5 mM MBCD. The amount of cholesterol in the non-buoyant fractions at the bottom of the sucrose gradient (fractions 19 and 20 corresponding to 40% (w/v) sucrose) also decreased, but the percentage of change was less (4.79 ± 0.96 to 3.96 ± 0.97 μg/10⁸ cells) and protein values were 231 ± 16 and 249 ± 26 μg/10⁸ cells, respectively (n = 3). Lower concentrations were effective but were more variable, and 5 mM MBCD had approximately the same effect as 2.5 mM MBCD. Treatment with 2.5 mM MBCD inhibited volume-expanded stimulation of taurine efflux by ~50% at both 30 and 60 min (Fig. 5). Although not shown, MBCD had no effect on taurine uptake in 940 medium, suggesting that at least over this time and at this concentration, nonspecific permeability to taurine was not affected by MBCD.

To determine the effect of MBCD on distribution of total and surface-expressed skAE1, skAE1 in DRMs and surface-expressed skAE1 in DRMs were analyzed as in Fig. 1. Samples were taken at 30 min after initiation of uptakes and, when appropriate, immediately chilled to 4 °C and biotinylated as previously described. Similar to results on taurine uptake, MBCD pretreatment partially inhibited the appearance of skAE1 on the surface and reduced the amount in the surface detergent-resistant membranes (Fig. 5).

**DISCUSSION**

Regulation of membrane proteins, including transporters, occurs through a variety of processes. These may include phosphorylation, regulation by association with or attachment to membrane lipids, or association with adaptor and accessory proteins. All of these possibilities appear to occur for the skate homolog of band 3, the human red blood cell anion exchanger...
allowing for efflux of solutes such as taurine, \(29\). Past results suggest that skAE1 participates in a permeability formed when cell volume is expanded hypoosmotically, allowing for efflux of solutes such as taurine, \(\beta\)-alanine, myo-inositol, and betaine (11–14). The present results suggest that one level of regulation of this activity may be due to exocytosis of skAE1 from an intracellular vesicular compartment, where it resides within lipid rafts, into the plasma membrane.

A significant percentage of skAE1 is found in DRMs in all, even isoosmotic, conditions. However, little DRM skAE1 from cells in isoosmotic medium is expressed on the surface, but this increases with hypoosmotic stimulated volume expansion. We hypothesize that skAE1 in an intracellular pool in DRM is altered and undergoes exocytosis to reside in DRM in the surface membrane. SkAE1 in DRM under isoosmotic conditions as well as hypoosmotic stimulated volume-expanded conditions is in both buoyant as well as non-buoyant membranes (on a sucrose gradient). We cannot prove that exchange between skAE1 in these two regions, buoyant and non-buoyant material regions, does not occur, but the simplest hypothesis is that it is skAE1 in a buoyant DRM region that is intracellular and is stimulated to fuse with the plasma membrane and thus is surface-expressed skAE1 in buoyant fractions of the DRM. We cannot exclude movement of skAE1 from detergent-soluble to detergent-resistant as there is a small increase upon hypoosmotic stimulated volume expansion. If this is the case, we believe that it accounts for only a small percentage of the increased surface skAE1 in buoyant material of the DRM.

SkAE1 in the detergent-resistant regions is tyrosine phosphorylated, and this increases upon hypoosmotic stimulated volume expansion. This may be due to the tyrosine kinase p72syk, which we have previously demonstrated is stimulated by hypotonic volume expansion (21). Additionally skAE1 in the lipid raft fractions that face outside the cell forms tetrameric complexes.

The role of specific membrane domains in cell signaling has received considerable attention over the past decade (30–33). Many proteins are preferentially directed to specific domains, and therefore functional events that require these proteins may only occur in these limited regions. The existence of lipid raft regions was first hypothesized by Simons and Ikonen (31) to explain the sorting of specific proteins into sphingolipid regions of the apical and basolateral membranes of epithelial cells. Subsequent investigations have demonstrated heterogeneity in the buoyant membrane regions (32, 33). We and others have used cholesterol-depleting agents to investigate this possibility. The lipid raft regions possess a high cholesterol/protein ratio, and their structure may be more easily disrupted than membrane domains with less cholesterol. Therefore, rapid inhibition of a functional response using cholesterol-binding agents such as methylcycloextrim may provide an early clue that lipid rafts play a pivotal role in one step of the process. Therefore, the ability of cycloextrim to inhibit taurine transport stimulated by volume expansion suggests that the lipid rafts of the plasma membrane are important in this functional response.

Depending on the cell type, diverse proteins preferentially associate with the raft regions including immunologic receptors such as the T-cell receptor or Fcy (34, 35), hormone receptors (36, 37), and transporters such as sodium/hydrogen exchangers and a number of types of ion channels (38, 39). In particular for the red blood cells, flotillin and stomatin as well are notably concentrated in the raft regions (28, 40, 41). It is tempting to speculate that the differential presence of these proteins in the lipid raft regions regulates their functions.

A number of proteins in lipid raft regions may form oligomers similar to skAE1, e.g. the T-cell receptor and the IgE receptor. Oligomerization and complex formation may be important steps in subsequent cell activation. In the lipid raft regions, it is possible that skAE1 comes into proximity to tyrosine kinases and other regulators that alter the structure sufficiently to allow changes in function. We speculate that modification by phosphorylation and/or association with adaptor proteins may participate in the ability of skAE1 to affect permeability for taurine. Previous work has demonstrated decreased band 4.1 binding and increased ankyrin binding with skAE1 upon hypoosmotic stimulated volume expansion (22, 24). Additionally the level of tyrosine phosphorylation increases (present studies and Ref. 21). However, the interaction of skAE1 with the cytoskeletal proteins and tyrosine kinases such as p72syk as well as the interaction of these proteins with membrane fractions under normal volume as well as expanded conditions is presently only partially understood.

The activity of a number of transport proteins, carriers and channels, may be modulated by the membrane lipid environment in which the transporter resides. The human homolog of skAE1, erythrocyte band 3, may be expressed on renal intercalated cells. Band 3, AE1, may be expressed on the apical side of these cells; however, when plated on a specific extracellular matrix, the band 3 is expressed on the basolateral side (42). The basolateral membrane of these cells is rich in sphingolipids, while the apical membrane is ganglioside-rich. The hypothesis arose whether band 3 had differential function based on its lipid environment in the basolateral versus apical membrane. Artificial membranes were used mimicking the apical or basolateral membrane lipid composition, band 3 was added, and activities were measured. When inserted into the sphingolipid-rich membranes, band 3 possessed exchanger activity that was sensitive to stilbenes, while band 3 expressed in ganglioside-enriched membranes is less active and not inhibited by stilbenes. Thus, the membrane environment appears to regulate band 3 function. Activity of a different Cl– transporting protein, the channel \(\text{I}_{\text{Cl,swell}}\) is modulated by expression of caveolin-1b (43). Using cells that express \(\text{I}_{\text{Cl,swell}}\) but not caveolins, it was demonstrated that introduction of the caveolin isoform 1b (expressed on surface membranes) but not 1a (expressed on internal membranes) increases the activity of \(\text{I}_{\text{Cl,swell}}\). \(\text{I}_{\text{Cl,swell}}\) appeared to associate with the newly formed caveolin-1b generated lipid rafts, thereby increasing its function.

In conclusion, hypoosmotically induced volume expansion stimulates the appearance of skAE1 on the plasma membrane of skate erythrocytes. A large percentage of the surface-expressed skAE1 is in buoyant regions of the plasma membrane. This skAE1 appears to be initially in an intracellular membrane compartment where it also resides in these buoyant membrane regions. Future work will determine whether the “exocytosis” of skAE1, which occurs under hypoosmotic stimulated but not volume expansion by permeant solutes, may be regulated by phosphorylation of skAE1 or potentially cytoskeletal interactions that may also be altered during this time.

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