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Electrogenic Cotransport of Na^+ and Sulfate in *Xenopus* Oocytes Expressing the Cloned Na^+/SO_4^{2-} Transport Protein NaSi-1*

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The Na^+/sulfate cotransporter cloned from rat kidney cortex (NaSi-1) has been expressed in oocytes of *Xenopus laevis* and subjected to electrophysiological analysis by current and voltage clamp methods. In current-clamped oocytes, superfusion with 1 mM sulfate resulted in a 12-mV depolarization of the cell membrane. Accordingly, in voltage-clamped oocytes sulfate induced an inward current (I_S), which was dependent on both the concentration of Na^+ and sulfate in the superfusate. Half-maximal I_S was observed at about 0.1 mM sulfate and 70 mM Na^+. The Hill coefficients were 1 and 2.8 for sulfate and Na^+, respectively. Thiosulfate and selenate created similar currents as sulfate with a similar K_m. At saturating concentrations of thiosulfate and selenate, addition of sulfate could not induce an additive current. Phosphate (1 mM) did not inhibit sulfate-induced currents. Finally, I_S was dependent on the holding potential being larger at more negative potentials. The results of this study strongly suggest an electrogenic cotransport of sulfate and Na^+ with a stoichiometry of 1:3.

Reabsorption of filtered sulfate in the proximal tubule of the mammalian nephron plays a major role in SO_4^{2-} homeostasis (for review, see Refs. 1 and 2). Transcellular transport is accomplished by Na^+-coupled uptake across the brush border membrane and probably by anion exchange across the basolateral cell membrane (2). The Na^+/sulfate cotransporter at the brush border membrane has been shown to interact with oxyanions such as thiosulfate and selenate, but not with phosphate (3–8). The Hill coefficient exceeding unity for Na^+ has been taken as evidence for the coupling of more than 1 Na^+ ion with sulfate at the brush border membrane (2).

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Recently, a Na^+-dependent sulfate transporter (NaSi-1)[1] has been cloned from rat kidney (9), which, upon expression in oocytes of *Xenopus laevis*, displays kinetics and substrate specificity characteristic for proximal tubule sulfate transport. The present experiments have been performed to test for electrogenic properties of this transport system.

EXPERIMENTAL PROCEDURES

cRNA encoding NaSi-1 was synthesized *in vitro* as previously described (9). Dissection of *X. laevis* and collection and handling of the oocytes have been described in detail elsewhere (10). Oocytes were injected with water or 1 or 10 ng of cRNA/oocyte. Two-electrode voltage-clamp recordings were performed 3–8 days after injection (10). The data were filtered at 10 Hz and recorded on a chart recorder. The external solution (superfusate) contained 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES. Sulfate, thiosulfate, and selenate were added to this solution at the indicated concentrations. Unless otherwise stated, the final solutions were titrated to pH 7.5 using HCl or NaOH, respectively. To study the Na^+ dependence of sulfate-induced current, NaCl was partially replaced by choline chloride. In those experiments KOH was used instead of NaOH for titration. The flow rate of the superfusion was 10 ml/min, and a complete exchange of the bath solution was reached within about 15 s. All data are given as means ± S.E., where n indicates the number of experiments. The size of the sulfate-induced current (I_S) varied 1–20 fold, depending on the time period after cRNA injection, the concentration of cRNA injected (see "Results") and on the different batches of oocytes (from different animals). Therefore, throughout the paper we show experimental data obtained at the same day for a particular set of experiments on multiple oocytes derived from one batch.

RESULTS

In oocytes injected with water, neither depolarizations (in current clamp) nor currents could be elicited by superfusion of 1 mM sulfate (in voltage clamp; $n = 10$). In oocytes injected with cRNA encoding NaSi-1, superfusion with sulfate induced an inward current (I_S) under voltage clamp conditions. I_S was dependent on both the amount of cRNA injected and the period of time after cRNA injection being maximal about 4–5 days after injection. Four days after injection of oocytes with 1 or 10 ng of NaSi-1 cRNA, superfusion with 1 mM sulfate induced an inward current of −2.34 ± 0.4 nA and −22.2 ± 4.3 nA, respectively ($n = 4$), at a holding potential of −50 mV. In current clamp measurements, an addition of sulfate to the superfusate caused a membrane depolarization of 12 mV (from −35.3 ± 2.0 mV to −23.3 ± 2.3 mV; $n = 3$; Fig. 1). For further studies, only currents carried by the NaSi-1 protein under voltage clamp studies were analyzed.

I_S was dependent on both Na^+ and sulfate concentration of the superfusate. Fig. 2 shows the analysis of the Na^+/transporter interaction. Applying the Hill equation, I_S was half-maximal at 71.1 ± 11.1 mM Na^+, with a Hill coefficient of 2.80 ± 0.38 at a holding potential of −50 mV ($n = 6$). At 100 mM Na^+, the analysis of the sulfate concentration dependence of I_S resulted in an apparent K_m of 0.093 ± 0.03 mM sulfate, with a Hill coefficient close to 1 (Fig. 3; $n = 4$). Selenate and thiosulfate also induced an inward current with an apparent K_m of 0.58 ± 0.09 mM ($n = 4$) for selenate and 0.084 ± 0.009 mM ($n = 4$) for thiosulfate (Fig. 3).

In previous studies, both selenate and thiosulfate have been shown to inhibit sulfate transport (6). In the experiment pre-

[1] The abbreviations used are: NaSi-1, Na^+-dependent sulfate transporter; I_S, sulfate-induced current.

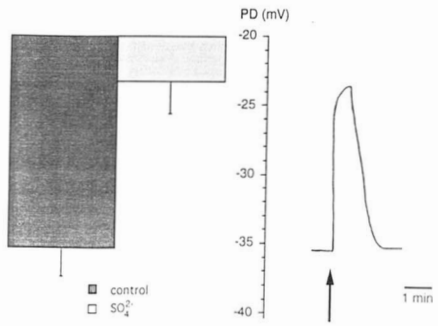
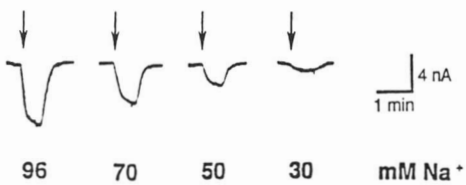


FIG. 1. **Sulfate-induced oocyte membrane depolarization.** On the *left* the data for the membrane potentials under control conditions or after superfusion with 1 mM sulfate are given as arithmetic means (± S.E.). The *right side* shows an original tracing of sulfate-induced membrane depolarization. The *arrow* indicates the start of a 30-s superfusion period with 1 mM sulfate.



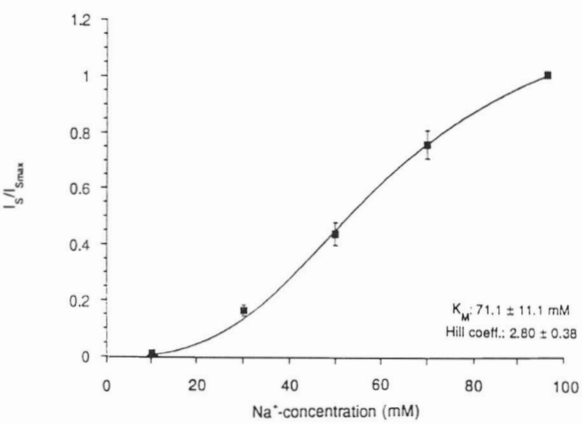


FIG. 2. **Currents induced by 0.5 mM sulfate as a function of ambient Na^+ concentration at a holding potential of −50 mV.** To lower Na^+ concentration, Na^+ was isoosmotically replaced by choline. The *upper panel* represents I_S obtained at different Na^+ concentrations for one characteristic oocyte. The *arrows* indicate the start of a 30-s sulfate superfusion period in the presence of the Na^+ concentrations (mM) indicated under the traces. The diagram illustrates the correlation between I_S and Na^+ concentration (arithmetic means ± S.E.; $n = 6$). The data were fitted using the equation $I_S = I_{S(max)} \times [Na^+]^n/([Na^+]^n + K_m)$, where n and $[Na^+]$ give the Hill coefficient and the Na^+ concentration, respectively.

sented in Fig. 4, 2 mM selenate (close to saturating concentration) induced an inward current of −15.8 ± 1.2 nA and 0.5 mM sulfate induced a current of −11.9 ± 1.3 nA (Fig. 4; $n = 4$). However, in the presence of 2 mM selenate, no significant additional current could be induced by sulfate (sum of selenate and sulfate-induced current was −16.8 ± 1.8 nA; $n = 4$). Sulfate (0.5 mM) did not induce an additional current in the presence of 2 mM thiosulfate ($n = 5$; data not shown). Superfusion with 5 mM phosphate could not inhibit I_S induced by 1 mM sulfate ($n = 5$; data not shown).

As has been reported for other Na^+-coupled transporters (12–15),[2] the current evoked by sulfate was dependent on the voltage across the cell membrane, being greater at more negative

[2] A. E. Busch, S. Waldegger, T. Herzer, J. Biber, D. Markovich, G. Hayes, H. Murer, and F. Lang, submitted for publication.

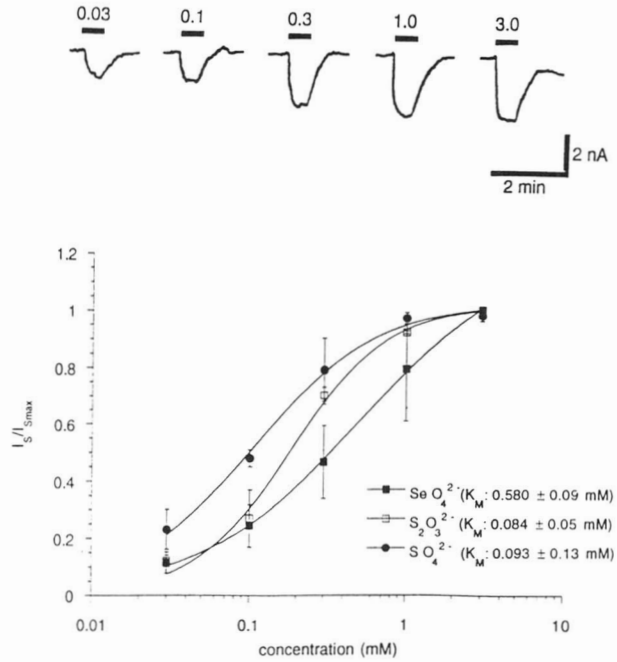


FIG. 3. **Sulfate concentration dependence of I_S.** The *upper panel* represents original tracings of I_S obtained at different sulfate concentrations in one characteristic oocyte (holding potential was −50 mV). The *bars* indicate a 30-s sulfate superfusion at the indicated sulfate concentrations (mM). I_S was normalized for each oocyte against the maximal induced current. Correlation between current (arithmetic means ± S.E.; $n = 4$) and sulfate concentration is given in the diagram. The data were fitted using the equation $I_S = I_{S(max)} \times [P_i]^n/([P_i]^n + K_m)$, where n and $[P_i]$ give the Hill coefficient and the superfusate sulfate concentration, respectively.

potentials (Fig. 5). Addition of 1 mM sulfate to the superfusate evoked at −90 mV an inward current of −48.8 ± 10 nA, which was reduced to −6.2 ± 1.2 nA at a holding potential of −10 mV ($n = 4$).

I_S was not altered at different superfusate pH. Superfusion of 1 mM sulfate at pH 6.3, 7.3, and 8.3 and a holding potential of −50 mV evoked an I_S of −10.3 ± 0.26 nA, −10.2 ± 0.16 nA, and −11.3 ± 0.13 nA, respectively. This is in strong contrast to transport of phosphate and current induced by phosphate in *X. laevis* oocytes expressing a cloned rat renal Na/P_i cotransporter (NaPi-2; see Refs. 11 and 16); in these studies, phosphate-induced transport and current was found to be magnified by alkaline pH values.

DISCUSSION

The present observations clearly show that sulfate transport via the rat renal brush border protein NaSi-1 is electrogenic, carrying positive charge into the cell. Thus, the carrier obviously does not simply transport 2 Na^+ ions with 1 divalent sulfate, as has been hypothesized in numerous previous studies (for review, see Ref. 2); therefore, several alternatives must be considered. Considering the dissociation constant of sulfate and the lack of pH dependence of I_S, it seems unlikely that monovalent sulfate is the substrate or that a co- or countertransport with H^+ or OH^- takes place. Instead, the most likely explanation for the electrogenicity of sulfate transport is a stoichiometry of 3:1 rather than 2:1 for Na^+/sulfate cotransport. In addition to the fact of an excess positive charge inward movement, a 3:1 stoichiometry is supported by the analysis for the Na^+/sulfate interaction with the transport protein, which revealed Hill coefficients of 2.8 and 1 for sulfate and Na^+, respectively.

In agreement with other electrogenic Na^+-coupled transport systems (12–15),[2] the substrate-induced current is a function of

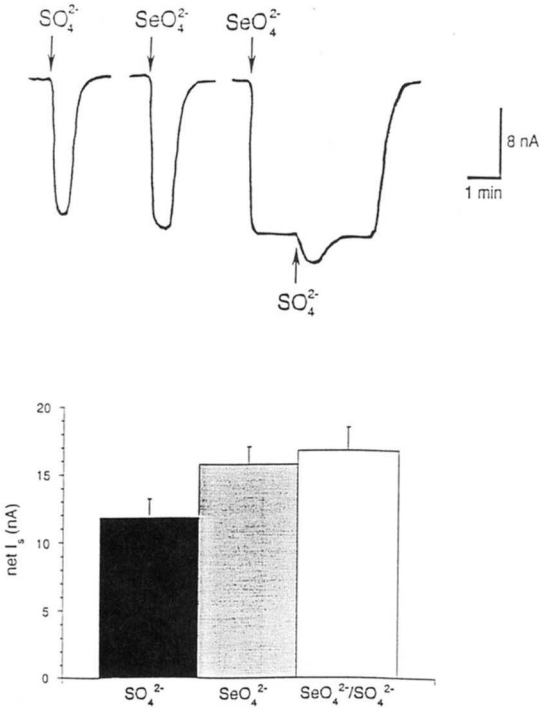


FIG. 4. **Interaction of sulfate and selenate on I_S.** The *upper panel* displays original tracings of I_S induced by sulfate (0.5 mM), by selenate (2 mM), or by sulfate (0.5 mM) in the presence of selenate (2 mM). The *arrows* indicate the start of a 30-s superfusion period of the substrates indicated. For the *right tracing*, selenate (2 mM) was applied for 3 min; after 1 min, sulfate was added for 30 s. This particular experiment shows the biggest increase of sulfate-induced additive inward current in the presence of selenate observed for the whole set of experiments. The diagram gives arithmetic means ± S.E. of the currents induced by sulfate (0.5 mM), selenate (2 mM), and sulfate combined with selenate. There was no significant difference between the selenate-induced current and the current induced after the addition of sulfate in the presence of selenate.

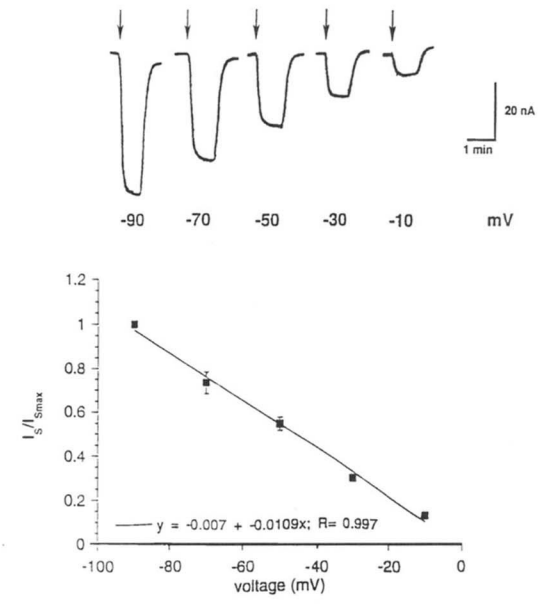


FIG. 5. **I_S as a function of potential difference across cell membrane.** The *upper panel* shows original tracings of I_S in one characteristic oocyte. At the *arrows*, 1 mM sulfate was added for 30 s at the holding potentials indicated. The diagram illustrates the correlation between I_S and membrane potential (arithmetic means ± S.E.; n = 4).

the potential difference across the cell membrane, which is likely to be a result of change in driving force and/or binding for Na^+, as has been reported for the Na^+-coupled glucose transporter (17). A depolarization-mediated decrease of sulfate transport could be the reason for increased sulfate excretion observed after increased glucose reabsorption, as such reabsorption is expected to depolarize the membrane (11). Depolarization-mediated decrease of sulfate transport could also account for the differences in Na^+ binding found by Markovich *et al.* (9) in sulfate-uptake studies for NaSi-1 compared to this study. Under non-voltage-clamp conditions, saturating concentrations of Na^+ and sulfate would cause larger depolarization of the oocyte membrane, consequently resulting in relative smaller uptake of sulfate compared to the sulfate uptake at low sulfate or Na^+. A more detailed kinetic analysis of NaSi-1-induced sulfate transport, including a study of membrane potential dependence of substrate interactions (Na^+; SO_4^{2-}), will be required to offer a satisfactory explanation for the apparent discrepancies in affinity for Na^+ and sulfate interaction as measured in oocytes by tracer techniques (9) and in the present electrophysiological study.

In renal brush border membrane vesicles, selenate and thiosulfate have been shown to compete for sulfate binding at the Na^+-coupled sulfate transport system (6). In the present study, both oxyanions also induced inward currents with similar kinetics. Furthermore, when saturating concentrations of thiosulfate or selenate were applied, addition of sulfate to the superfusate did not cause an additive current, in agreement with recent sulfate uptake studies on the same system (9). These results strongly suggest that selenate and thiosulfate are transported by NaSi-1 after binding to a common binding site for oxyanions.

In conclusion, the present observations reveal that sulfate transport via a recently cloned rat renal brush border membrane transporter (NaSi-1) is electrogenic. The observations strongly suggest cotransport of 1 molecule of sulfate with 3 Na^+ ions.

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