A major function of the kidney is the reabsorption of salt and water from the glomerular filtrate. The mechanism of water transport is not well understood, although it is generally accepted that water transport is secondary to active solute transport (23). Of the 180 liters of plasma filtered by the glomerular apparatus every day, >80% is reabsorbed in the proximal tubule. Because aquaporins have been shown to be present both in the apical and basolateral membranes of proximal tubular cells, transcellular water transport is thought to be mediated through these water channels. However, in a recent study on aquaporin-1 knockout mice (21), the transepithelial water permeability of the proximal tubule was reduced 78%, whereas the fluid reabsorption was reduced only 50%. One interpretation of this result is that other membrane proteins are involved in fluid reabsorption. It has been proposed that ion-coupled solute transporters can mediate the transport of water, and there is evidence for the involvement of the intestinal Na+-glucose cotransporter (SGLT1) in transport of water (10, 11, 14, 31). We speculate that renal cotransport proteins may contribute to water reabsorption in the proximal tubule.

The goal of this study was to investigate whether, in general, cotransport proteins could play a role in renal water reabsorption. Specifically, we tested the renal Na+-dicarboxylate cotransporter (NaDC-1) for water transport. NaDC-1 belongs to a family of Na+-dependent anion transporters, which includes the Na+-dependent dicarboxylate transporters (1, 3, 16, 17) and the renal Na+-sulfate cotransporter (13). NaDC-1 is primarily found in the apical membrane of the kidney proximal tubules where it reabsorbs tricarboxylic acid cycle intermediates (24). Defects in this transporter may play a role in the development of idiopathic hypocitraturia (see Ref. 18). NaDC-1 is electrogenic, with a stoichiometry of 3 Na+ transported/divalent carboxylate ion (6, 19). We measured substrate transport using the two-electrode voltage clamp and water transport as the changes in cell volume by an optical method on the cloned NaDC-1 expressed in Xenopus laevis oocytes. Our results indicate that NaDC-1 mediates both passive and solute-coupled water transport, and under physiological conditions, the cotransporter could contribute to fluid reabsorption across the proximal tubule.

MATERIALS AND METHODS

X. laevis oocytes were defolliculated, injected with 50 ng rabbit NaDC-1 cRNA (19), and maintained in Barth's medium supplemented with 50 mg/ml gentamycin at 18°C for 3–8 days before use (11, 20). During the experiments, the oocyte was normally perfused with a NaCl control solution containing (in mM) 90 NaCl, 20 mannitol, 2 KCl, 1 MgCl2, 1 CaCl2, and 10 HEPES (pH 7.5, 212 mosM). In some experiments, 90 mM NaCl was exchanged with 90 mM LiCl or 90 mM choline chloride. Solutions containing substrates were made by isosmotic replacement of mannitol in the control solution with either 10 mM sodium citrate or 10 mM sodium succinate. In experiments studying lithium inhibition of water transport, the test solution contained 80 mM NaCl, 10 mM LiCl, and 10 mM choline citrate; the osmolality was the same as the control solution (212 mosM). Hyperosmotic solutions were prepared.
by addition of 20 mM mannitol to the appropriate solutions. The osmolarity of the solutions was measured using a vapor pressure osmometer (Wescor, Logan, UT).

The experimental protocol allowed simultaneous measurements of substrate transport and the oocyte volume. The experiments were performed under continuous superfusion of the chamber (functional volume, 15 µl), and the half time for solution exchange was 1 s. Electrogenic substrate transport by NaDC-1 was studied using the two-electrode voltage-clamp method (8, 19). Because 3 Na⁺ are transported with each divalent carboxylate ion, the activity of NaDC-1 can be monitored as the inward current evoked by the substrate. The number of positive charges (Q) transported into the oocyte was obtained from the integral of the substrate-induced inward current.

Two general protocols were used to measure water fluxes. The osmotic water flux was estimated from the rate of change of cell volume when an osmotic gradient (−20 mosM) was imposed by removal of mannitol from the superfusing solution. These experiments were performed in the absence of substrates (citrate and succinate) in the external solution. To measure water flow during Na⁺–succinate (or citrate) cotransport, the oocyte membrane potential was held at −110 mV and the external solution was abruptly exchanged with a test solution in which mannitol in the control solution was isosmotically replaced by a transported substrate (succinate or citrate). Water fluxes across the oocyte membrane were calculated from the changes in cell volume. The oocyte cross-sectional area was measured every 0.5 s from bright-field images by a charged coupled device camera, and the images were digitized (11, 14, 31). The relative volume of the oocytes (V/V₀) is related to the relative cross-sectional area (A/A₀) by the following equation: V/V₀ = (A/A₀)², where V is the volume, V₀ is the initial volume, A is the cross-sectional area, and A₀ is the initial cross-sectional area. Volume flow (Jᵥ), was obtained from the following relation: Jᵥ = V/dt/V/V₀, where (d/dt)(V/V₀) is the relative volume change with time. Osmotic water permeability (Lᵥ) of the oocyte is related to the volume flow by the following equation: Lᵥ = Jᵥ/ΔV, where ΔV is the partial molar volume of water (18 cm³/mol), and Δπ is the osmotic difference. All experiments were repeated on at least three oocytes from different donor frogs. Statistics were given as means ± SE, and n is the number of experiments. When values were obtained from linear regression of the data, their error was obtained as the SE of the fits.

RESULTS

Osmotic water transport. Oocytes injected with NaDC-1 cRNA showed an increase in osmotic water permeability compared with control noninjected oocytes. This is illustrated in Fig. 1A where the volume changes in response to an osmotic challenge of −20 mosM (created by removal of mannitol from the superfusing control solution) to an oocyte injected with NaDC-1 cRNA and a control oocyte from the same batch are shown. Upon imposing the osmotic gradient in the absence of substrate, the NaDC-1 cRNA injected oocyte swelled at the rate of 53 pl/s. This rate was three times greater than that of the control oocyte (18 pl/s). When the osmotic gradient was removed, the swelling in both oocytes stopped. The osmotic water permeability Lᵥ of the oocyte injected with NaDC-1 cRNA was 3.6 ¥ 10⁻⁴ cm/s, compared with 1.3 ¥ 10⁻⁴ cm/s for the control oocyte. Figure 1B shows that, on average, oocytes injected with NaDC-1 cRNA had an Lᵥ of 2.8 ± 0.1 ¥ 10⁻⁴ cm/s (n = 20 oocytes), which was twofold higher than the Lᵥ of control oocytes (1.2 ± 0.1 ¥ 10⁻⁴ cm/s, n = 6 oocytes).

The increase in Lᵥ of oocytes injected with NaDC-1 cRNA was proportional to the number of transporters in the oocyte plasma membrane. In a series of experiments, the expression levels of NaDC-1 and osmotic water transport (expressed as Lᵥ) were measured in the same oocyte (Fig. 2). Expression level of NaDC-1 was
determined as the total number transporters in the oocyte plasma membrane. This was estimated by measuring the maximal inward current ($I_{\text{max}}$) induced by a saturating concentration (10 mM) of citrate in the NaCl solution at –110 mV membrane potential. The number of transporters in the oocyte plasma membrane was obtained by dividing maximal transport rate ($I_{\text{max}}$) by the turnover rate of NaDC-1, which was assumed to be 50/s. The volume flow experiments were performed as described in Fig. 1A, with the external solution containing Na$^+$ (●), Li$^+$ (○), or choline (▼). The background $L_p$, which was determined in control oocytes (1.2 ± 0.1 × 10⁻⁴ cm/s), has been subtracted. All data points were used to determine the slope of the regression line: 1.5 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹. The $L_p$ obtained in choline (2.2 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹) was higher than those obtained in Na$^+$ (1.4 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹) or Li$^+$ (1.4 ± 0.2 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹).

Solute-coupled water transport. We tested whether NaDC-1 exhibited solute-coupled water transport. The experiments were performed in the absence of an osmotic gradient, so the only driving force for water transport would be derived from the NaDC-1-dependent solute flux. In these experiments, transporter activity, which was measured as the inward current induced by citrate, and the oocyte volume were monitored simultaneously. Figure 3 shows the results of such an experiment. Initially, the oocyte was perfused with the control solution, and the membrane potential was held at –110 mV. The solution was abruptly

![Graph showing relationship between osmotic water permeability and expression level of NaDC-1](http://ajprenal.physiology.org/)

**Fig. 2.** Relationship between passive (osmotic) water permeability $L_p$ and the expression level of NaDC-1. The expression level was determined from the maximal transport rate ($I_{\text{max}}$) generated by a saturating concentration (10 mM) of citrate in the NaCl solution at –110 mV membrane potential. The number of transporters in the oocyte plasma membrane was obtained by dividing maximal transport rate ($I_{\text{max}}$) by the turnover rate of NaDC-1, which was assumed to be 50/s. The number of transporters in the oocyte plasma membrane was obtained by dividing maximal transport rate ($I_{\text{max}}$) by the turnover rate of NaDC-1, which was assumed to be 50/s. The volume flow experiments were performed as described in Fig. 1A, with the external solution containing Na$^+$ (●), Li$^+$ (○), or choline (▼). The background $L_p$, which was determined in control oocytes (1.2 ± 0.1 × 10⁻⁴ cm/s), has been subtracted. All data points were used to determine the slope of the regression line: 1.5 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹. The $L_p$ obtained in choline (2.2 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹) was higher than those obtained in Na$^+$ (1.4 ± 0.1 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹) or Li$^+$ (1.4 ± 0.2 × 10⁻¹⁴ cm·s⁻¹·protein⁻¹).

![Graph showing NaDC-1-coupled water transport](http://ajprenal.physiology.org/)

**Fig. 3.** NaDC-1-coupled water transport measured under isosmotic conditions. In an NaDC-1-expressing oocyte, water transport (volume change; $ΔV$) and substrate transport (inward current) were measured concurrently during isosmotic solution changes. The membrane potential was held at –110 mV during the experiment. At the arrow (+ citrate), the superfusing control solution was rapidly changed to an isosmotic citrate-containing solution, by replacement of 20 mM mannitol with 10 mM citrate. Addition of citrate immediately evoked a linear increase in oocyte volume (jagged line in A) at a rate 40 pl/s and an inward citrate-dependent current (B), reaching a maximum of 1280 nA. Removal of citrate (–citrate) resulted in a decrease in current to the initial value and a stop in swelling of the oocyte. The number of charges (Q) transported into the oocyte (obtained by integration of the current) is plotted as a function of time (smooth line in A). The relationship observed between water and charge transport implied that transport of 1 inward positive charge is accompanied by transport of 165 H₂O molecules.
exchanged with a test solution, in which mannitol in the control solution was isosmotically replaced by 10 mM citrate. The isosmotic addition of citrate evoked an inward current, reaching a maximum of 1,280 nA (Fig. 3B). Concurrent with the increase in citrate-induced current, there was a linear increase in the volume of the oocyte (Fig. 3A, jagged trace) at a rate of 40 pl/s. The initial volume change was directly correlated with the inward charge (Q) obtained as the integral of the citrate-induced inward current. The smooth curve of Fig. 3A was drawn with each inward-positive charge accompanied by the transport of 165 water molecules (see below and Fig. 4). The curve, which represents the water flow coupled to Na\(^+\)-citrate cotransport, superimposed on the curve describing the volume change of the oocyte. The ratio between water and substrates (3 Na, 1 dicarboxylate, 165 H\(_2\)O) resulted in the transport of 12 H\(_2\)O/charge (41 trials on 14 oocytes). Experiments performed with citrate showed a linear relationship between volume flow and Na\(^+\)-dicarboxylate cotransport with a slope of 33 ± 6 pl·s\(^{-1}\)·nA\(^{-1}\). Presumably, Na\(^+\) and Li\(^+\) are competing for the same binding site, and Li\(^+\) is a poor activator of substrate transport (19, 27). The presence of 10 mM Li\(^+\) (together with 80 mM Na\(^+\) and 10 mM citrate) reduced both the substrate-evoked inward current and the water flux to 33 ± 2 and 31 ± 3%, respectively (8 trials on 4 oocytes) (data not shown). The relationship between water flow and NaDC-1 transport rate is shown in Fig. 4 (C). The coupling ratio between water and current in the presence of 10 mM Li\(^+\) was 34 ± 3 × 10\(^{-3}\) pl·s\(^{-1}\)·nA\(^{-1}\) (8 trials on 4 oocytes).

The data from the experiments with citrate, succinate, and Li\(^+\) could be described by the same linear function (Fig. 4). The slope of the regression line, 33 ± 2 × 10\(^{-3}\) pl·s\(^{-1}\)·nA\(^{-1}\), corresponds to the transport of 176 ± 12 H\(_2\)O per inward positive charge, or 176 H\(_2\)O:3 Na\(^+\)·1 dicarboxylate (since the stoichiometry of NaDC-1 is 3 Na\(^+\) per divalent dicarboxylate, Refs. 6 and 19).

Uphill water transport. NaDC-1 was capable of transporting water uphill, against the osmotic driving force (Fig. 5). In Fig. 5A, bottom trace, an oocyte expressing NaDC-1 was subjected to an osmotic challenge of +20 mosM, obtained by addition of 20 mM mannitol to the control solution. The oocyte volume immediately started to decrease at a rate (J \(_p\)) predicted by the osmotic water permeability (L\(_w\)). When the same osmotic gradient was applied but under the condition that NaDC-1 was transporting citrate (addition of 20 mM of mannitol to the isosmotic citrate solution), then the rate of shrinkage of the oocyte and the volume flow \(J_{\text{v,citrate}}\) was decreased (Fig. 5A, top trace). The difference between the two volume flows (NaDC-1-coupled volume flow) is represented by the jagged line in Fig. 5B and corresponds to the water transport that was coupled to the influx of Na\(^+\) and citrate by NaDC-1. The smooth curve of Fig. 5B was obtained from the integral of the citrate-induced inward current with a coupling ratio of 245 water molecules/inward charge. The smooth curve superimposed on the volume curve, indicating that NaDC-1 transported water together with Na\(^+\) and citrate against the osmotic gradient. On average, the coupling ratio was 182 ± 12 water molecules/inward charge (7 trials on 4 oocytes). Thus the stoichiometry for uphill water transport is 182 H\(_2\)O:3 Na\(^+\)·1 dicarboxylate.

DISCUSSION

The increase in \(L_p\) of oocytes expressing NaDC-1 and the linear relationship between expression level and
Fig. 5. Uphill water transport by NaDC-1. Volume changes and inward current were measured during hyperosmotic solution changes in an NaDC-1-expressing oocyte. Introduction of an osmotic gradient of +20 mosM (first arrow) induced a volume change of −56 pl/s (A, mannitol). There were no changes in inward current (data not shown). Introduction of an osmotic gradient of +20 mosM in the presence of substrate (10 mM citrate) induced both a volume change of −18 pl/s (A, citrate) and an inward current, with I\text{max} of 820 nA (data not shown). NaDC-1-coupled water flow was obtained from the difference between the two volume changes (ΔV\text{citrate} − ΔV\text{mannitol}). The volume change was 46 pl/s, shown as the jagged trace in B. The number of charges transported into the oocyte (the integral of the inward current) was plotted in B as a smooth line. The relationship between the inward charge and the volume flow suggested cotransport of 246 H\textsubscript{2}O molecules with 1 charge.

The L\text{p} is due to a NaDC-1-specific water permeability. This increase is not simply due to an increase in protein density in the plasma membrane because specific inhibitors block passive transport through other cotransporters, for instance, phlorizin blocks the SGLT1 L\text{p} and SKF blocks the GAT1 (Na\textsuperscript{+}–Cl\textsuperscript{−}–GABA transporter) L\text{p} (10). The rate of water transport through the passive pathway is determined by the direction of the imposed osmotic gradient (Figs. 1A and 5A). The passive water permeability per protein can be obtained from the turnover rate of the cotransporter. In general, Na\textsuperscript{+}-coupled cotransporters have turnover rates in the range of 10–100 s\textsuperscript{−1} (12). Assuming a turnover rate for NaDC-1 of 50 per s would give an L\text{p} of 1.5 × 10\textsuperscript{−14}, cm\textsuperscript{−1}·protein\textsuperscript{−1}. This is comparable to the L\text{p} per protein found for the Na\textsuperscript{+}–glucose cotransporter SGLT1 (1.4 × 10\textsuperscript{−14} cm\textsuperscript{−1}·protein\textsuperscript{−1}) (10, 28). The L\text{p} per protein found for cotransporters is ∼100 times lower than that of the aquaporins (15). The activation energy for passive water transport by SGLT1 is 5 kcal/mol and is comparable to those of the aquaporins (10). Therefore, Na\textsuperscript{+}-dependent cotransporters may be described as being low-conductance water channels (10, 11, 28).

Solute-coupled water flow by NaDC-1 is summarized by the following observations: 1) secondary active transport of Na\textsuperscript{+} and citrate was accompanied by an increase in oocyte volume (Fig. 3); 2) the time courses of volume change and citrate-induced current (inward charge) were superimposable (Fig. 3); 3) there was a strict stoichiometric relationship between Na\textsuperscript{+}, citrate (or succinate), and water, and the coupling ratio was 3 Na\textsuperscript{+}, 1 dicarboxylate, and 176 water molecules per transport cycle (Figs. 3 and 4); 4) Li\textsuperscript{+} inhibited both water flow and NaDC-1 transport to the same extent, with no change in the coupling ratios (Fig. 4); and 5) the solute-coupled water flow occurred in the absence of, and even against, an osmotic gradient (Figs. 3 and 5). These findings suggest that there is a direct coupling between water flow and secondary active transport of the substrates via NaDC-1. Coupling may be related to conformational changes of the protein during the transport cycle (11, 14).

Solute-coupled water transport mediated by NaDC-1 shares a number of properties with that observed in SGLT1 (11, 14, 31): 1) independence from osmotic gradients, 2) strict stoichiometric relationship between water and substrates, and 3) both components of water transport (osmotic and solute coupled) are present under substrate transporting conditions. Water transport has also been observed in cotransport proteins from native tissue, such as the choroid plexus K\textsuperscript{+}–Cl\textsuperscript{−} and the retinal pigment epithelium H\textsuperscript{+}–lactate cotransporters (29, 30), and in the cloned human brain Na\textsuperscript{+}–Cl\textsuperscript{−}–GABA, rat thyriod Na\textsuperscript{+}–iodide, and the plant H\textsuperscript{+}–amino acid cotransporters expressed in oocytes (11). This is consistent, in view of the hypothesis that cotransporters from different families share common kinetic mechanisms (26), and suggests that there may be an essential role for water in the cotransport process.

The following observations support the hypothesis that solute-coupled water flow is not due to a local osmotic mechanism: 1) transport through ion channels,
such as connexin 50, nystatin, and gramicidin, does not result in initial solute-coupled water flow (14, 25, 31); 2) the osmotic effects have also been ruled out by theoretical considerations (11); and 3) if solute-coupled water transport were driven by local osmotic gradients, then the ratio of water molecules to solutes would be the same for all transporters. In fact, this ratio varies from transporter to transporter. In human SGLT1, 210 water molecules are coupled to 3 solutes (2 Na\(^+\) and 1 glucose) (14), whereas in rabbit SGLT1, the ratio was 360 H\(_2\)O:2 Na\(^+\):1 glucose (31). In both the K\(^+-\)Cl (29) and in the H\(^-\)lactate cotransporter (30), the water-to-solute ratio was 500:2. The ratio for NaDC-1 was 175 water coupled to 4 solutes: 3 Na\(^+\) and 1 dicarboxylate. In preliminary experiments, the plant H\(^+\)-amino acid cotransporter had a ratio of 50:2 (1 H\(^+\) and 1 amino acid) (unpublished data).

Does NaDC-1 play a role in renal proximal tubular water transport? We note that citrate stimulates fluid absorption in rabbit isolated proximal tubule (2). In the case of human, the plasma concentration of all tricarboxylic acid cycle intermediates is \(~0.25\) mM (7), and on the basis of a glomerular filtration rate of 180 liters/day and a reabsorption efficiency of 90% (5), 40 mmol are the basis of a glomerular filtration rate of 180 liters/day.

The sodium/dicarboxylate cotransporter (NaDC-1) transports sodium across the apical membrane to the lumen. The cotransporter utilizes the sodium gradient (at least 20 mmol/l) as an energy source for transport (4). Active movement of sodium is required to maintain the osmotic gradient within the lumen and thus drive the transport of water. The amount of water that can be transported across the apical membrane is limited by the number of sodium/dicarboxylate cotransporters and the rate of sodium uptake. The sodium/dicarboxylate cotransporter transports sodium and dicarboxylates, such as succinate, fumarate, malate, and citrate, across the apical membrane. The ratio of water to solute transported is typically 1:3, indicating that sodium is transported with three dicarboxylates.

\section*{REFERENCES}


