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Expression of the Skate (*Raja erinacea*) AE1 Osmolyte Channel in *Xenopus laevis* Oocytes: Monovalent Cation Permeability

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Abstract. The aim of this study was to express the cloned skate anion exchanger 1 (skAE1) in *Xenopus* oocytes and determine whether the differences in monovalent cation permeabilities in hypotonically stimulated skate and trout erythrocytes could be due to differences in the presence or absence of intracellular channel regulators between the two species or in the intrinsic permeability properties of the channels themselves. The expressed protein (skAE1) was inserted into the oocyte cell membrane and facilitated both Cl^- exchange and taurine transport. Expression of skAE1 in oocytes showed similar monovalent cation permeabilities as previously reported for skate erythrocytes and different from both trout erythrocytes and trAE1 expressed in *Xenopus* oocytes. These results show that the skAE1 expressed in oocytes functions in a manner similar to that of the osmolyte channel in hypotonically activated skate erythrocytes and supports the hypothesis that differences in the monovalent cation permeabilities of the osmolyte channels in skate and trout RBCs resides in the differences in permeability properties of the channels between the two species.

Key words: Taurine — Electrolytes — Transport — skAE1

Introduction

The ability to regulate cell volume is a fundamental property in many cells, as deviations in cell volume may affect many critical cellular functions [12, 17]. Hypotonic media cause cells to swell, which in turn activates compensatory mechanisms that increase the transport of osmotically active particles followed by entrained water, effectively restoring cell volume. This process is called regulatory volume decrease (RVD). Bony as well as cartilagenous fish erythrocytes are useful models for studying this regulatory process as the survival of these fishes, which face changing osmotic environments, depend largely on their ability to regulate cell volume. During RVD, electrolytes and organic osmolytes are transported via a swelling-activated, sodium-independent, bidirectional, nonselective channel. The β-amino acid taurine is a major organic osmolyte that is transported by fish red blood cells during cell volume regulation. It has been shown that in skate and trout red blood cells, hypotonically induced cell volume increase stimulates the transport of taurine significantly [5, 6, 10].

Previous studies showed that in skate and trout erythrocytes, the swelling-activated organic osmolyte channel is blocked by inhibitors of the anion exchanger (AE1) band 3 [2, 5]. This and other evidence suggested that AE1 mediates taurine transport via a volume-activated organic osmolyte channel either by regulating the channel or by acting as the channel itself. Trout AE1 (trAE1) has been cloned and when it was expressed in *Xenopus laevis* oocytes, trAE1 induced both anion exchange and channel activities [3, 4]. Also, when expressed in *Xenopus* oocytes, the characteristics and properties of the trAE1-mediated organic osmolyte channel was different from that of the endogenous swelling-activated channel that produces the swelling-activated Cl^- current, $I_{Clswell}$, suggesting that trAE1 forms a new channel in the oocyte membrane [11].

The swelling-activated organic osmolyte channel's substrate specificity in the skate erythrocytes

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differs from that seen in trout erythrocytes. A major difference is that the swelling-activated channel is permeable to Na^+, K^+ and Rb^+ in trout [5, 8, 13], but is poorly permeable to these cations in skate [2] erythrocytes. The differences in cation permeabilities in hypotonically stimulated skate and trout erythrocytes could be due to differences in the presence or absence of intracellular channel regulators between the two species or in the intrinsic permeability properties of the channels themselves. To answer this question, in the present study, we express skate AE1 (skAE1), which has been recently cloned [9], in *X. laevis* oocytes. We show that skAE1 expression induces anion exchange activity and the formation of an osmolyte (taurine) channel in oocytes that resembles the channel in skate RBC. We then tested the channel's permeability to Na^+, K^+, and Rb^+. We found that the channel induced by skAE1 expression is poorly permeable to these three cations. These findings support the alternative that differences between the monovalent cation permeabilities of the osmolyte channels in skate and trout RBCs resides in the differences in permeability properties of the channels themselves and not in the intracellular channel regulators.

Materials and Methods

In vitro cRNA Transcription

The skate (*Raja erinacea*) RBC anion exchanger (AE1) mRNA was prepared from full-length cDNA from skate erythrocytes supplied by Dr. Helene Guizouarn. The skAE1 cDNA was cloned in pGEM T-easy vector (Promega, Madison, WI). The skAE1 cDNA was linearized by SacII downstream of the cDNA before in vitro transcription. Two μg of linearized DNA was transcribed for 2 h at 37°C with a mixture of SP6 RNA polymerase, RNase-free H_2O (Promega), capping agent GpppG and premixed rNTPs [rGTP, rATP, rCTP, rUTP]. RNAse-free DNAse was added and the mixture was incubated for 20 min at 37°C. The capped RNA was extracted with phenol/chloroform. Isopropanol was added to the extract and the mixture was incubated at −80°C for 15 min, microcentrifuged for 5 min and then the isopropanol was poured off. The pellet was washed twice in 70% EtOH, air dried, then resuspended in RNAse free H_2O with RNAsin. RNA was stored at −80°C. An aliquot was analyzed by agarose-formaldehyde gel electrophoresis.

Oocyte Injection

The oocytes were removed from adult female *X. laevis* anesthetized with 0.3% 3-aminobenzoic acid ethyl ester. The oocytes were maintained at 18°C in ND 96 (in mM: 96.0 NaCl; 2.0 KCl; 1.8 $CaCl_2$; 1.0 $MgCl_2$; 5.0 HEPES; pH 7.5; 210 mOsm, filtered). The isolated oocytes were defolliculated by treatment with 2 mg/mL collagenase and 1.0 mg/mL trypsin inhibitor in calcium-free OR2 (oocyte Ringers, in mM: 82.5 NaCl, 2.5 KCl, 5.0 HEPES, 1.0 $MgCl_2$) for 40 minutes. The collagenase and trypsin inhibitor were aspirated off and the oocytes were washed in calcium-free OR2 three times. Stage V–VI oocytes were then selected and maintained in ND96 supplemented with penicillin (10 U/mL) and streptomycin (10 μg/mL) and sodium pyruvate (2.5 mM) at 18°C overnight and then injected with skAE1 cRNA (50 nL at a concentration of 6 ng/50 nL). The control group consisted of oocytes that were defolliculated, selected and maintained as mentioned above, then injected with 50 nL RNAse-free water.

Taurine Uptake Measurements

For taurine uptake experiments, 8–12 oocytes injected with skAE1 were first washed in ice-cold ND 96 then transferred to wells containing 0.4 mL ND96, 1 mM taurine and ^{3}H-taurine with a specific activity of 40,000 cpm/nmol taurine. Control groups were water-injected oocytes incubated in ND96, 1.0 mM taurine and ^{3}H-taurine. After incubating for a designated time, the oocytes were washed twice in ice-cold media and quickly transferred to scintillation vials. Excess extracellular fluid was quickly aspirated and 20 μL of 20% SDS were added to all the tubes. 10 μL of the incubation medium at time $t = 0$ from each group was counted in duplicate. Liquid scintillation fluid was added to scintillation vials, vortexed, then placed in a liquid scintillation spectrometer and analyzed for ^{3}H-taurine. Results are presented as pmol taurine/h/oocyte. 96 mM N-methyl-D-glucamine was substituted for 96 mM NaCl (in ND96) to determine Na^+ dependence of taurine transport; the former solution was adjusted to the same osmolarity as the latter, 210 mOsm.

Measurement of ^{36}Cl Exchange

^{36}Cl exchange experiments were performed in skAE1-injected oocytes. The skAE1 oocytes were injected with ^{36}Chloride (50 nL of 130 mM Na ^{36}Cl, 25 mM HEPES) then quickly washed in ice-cold ND96. The oocytes were then placed in wells with 1.0 mL of ND96. At $t = 0$ and at 3-minute intervals, for 12 minutes, 950 μL of media were taken out of the wells and placed in liquid scintillation vials and replaced with 950 μL fresh ND96. At the 12-minute time point, the ND96 was replaced with gluconate medium (replacing all chloride with gluconate, molar concentrations of sodium, potassium, magnesium, calcium and HEPES remained the same as the concentrations found in ND96; osmolarity was adjusted to 210 mOsm) and at each time point, up to 24 minutes, gluconate medium was used. At the 24-minute time point, the gluconate medium was removed and replaced with ND96 and at each time point, for the remainder of the experiment, ND96 was used. After the last time point, oocytes were removed from the wells, washed in ice cold medium and transferred to scintillation vials. The excess extracellular fluid was quickly removed and 20 μL of 20% SDS were added to all tubes. Liquid scintillation fluid was added to all the tubes. The tubes were then vortexed and placed in a liquid scintillation analyzer to determine ^{36}Cl activity for each oocyte and the ^{36}Cl that was present in the medium at each time point. Results are presented as log % ^{36}Cl remaining in the oocyte.

Determination of Oocyte Monovalent Cation Content

Oocytes were extracted, defolliculated and injected as described above. Oocytes were allowed to incubate at 18°C for 72 hours. Fifteen oocytes were quickly washed three times in 15 mL of milliQ water (Millipore) and placed in microcentrifuge tubes. The excess water was removed using a micropipette and the oocytes were allowed to dry overnight (~9 hours) in an oven set at 80°C. Dried oocytes were weighed to determine dry cell solids. Intracellular ions were extracted by suspending dried oocytes in 1 mL of milliQ water

overnight at 4°C. Perchloric acid (20 μL of 70% v/v PCA) was added to the suspension. After centrifugation, the clear supernatant was saved for analysis of cations. Measurements of sodium and potassium were done with a flame spectrophotometer and results were expressed as μmol/g dry weight oocyte.

^{86}Rubidium Uptake

Rubidium uptake was performed as described for taurine uptake, in NO_3^- ND96 (ND96 with $NaNO_3$ and KNO_3 replacing NaCl and KCl, respectively) and supplemented with ^{86}RbCl (specific activity 36,000 c.p.m./nmol) using an incubation time of 30 minutes.

Pharmacological Inhibitors

To determine the inhibitor sensitivity of skAE1, the procedure was followed as described above, in the presence of one of the following inhibitors/blockers: 0.1 mM DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid), 0.1 mM niflumic acid and 0.5 mM DNDS (4,4′-dinitrostilbene-2,2′-disulfonic acid). A ten-times stock solution was made in ND96 for each of the inhibitors and addition of the stock solution to the incubation medium diluted the inhibitors to the desired concentrations.

Membrane Protein Preparation and Western Blotting

The method used to determine the cellular localization of skAE1 in injected oocytes is described by Schmieder et al. [18]. A group of water- or skAE1 cRNA-injected oocytes was incubated for 72 h in ND96 medium as above. Fifteen oocytes were combined and homogenized in 5 mL lysis buffer (50 mM Tris, pH 7.4, 66 mM ethylenediamine tetraacetic acid, 1% vol/vol Triton X-100, 0.4% wt/vol deoxycholic acid, with the Complete protease inhibitor cocktail (Roche Molecular, Indianapolis, IN). Samples were homogenized with thirty strokes in a tight-fitting Teflon pestle homogenizer and centrifuged at 1000 ×*g* for 5 min at 4°C. The pellets were brought up to 500 μL in lysis solution, homogenized again, and centrifuged at 1000 ×*g* for 5 min at 4°C. This process was repeated a third time to clear the supernatant. The pellet was then homogenized in 500 μL lysis buffer and centrifuged at 70,000 ×*g* for 30 min at 4°C to obtain the microsomal pellet. The pellet was resuspended in lysis buffer, the protein concentration measured by the bicinchoninic-acid procedure, and 20 μg loaded on 10% SDS-PAGE. Samples were resolved and immediately transferred to a polyvinylene difluoride membrane. Blots were blocked in 5% Blotto (5% wt/vol nonfat dry milk in T-TBS (10 mM Tris pH 7.4, 140 mM NaCl, 5 mM KCl with 0.5% vol/vol Tween 20)) and incubated overnight with rabbit polyclonal anti-skAE1 (prepared by Alpha Diagnostic International) or Na^+/K^+-ATPase α subunit. The blots were washed five times in T-TBS, incubated with the appropriate peroxidase-conjugated secondary antibodies, washed four times with T-TBS and a last wash in TBS and developed using an enhanced chemiluminescent system (Pierce, Rockford, IL).

Materials

Female *X. laevis* were purchased from Nasco (Madison, WI) and maintained at room temperature in chlorine-free water. Sodium pyruvate, inhibitors and salts were purchased from Sigma-Aldrich Chemical. Collagenase and trypsin inhibitor was purchased from Life Technologies. $Na^{36}Cl$ was purchased from ICN Biomedicals, ^{3}H-Taurine and ^{86}Rb were purchased from NEN Life Science.

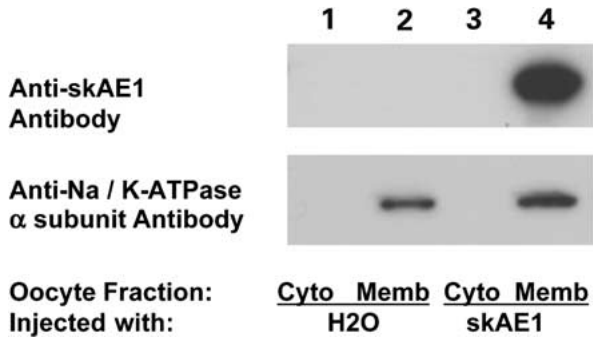

Fig. 1. Cellular localization of skAE1 expressed in *Xenopus* oocytes. A cytosolic and crude membrane preparation from control (water-injected) oocytes (lanes *1* and *2*, respectively) and skAE1-injected (lanes *3* and *4*, respectively) oocytes were tested for immunoreactivity with polyclonal antibodies raised against the C-terminus of skAE1 (*top panel*) and Na^+/K^+-ATPase α subunit (*lower panel*).

Results

Expression of skAE1 in *Xenopus* Oocytes—skAE1 Cellular Localization

Previous studies by Guizouarn and coworkers [4] showed that expression of skAE1 in *X. laevis* oocytes induces an uptake of ^{36}Cl into the oocytes. We now show that the increased uptake is due to stimulation of Cl-exchange as a result of the insertion of skAE1 into the oocyte cell membrane.

In order to show that skAE1 was expressed in *Xenopus* oocyte cell membranes, skAE1 cRNA was injected into *Xenopus* oocytes. Western blots were performed on cytosol and membrane fractions of the oocytes in order to demonstrate that skAE1 was expressed in the oocyte cell membrane. Cytosolic and crude membrane fractions were prepared from water- and skAE1 cRNA-injected *Xenopus* oocytes. These samples were examined for immunoreactivity by Western blot with polyclonal C-terminal skAE1 antibody. There was a large band (predominantly at ~95 kda) in the membranes from oocytes injected with the skAE1 cRNA, but there was no immunoreactivity in the cytosolic preparations from skAE1-injected or water-injected oocytes, nor in the membrane preparations of water-injected oocyes (Fig. 1). The membrane preparations from both water- and skAE1-injected oocytes all tested positive for the Na^+/K^+-ATPase α subunit, confirming the presence of plasma membrane in the crude membrane preparations, while the cytosolic preparations tested negative for the Na^+/K^+-ATPase α subunit. These results show that when skAE1 cRNA is injected into the *Xenopus* oocytes, the exogenous cRNA is translated by the oocytes and the protein is inserted into the cellular membrane.

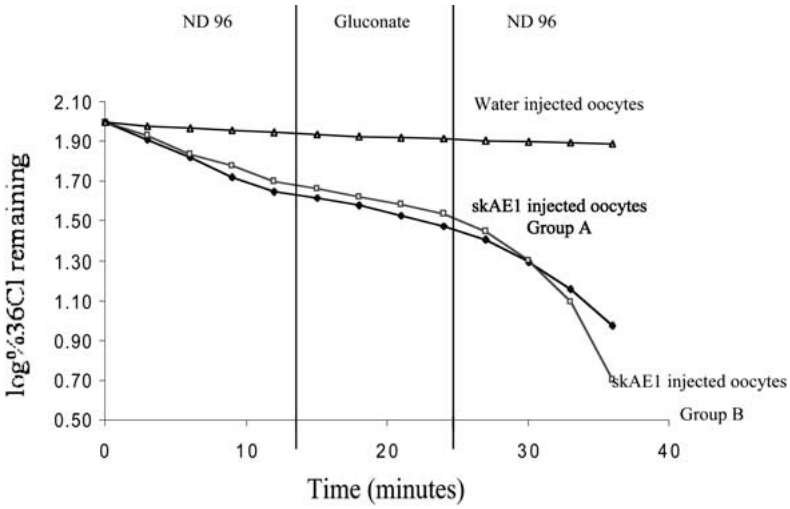

Fig. 2. Time course of chloride exchange (efflux) across the *Xenopus* oocyte plasma membrane in oocytes expressing skAE1 compared to control (water-injected) oocytes. At 12 minutes, oocytes were transferred from ND96 (210 mOsm) to Cl^--free gluconate medium (210 mOsm) and at 24 minutes, back to ND96. Values are expressed as means ($n = 10$). SE are smaller than symbols.

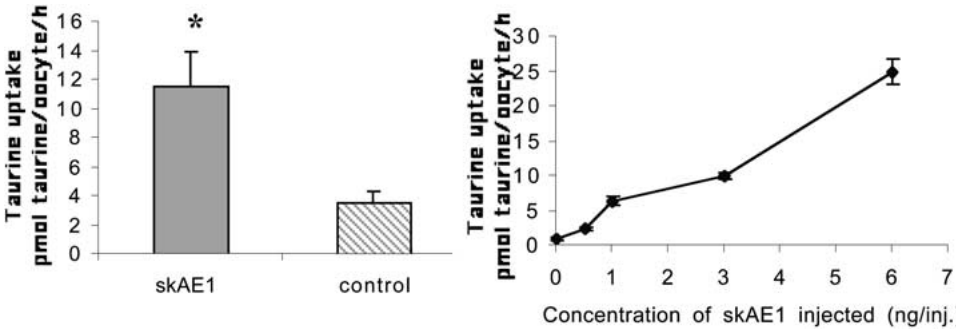

Fig. 3. Taurine uptake in *Xenopus* oocytes (post 72 h) injected with skAE1 cRNA or water. Values are expressed as mean ± SE ($n = 10$). Asterisk: data significantly different from control ($P < 0.01$) (*left panel*). Dose-response curve of increasing concentrations of skAE1 cRNA on taurine uptake. Values are expressed as mean ± SE ($n = 10$ for each concentration) (*right panel*).

Anion Exchange Activity

As shown in Fig. 2, chloride exchange activity was induced by expression of skAE1 in the oocytes. The chloride efflux was significantly greater in skAE1-injected oocytes than in water-injected oocytes in ND96, and when chloride-free gluconate medium replaced the Cl-containing ND96, chloride efflux decreased significantly and then was restored when ND96 replaced the gluconate medium. The small chloride efflux remaining in the gluconate medium may have been due to $H+/Cl^-$ cotransport, which has been shown to be present in skate erythrocytes [2]. Thus, when skAE1 mRNA was injected into *Xenopus* oocytes, a functional skAE1 protein was formed and inserted into the oocyte membrane, stimulating the exchange of Cl^- across the cell membrane.

Skate AE1-mediated Taurine Transport

In the absence of skAE1, oocytes are almost impermeable to taurine. However, skAE1 expression in *Xenopus* oocytes significantly increased taurine transport (10.7 ± 2.6 (mean ± SE) pmol taurine/oocyte/h) compared to water-injected oocytes (2.6 ± 0.7 (mean ± SE) pmol taurine/oocyte/h) (Fig. 3). Thus, the expression of skAE1 in oocytes confers the transport of the organic osmolyte, taurine.

The taurine transport was more than 80% independent of sodium. Taurine uptake in ND96 was 8 ± 0.2 pmol taurine/oocyte/h and 6.5 ± 0.2 pmol taurine/oocyte/h (mean ± SE) when sodium was replaced by N-methyl-D-glucamine. The difference between the means was not statistically significant ($P > 0.2$). In order to determine the optimal dose of mRNA for skAE1 expression, the transport of taurine was measured in oocytes injected with increasing amounts of skAE1 cRNA (0.5 ng/injection, 1.0 ng/injection, 3.0 ng/injection and 6.0 ng/injection). Figure 3 shows that there was an increase in taurine transport that correlated with the increased amount of cRNA injected. There was a significant increase of taurine transport in oocytes injected with 6.0 ng of skAE1 cRNA compared to the oocytes injected with lower concentrations of cRNA. Therefore, all

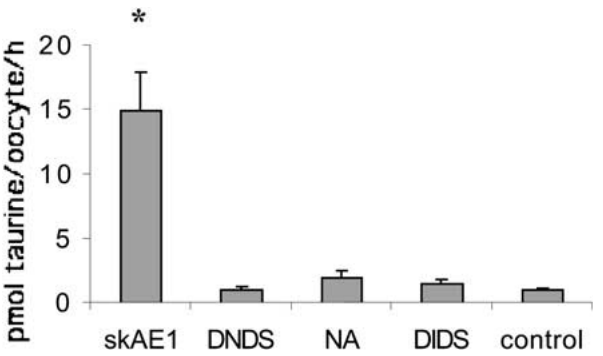

Fig. 4. Anion transport inhibition of taurine transport by skAE1 in *Xenopus* oocytes: 0.5 mM DNDS, 0.1 mM niflumic acid, 0.1 mM DIDS. Experimental oocytes were injected with skAE1 cRNA. Control oocytes were water-injected. Values are expressed as mean ± SE ($n = 10$). Asterisk: data significantly different from untreated, skAE1 injected oocytes ($P < 0.01$).

subsequent experiments were performed on oocytes injected with 6.0 ng skAE1 cRNA/injection, since this dose gave consistent and reliable results.

Pharmacological Blockers and Anion Transport Inhibitors

The aim of the following studies was to determine whether the skAE1-induced taurine uptake showed the same sensitivities to transport inhibitors as did the hypotonically stimulated skate RBC. Previous studies showed that niflumic acid and DIDS inhibit the AE1-mediated volume-sensitive transport of taurine in both skate and trout erythrocytes. Therefore, the effects of these inhibitors on skAE1-mediated taurine transport were investigated in *Xenopus* oocytes.

The results show that the anion transport inhibitors, DIDS (0.1 mM), DNDS (0.5 mM) and niflumic acid (0.1 mM) significantly inhibited skAE1-mediated taurine uptake by 94%, 95% and 93%, respectively (Fig. 4). Interestingly, DIDS was shown to have no effect on trAE1-induced taurine transport in oocytes [2]. Also, the concentration dependence of niflumic acid inhibition on taurine uptake and chloride efflux demonstrated that niflumic acid inhibited both taurine uptake and chloride exchange at similar concentration ranges (Fig. 5), possibly suggesting a similar transporter for both.

Sodium, Potassium and Rubidium Permeabilities

In order to determine whether or not the expression of skAE1 increases the permeability of the oocytes to the electrolytes sodium and potassium, we measured the sodium and potassium concentrations in skAE1- and water-injected oocytes that had been incubated for 72 hours in ND96 medium. As shown in Fig. 6, the sodium concentration was similar in water- and skAE1-injected oocytes and the small difference between the two was not statistically significant. The potassium concentration was not significantly different in skate- and water-injected oocytes. However, the potassium concentration was significantly lower in trAE1-injected oocytes than either water- or skAE1-injected oocytes and the sodium concentration was significantly higher in trAE1-injected oocytes than either water- or skAE1-injected oocytes. As an additional method to compare potassium permeability in skAE1 and water-injected oocytes, we used radioactive rubidium as a marker for the transport of potassium ions in the oocytes. Rubidium uptake was only slightly lower in skAE1- than in water-injected oocytes (Fig. 7), and the difference is not statistically significant. However, rubidium uptake in trAE1-injected oocytes was significantly higher than skAE1- or water-injected oocytes. When the uptake of rubidium in skAE1-, trAE1- and water-injected oocytes was tested in the presence of ouabain, there was a slight decrease in rubidium uptake in all three groups. However, the difference was not statistically significant.

Discussion

Previous studies have suggested that in skate and trout red blood cells, the anion exchanger (AE) functions not only in anion exchange but also as an osmolyte channel [5, 6, 10]. Expression of the trout anion exchanger protein (trAE1) in *X. laevis* oocytes [3, 4] showed that the expressed protein functioned in both anion exchange and as an organic osmolyte (taurine) channel in a manner similar to that observed in intact trout red blood cells [5]. The transport properties of the osmolyte channel in skate red blood cells, while similar in several respects to that in the trout, does differ significantly in one respect. The channel in skate red blood cells transports sodium, potassium and rubidium ions relatively poorly [2] compared to the channel in the trout red blood cells [5]. Therefore, the question arises as to whether the different channel cation permeability properties observed in the skate and trout red blood cells are due to differences in the channel properties of the skate and trout AE1 or to other differences, e.g., regulatory factors, in the two red blood cells. Thus, we expressed skAE1 in *X. laevis* oocytes to help answer this question.

First, we showed that the expressed skAE1 protein was inserted into the cell membrane of the *Xenopus* oocytes and the protein functioned as an anion exchanger, facilitating the exchange of chloride ion across the cell membrane. Then we showed that the protein functioned as an osmolyte (taurine) channel in the oocytes and showed the same anion transport inhibitor sensitivities as in the skate erythrocyte. Finally, we compared the cation transport

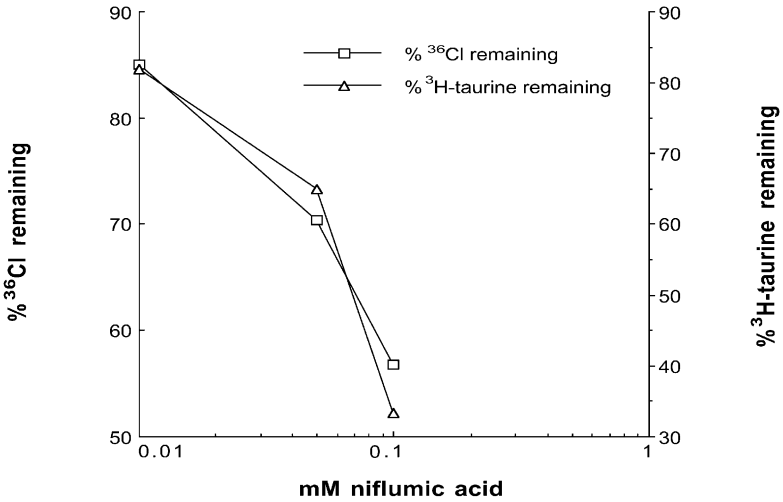

Fig. 5. Dose response curve for niflumic acid (0.01, 0.05 and 0.1 mM niflumic acid) on ^{3}H-taurine uptake and $^{36}Cl^{-}$ efflux on skAE1 cRNA- or water-injected *Xenopus* oocytes. Values for taurine uptake experiments and $^{36}Cl^{-}$-efflux experiments are means (n = 10) of the % taurine and %^{36}Cl efflux.

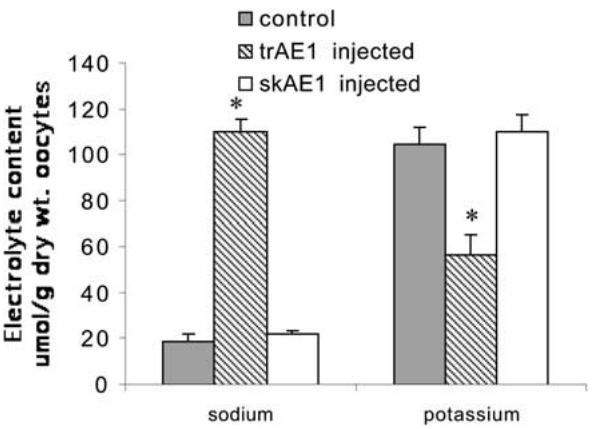

Fig. 6. SkAE1 does not change sodium and potassium contents of *Xenopus* oocytes injected with skAE1 cRNA. Oocytes were injected with skAE1 cRNA or water and incubated for 72 hours at 18°C before oocyte monovalent cation contents were measured. Cation contents are expressed as micromoles per gram dry-weight oocytes. Hatched bars are trAE1 injected, light bars are skAE1 and solid bars are water injected oocytes. Values are expressed as means ± SE (n = 15), $P < 0.05$.

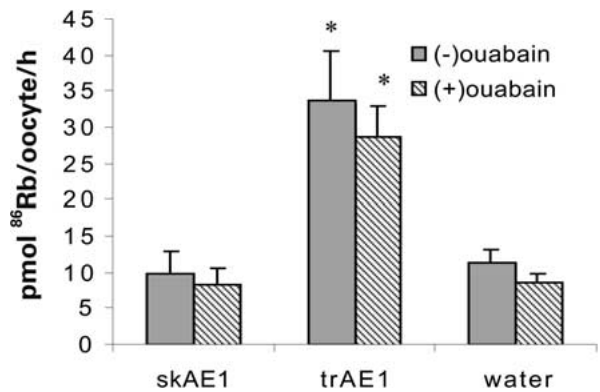

Fig. 7. SkAE1 does not change rubidium uptake in *Xenopus* oocytes. Oocytes were injected with skAE1 cRNA, trAE1 cRNA or water, allowed to equilibrate for 72 hours before ^{86}Rb uptakes were measured. Values are expressed as mean ± SE (n = 8), $P < 0.05$.

properties of skAE1 expressed in *Xenopus* oocytes with those of the osmolyte channel in skate red blood cells as well as those of the trAE1 expressed in *Xenopus* oocytes (shown in Table 1). The cation transport properties of the expressed skAE1 channel are similar to those observed previously for the osmolyte channel in skate red blood cells [2, 6, 19]. Both systems transport taurine readily, but Na^{+}, K^{+} and ^{86}Rb are transported relatively slowly by the skAE1 channel compared to trAE1. The difference in cation transport properties in skAE1 and trAE1 mimic the different properties of the osmolyte channels seen between skate and trout erythrocytes. For example, K^{+} is readily released by hypotonically expanded trout erythrocytes [5] and in *Xenopus* oocytes expressing trAE1 [3], whereas skAE1 expressed in *Xenopus* oocytes (present study) and hypotonically stimulated skate erythrocytes both transport K^{+} relatively slowly [2]. Thus, the cation transport properties of the channel induced by expression of skAE1 are identical to those in the skate erythrocyte, which strongly suggests that skAE1 can account for the properties of the osmolyte channel in skate erythrocytes.

The differences in cation transport properties of the two expressed channels may be due to the differences in the structures or the folding of the two proteins in the cell membrane. For example, comparison of the topological structure of trAE1 and skAE1 shows that trAE1 has a long extracellular Z-loop between transmembrane segments 5 and 6, which is absent in skAE1 [9]. In addition, there are different N-glycosylation sites on skAE1 (in the extracellular loop between transmembrane segments 5 and 6) compared to trAE1. It is also possible, however, that other factors, such as oligomerization and phosphorylation, known to affect the function of AE1 in skate red blood cells [14, 15], may account for

Table 1. Comparison of the osmolyte channel properties of the skate AE1, skate RBC and trout AE1

Substrates	Skate RBC[1]	skAE1 oocytes[2]	trAE1[3] oocytes
Taurine	+	+	+
K+	–	–	+
86Rb+	+/–	–	+
Na+	–	–	+

[1] Data from ref. 1, 2, and 7
[2] Present study
[3] Data from ref. 2, 3, 7, 8 and 10
+ Indicates rapid permeability,
(±) indicates low permeability,
– indicates permeability not significant.

the different transport properties of trAE1 and skAE1 in oocytes.

Most cells possess osmolyte channels, which are activated when the cells are hypotonically stressed. In the non-erythroid cells examined, electrophysiological and radioactive flux studies have shown that these channels are swelling-activated anion channels ($I_{clswell}$) [16]. However, the molecular identity of these channels is, as yet, unknown. In contrast, confirming previous indirect evidence obtained in skate and trout red blood cells, Motais and collaborators, working with trAE1 expressed in oocytes [3, 4], and we, working with skAE1 also expressed in oocytes, have now shown that both trAE1 and skAE1 can account for the properties of the osmolyte channels in the respective intact red blood cells. Therefore, we now have the ability to determine both the functional and structural properties of the channel proteins, as well as possible regulatory factors involved in the operation of the channel in expression systems that allow the manipulation of the multiple factors that are known to affect the function of the channel in the intact red blood cell. In addition, these studies could serve as a model for elucidating the nature and properties of the proteins that act as the channels in non-erythroid cells.

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