

Rapid Report

Urea transport by cotransporters

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1. The rabbit Na⁺–glucose cotransporter (rbSGLT1) was expressed in *Xenopus laevis* oocytes and urea transport in rbSGLT1 and non-injected (control) oocytes was studied using [¹⁴C]urea as a tracer. The level of rbSGLT1 expression in these batches of oocytes was monitored by measuring the uptake of α -methyl-D-[¹⁴C]glucopyranoside ([¹⁴C] α MDG).
2. In rbSGLT1-expressing oocytes, there was a 4-fold increase in urea transport in the absence of sugar relative to that in control oocytes. Urea uptake was not Na⁺ dependent and was linear with both time of incubation (5–120 min) and increasing urea concentration (50 μ M to 100 mM) in the bathing medium. rbSGLT1 urea uptake was blocked by the rbSGLT1-specific inhibitor phlorizin (K_i 1 μ M) in 100 mM NaCl buffer, but was not affected in 100 mM choline chloride buffer. Phloretin inhibited rbSGLT1 urea uptake with a low affinity (K_i > 1 mM) in the presence and absence of Na⁺. The uptake of 55 μ M urea through rbSGLT1 was not blocked by 100 mM urea analogues including thiourea, 1,3-dimethyl urea, 1,1-dimethyl urea and acetamide.
3. The activation energies (E_a) of urea transport for control and rbSGLT1-expressing oocytes were 14 ± 3 and 6 ± 1 kcal mol⁻¹, respectively. The low E_a for urea transport through rbSGLT1 is comparable to the E_a of passive water transport through rbSGLT1.
4. Urea transport through rbSGLT1 was further increased when the cotransporter was activated by the addition of sugar to the external medium. The rate of sugar-dependent urea uptake was directly proportional to the rate of Na⁺–glucose–H₂O cotransport such that the amount of urea transport was approximately proportional to the molar concentration ratio of urea to H₂O (55 μ M/55 M).
5. The low affinity Na⁺–glucose (pSGLT3), the Na⁺–iodide (rNIS) and the Na⁺–Cl⁻–GABA (hGAT1) cotransporters expressed in oocytes demonstrated similar urea transport properties.
6. These observations suggest that cotransporters behave as urea channels in the absence of substrates. Furthermore, under substrate-transporting conditions, the same cotransporters serve as urea cotransporters. This could account for urea transport in cells that appear not to have urea uniporters or channels.

Urea is a major metabolic product of mammalian biopathways and its transport plays a vital role in nitrogen elimination and osmotic homeostasis. Urea, which is transported via the blood to the kidneys for excretion, is integral to the urinary concentration mechanism in the kidney. Although there has been some evidence that urea is transported actively (Kawamura *et al.* 1976; Kato & Sands, 1998), it is generally considered that urea is passively transported across biological

membranes by diffusion. In recent years, specific transporters for urea have been identified in the renal medulla and cloned (for a review see Tsukaguchi *et al.* 1998). Subsequent studies have shown that some water channels, such as aquaporins AQP3, AQP7 and AQP9, are permeable to urea (for a review see Borgnia *et al.* 1999). In both the facilitated urea transporters and aquaporins, urea is passively transported down its concentration gradient.

To date, no urea uniporters or channels have been identified in the renal proximal tubule, where about 40% of the urea filtered by the glomerulus is reabsorbed. However, given that the proximal tubule contains a significant number of cotransporters that behave as water channels and pumps (Zeuthen, 1991, 1994; Zeuthen *et al.* 1996, 1997; Loo *et al.* 1996, 1999; Meinild *et al.* 1998, 2000), we investigated whether cotransporters can also transport urea. We tested transporters with demonstrated water transport properties, such as the pig low affinity Na⁺-glucose (pSGLT3), rat Na⁺-iodide (rNIS) and human Na⁺-Cl⁻-GABA (hGAT1) transporters, but primarily focused on the rabbit Na⁺-glucose cotransporter (rbSGLT1). Our findings indicate that cotransporters act as urea channels in the absence of substrate and as urea cotransporters in the presence of substrate. These results may have further implications for the mechanism of urea transport in the renal proximal tubule and other tissue cells.

METHODS

The rabbit Na⁺-glucose (rbSGLT1), rat Na⁺-iodide (rNIS), human Na⁺-Cl⁻-GABA (hGAT1), and pig low affinity Na⁺-glucose (pSGLT3) cotransporters were overexpressed in *Xenopus laevis* oocytes as previously described (Hediger *et al.* 1987; Eskandari *et al.* 1997; Loo *et al.* 1999). Mature oocytes were collected from adult female frogs anaesthetized with 0.1% Tricaine. Frogs were killed with an overdose of anaesthetic after the final collection. These protocols were approved by the UCLA Animal Research Committee, and meet all the NIH guidelines for the humane treatment of animals used for research. Oocytes were treated with 0.4% collagenase-B (Boehringer-Mannheim, Indianapolis, IN, USA) for 1 h and defolliculated in a 150 mM K₂HPO₄ medium for 1 h. Oocytes were then incubated at 18°C in Barth's medium. Oocytes were injected with 50 ng of capped cRNA (MegaScript, Ambion, Austin, TX, USA) or not injected (control) and 5–6 days were allowed for expression prior to use in experiments. Reagents were purchased from Sigma unless stated otherwise.

Sugar uptake was measured using α -methyl-D-glucopyranoside (α MDG), a non-metabolizable sugar analogue which is transported by rbSGLT1. The initial rates of urea and sugar uptake into oocytes were measured at 22°C (unless stated otherwise) using radioactive tracers (Ikeda *et al.* 1989). [¹⁴C]urea (specific activity, 57 mCi mmol⁻¹) and methyl- α -D-[U-¹⁴C]-glucopyranoside (specific activity, 293 mCi mmol⁻¹) (Amersham Life Sciences, Elk Grove, IL, USA) uptake was usually carried out at final urea and α MDG concentrations of 55 and 50 μ M, respectively. Radiotracer experiments were performed on oocytes obtained from the same donor frog, incubated in either 100 mM NaCl or 100 mM choline chloride medium. Experiments were repeated at least twice on oocytes isolated from different donor frogs. Uptake was expressed as a function of the amount (in pmol) per oocyte per minute (mean \pm S.E.M.).

The urea permeability, P_{urea} , was determined according to the equation: $P_{\text{urea}} = J_{\text{urea}}/[\text{urea}] \times S$ where J_{urea} is the rate of urea flux (pmol oocyte⁻¹ s⁻¹), [urea] is the urea concentration (mol cm⁻³), and S is the oocyte membrane surface area (0.44 cm²; Zampighi *et al.* 1995).

The number of transporters (N) in the plasma membrane of oocytes expressing rbSGLT1 was determined from the maximal rate of sugar uptake, J_{max} , and the turnover number of rbSGLT1 (25 s⁻¹, Panayotova-Heiermann *et al.* 1994) using the relationship:

$N = J_{\text{max}} \times A/\text{turnover number}$, where A is Avogadro's number (6.02 $\times 10^{23}$ molecules mol⁻¹). J_{max} was estimated from the α MDG uptake at 50 μ M and the $K_{0.5}$ for α MDG uptake (0.2 mM) using the equation: $J = (J_{\text{max}}[\alpha\text{MDG}])/(K_{0.5}^{\alpha\text{MDG}} + [\alpha\text{MDG}])$, where $K_{0.5}$ is the half-maximal concentration (mol l⁻¹), $[\alpha\text{MDG}]$ is the α MDG concentration (mol l⁻¹), and J is the rate of sugar uptake in oocytes (pmol oocyte⁻¹ s⁻¹).

RESULTS

Passive urea transport

Figure 1A illustrates the results from an experiment measuring the urea uptake in the absence of α MDG in the bathing medium by control and rbSGLT1-expressing oocytes. Urea uptake in oocytes expressing rbSGLT1 (7.6 \pm 0.5 pmol oocyte⁻¹ (30 min)⁻¹) was 4-fold higher than in control oocytes (1.7 \pm 0.03 pmol oocyte⁻¹ (30 min)⁻¹). Urea uptake in both control and SGLT1-expressing oocytes was linear with the time of incubation (5–120 min), and the rate of uptake was linear with urea concentration from 10 μ M to 100 mM. In one experiment the uptake at 55 μ M and 100 mM urea was 3.1 \pm 0.2 and 5501 \pm 65 pmol oocyte⁻¹ h⁻¹, respectively, in control oocytes ($n = 4$ oocytes), and 13.3 \pm 1.3 and 25 500 \pm 1000 pmol oocyte⁻¹ h⁻¹, respectively, in rbSGLT1-expressing oocytes ($n = 6$). The rate of urea uptake in control oocytes corresponds to a urea permeability, P_{urea} , of 4 $\times 10^{-8}$ cm s⁻¹. This is comparable to the urea permeabilities reported by others (e.g. Zhang & Verkman, 1991) after correcting for the increase in the oocyte plasma membrane area due to folding (Zampighi *et al.* 1995). Thus, the urea permeability due to rbSGLT1, which was obtained by subtraction of the background urea permeability of control oocytes from the total urea permeability of rbSGLT1-expressing oocytes, was 1.3 $\times 10^{-7}$ cm s⁻¹. The increase in urea permeability was directly proportional to the level of SGLT1 expression. The level of expression was gauged in six oocytes by the magnitude of the maximum Na⁺-glucose current (Mackenzie *et al.* 1998) immediately before measuring the radioactive urea tracer uptake, and the urea flux (pmol oocyte⁻¹ (30 min)⁻¹) was directly proportional to the Na⁺-glucose current (nA) over a 3-fold range (regression coefficient, 0.7).

The rate of α MDG uptake (246 \pm 9 pmol oocyte⁻¹ (30 min)⁻¹) in the same batch of oocytes as shown in Fig. 1A was 65-fold higher than in control oocytes (3.8 \pm 0.2 pmol oocyte⁻¹ (30 min)⁻¹), corresponding to values obtained previously (Hediger *et al.* 1987; Ikeda *et al.* 1989). Using these fluxes we estimate that for the oocytes used in Fig. 1A, approximately 2 $\times 10^{10}$ copies of rbSGLT1 were located in the plasma membrane. Given this density of cotransporters, we obtained a P_{urea} of 7 $\times 10^{-18}$ cm s⁻¹ per rbSGLT1 cotransporter.

The presence of urea analogues did not affect urea uptake into control and rbSGLT1-expressing oocytes. Uptake of 55 μ M urea into control and rbSGLT1-expressing oocytes

was insensitive to the addition of excess concentrations (100 mM) of the urea analogues. For example, in one experiment where the rbSGLT1-specific uptake of 55 μM urea was 9.8 ± 1.1 pmol oocyte⁻¹(30 min)⁻¹ ($n = 5$), the uptake in the presence of 100 mM thiourea, *N*-methylurea, 1,3-dimethylurea, acetamide and 1,1-dimethylurea was 8.6 ± 0.9 ($n = 5$), 9.5 ± 0.9 ($n = 5$), 8.9 ± 1.0 ($n = 5$), 9.5 ± 1.9 ($n = 5$) and 9.0 ± 1.7 pmol oocyte⁻¹(30 min)⁻¹ ($n = 5$), respectively. This is consistent with the linearity of urea uptake up to 100 mM urea.

Urea uptake by rbSGLT1 was independent of Na⁺; for example, in two pairs of experiments the specific uptake was 14.2 ± 0.7 ($n = 5$) and 10.1 ± 0.5 pmol oocyte⁻¹(30 min)⁻¹ ($n = 6$) in Na⁺, and 15.6 ± 0.6 ($n = 6$) and 10.3 ± 0.7 pmol oocyte⁻¹(30 min)⁻¹ ($n = 6$) in choline. However, urea uptake was inhibited by phlorizin, a specific inhibitor of Na⁺-dependent glucose transport by rbSGLT1 (Fig. 1*B*). The addition of 1 μM phlorizin reduced the rbSGLT1 uptake of 55 μM urea by ~50%. Increasing the concentration of phlorizin to 50 μM

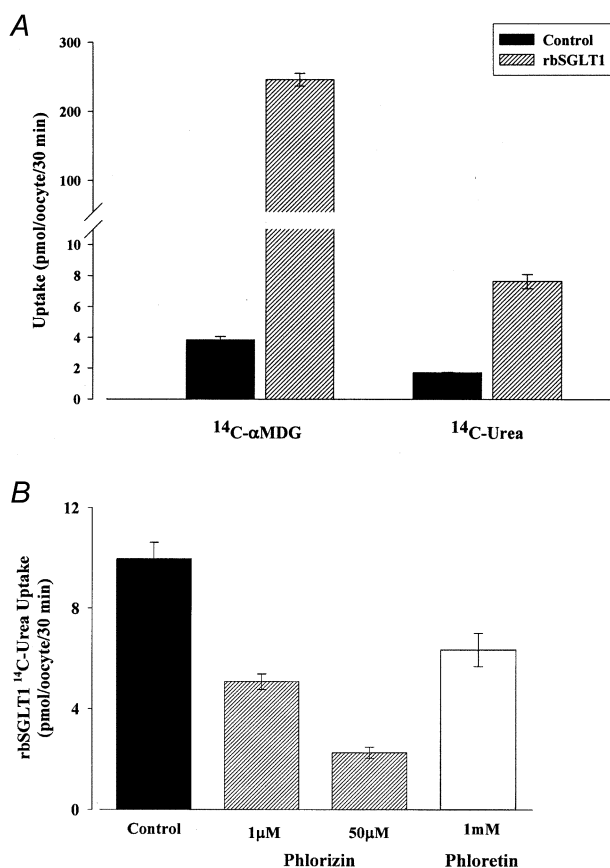


Figure 1. Passive urea transport by rbSGLT1

A, 50 μM [¹⁴C] α MDG and 55 μM [¹⁴C]urea uptake in control and rabbit SGLT1 (rbSGLT1)-expressing oocytes. Oocytes obtained from the same frog were either injected with 50 ng of rbSGLT1 cRNA or not injected (Control). Uptake was studied in oocytes after 5 days incubation in Barth's medium. For transport experiments, oocytes were incubated in 100 mM NaCl and transport of sugar or urea was measured as a function of the amount (in pmol) per oocyte per 30 min. Each bar in the graph represents the mean α MDG or urea uptake by 4–5 oocytes. Left, ~250 pmol of α MDG is transported by rbSGLT1-expressing oocytes, 65-fold higher than by control oocytes. Right, ~8 pmol of urea is transported by rbSGLT1-expressing oocytes, 4-fold higher than by control oocytes. *B*, inhibition of rbSGLT1 urea transport by phlorizin and phloretin. Uptake of 55 μM [¹⁴C]urea was studied after 6 days incubation in non-injected oocytes and in rbSGLT1-injected oocytes. Urea flux was measured in the presence of 100 mM NaCl buffer and in the absence of sugar. Uptake was calculated as the amount of urea transported (in pmol) per oocyte per 30 min. Each bar represents the mean urea uptake by 4–6 rbSGLT1 oocytes from the same batch either in the absence (Control) or in the presence of phlorizin or phloretin. All values have been corrected for endogenous urea uptake. Addition of 1 or 50 μM phlorizin or 1 mM phloretin to the transport buffer had no effect on non-injected oocytes. In rbSGLT1 oocytes, the presence of 1 μM phlorizin in the transport buffer resulted in a 50% decrease in urea transport. Addition of 50 μM phlorizin further inhibited urea transport to ~20% of that by control oocytes. Identical experiments performed with 1 mM phloretin also reduced rbSGLT1 urea uptake by ~35% compared to control.

reduced urea uptake to 23% of that in the absence of phlorizin (Fig. 1B, Control). The apparent phlorizin inhibition constant, K_i , was $1 \mu\text{M}$ in the presence of 100 mM external Na^+ , but was greater than 1 mM in choline. Phloretin also inhibited urea uptake by rbSGLT1. The K_i for phloretin was greater than 1 mM in the absence and presence of Na^+ . Neither phlorizin nor phloretin inhibited urea uptake into non-injected oocytes.

The specificity of the increase in urea permeability of rbSGLT1-expressing oocytes was examined by measuring the uptake of radiolabelled mannitol, glycerol, sulphate, chloride and L-alanine. With these probes there was no observable increase in permeability. For example, in one experiment where the $55 \mu\text{M}$ urea uptake increased from $1.7 \pm 0.3 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$ ($n = 5$) in control oocytes to $7.6 \pm 0.5 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$ ($n = 5$) in rbSGLT1-expressing oocytes, the uptake of $55 \mu\text{M}$ mannitol was $1.4 \pm 0.1 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$ ($n = 5$) in control and $1.8 \pm 0.2 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$ ($n = 5$) in rbSGLT1-expressing oocytes. In the case of $55 \mu\text{M}$ L-alanine the uptake was 24 ± 1 ($n = 6$) and $22 \pm 1 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$ ($n = 6$), respectively.

The temperature sensitivity of urea uptake was determined over the range $14\text{--}30^\circ\text{C}$ and the corresponding Arrhenius plots are shown in Fig. 2. In control oocytes, the activation energy, E_a , for urea transport was $14 \pm 3 \text{ kcal mol}^{-1}$, a value comparable to

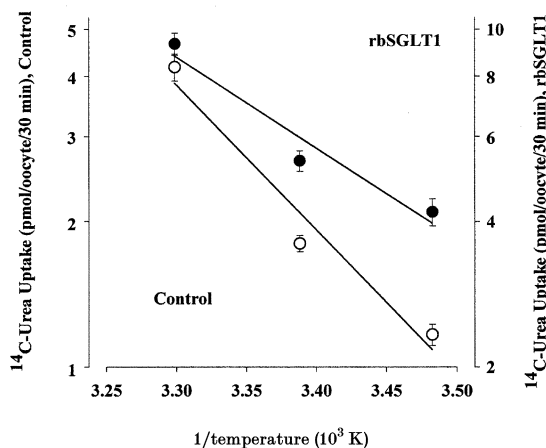


Figure 2. Arrhenius plots of urea uptake by rbSGLT1 (●) and control (non-injected, ○) oocytes

Urea uptake was measured in oocytes incubated in $55 \mu\text{M}$ [^{14}C]urea for 30 min at 14 , 22 and 30°C . The experiment was performed on 29 oocytes obtained from the same batch and after 5 days expression. Each data point with error bars indicates the mean urea uptake by 4–6 oocytes (\pm S.E.M.). The lines were drawn by linear regression. For control oocytes, a slope of -3.0 ± 0.6 corresponds to an activation energy, E_a ($E_a = -2.3R \times \text{slope}$, where R is the gas constant), of $14 \pm 3 \text{ kcal mol}^{-1}$. For rbSGLT1-expressing oocytes, the slope of -1.3 ± 0.3 corresponds to an E_a of $6 \pm 1 \text{ kcal mol}^{-1}$.

that reported previously for the efflux of urea (10 kcal mol^{-1} , Zhang & Verkman, 1991) and to that of passive water diffusion through the plasma membrane (12 kcal mol^{-1} , Loo *et al.* 1996; 10 kcal mol^{-1} , Zhang & Verkman, 1991). The E_a for urea transport in rbSGLT1 oocytes was $6 \pm 1 \text{ kcal mol}^{-1}$.

Urea uptake was also higher in oocytes expressing the low affinity Na^+ -glucose (pSGLT3), the Na^+ -iodide (rNIS) and the Na^+ - Cl^- -GABA (hGAT1) cotransporters (Fig. 3). The values for urea uptake obtained for rNIS- and hGAT1-expressing oocytes were 3-fold higher ($8.5 \pm 0.5 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$) than when compared to that of control oocytes ($3.2 \pm 0.2 \text{ pmol oocyte}^{-1} (30 \text{ min})^{-1}$). Urea uptake by pSGLT3 was also significantly higher than in control oocytes ($P \leq 0.05$; Fig. 3, right). The increase in urea transport was blocked by specific inhibitors, i.e. $100 \mu\text{M}$ phlorizin blocked pSGLT3 urea uptake by 82%, and $20 \mu\text{M}$ SKF 89976A reduced hGAT1 urea uptake by 20% (not shown).

Urea transport in the presence of substrates

The addition of substrates to the bathing medium further increased urea transport by cotransporters. To minimize complications due to the voltage dependence of cotransport activity, we used substrate concentrations below the $K_{0.5}$ for transport. This decreased the depolarization of the membrane potential and the concomitant reduction in the rate of Na^+ -substrate cotransport. Figure 4 summarizes the results of one set of experiments with rbSGLT1, hGAT1 and pSGLT3. Urea

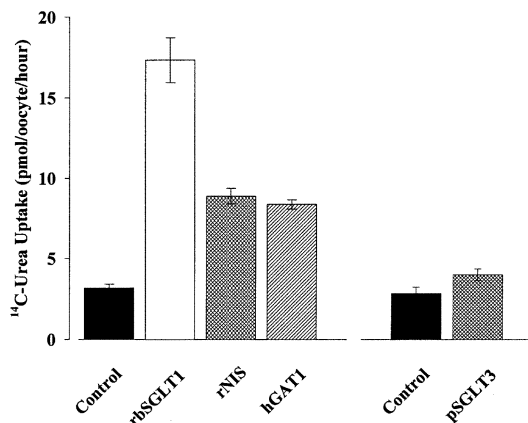


Figure 3. Urea transport by different cotransporters

Uptake of $55 \mu\text{M}$ [^{14}C]urea was measured for 30 min in oocytes after 5 days of expression of different cotransporters. Oocytes from the same batch were injected with 50 ng cRNA of rabbit Na^+ -glucose cotransporter (rbSGLT1), rat Na^+ -iodide cotransporter (rNIS), human Na^+ - Cl^- -GABA transporter (hGAT1) or pig low affinity sugar transporter (pSGLT3), or were not injected (Control). Each bar represents the mean uptake by 4–6 oocytes and was tested for significance ($P \leq 0.05$).

transport by rbSGLT1 increased from 4.0 ± 0.3 to 8 ± 0.5 pmol oocyte⁻¹ h⁻¹ in the presence of 0.1 mM α MDG. Phlorizin (100 μ M) inhibited this urea transport by rbSGLT1. For pSGLT3, the urea uptake increased from 1.1 ± 0.3 to 2.2 ± 0.01 pmol oocyte⁻¹ h⁻¹ in the presence of 1 mM α MDG. Addition of 5 mM α MDG increased the urea uptake by pSGLT3 from 4.0 ± 0.4 to 6.2 ± 0.4 pmol oocyte⁻¹ (30 min)⁻¹. Urea transport measured in the presence of 1 μ M GABA by hGAT1 also increased from 7 ± 0.5 to 14 ± 1 pmol oocyte⁻¹ h⁻¹.

We determined the stoichiometry between urea and Na⁺-glucose cotransport by rbSGLT1 by measuring the amount of [¹⁴C]urea uptake (55 μ M) in the presence and absence of 0.2 mM α MDG. We also determined the amount of α MDG transported by rbSGLT1 by measuring the amount of Na⁺-dependent [¹⁴C] α MDG (0.2 mM) uptake in the same batch of rbSGLT1-expressing oocytes. The substrate-dependent urea uptake by rbSGLT1 was 1.6 ± 0.4 pmol oocyte⁻¹ (30 min)⁻¹ ($n = 10$) and the Na⁺-dependent α MDG uptake was 776 ± 28 pmol oocyte⁻¹ (30 min)⁻¹ ($n = 10$). The results from this experiment show that the urea to α MDG transport ratio of rbSGLT1 is approximately 2.1×10^{-3} urea/ α MDG.

DISCUSSION

Recent studies have indicated that cotransporters can mediate the transport of water across cell membranes in addition to their usual substrates; for example, the rabbit Na⁺-glucose cotransporter (rbSGLT1) acts as a water channel and cotransports 260 water molecules along with 2 Na⁺ and 1 sugar molecule (Loo *et al.* 1996). In the present study, we have found that cotransporters can also mediate the transport of urea via two distinct pathways: a passive channel and an active cotransport pathway.

Cotransporters can passively transport urea in the absence of a cosubstrate. Cotransporters also have urea transport properties in common with those of urea uniporters and aquaporins known to transport urea. Passive urea transport by rbSGLT1-expressing oocytes: (1) was non-saturable up to 100 mM of urea; (2) was independent of cations, occurring in both NaCl and choline chloride medium; (3) was inhibited by the presence of phloretin (1 mM); and (4) in the presence of Na⁺, was highly sensitive to phlorizin (K_i 1 μ M), a specific inhibitor that blocks conformational changes of the protein allowing for sugar and water transport. In addition, the E_a of passive urea transport through rbSGLT1 is low ($\leq 6 \pm 1$ kcal mol⁻¹) compared to that of control oocytes (14 ± 3 kcal mol⁻¹). This low activation energy corresponds to that of passive water transport through rbSGLT1 (5 kcal mol⁻¹, Meinild *et al.* 1998; Loo *et al.* 1999) and aquaporins (≤ 4 kcal mol⁻¹, Preston *et al.* 1992) suggesting that the urea pathway is a channel. Given the similarities in kinetic properties and activation energies to passive water transport through cotransporters and aquaporins and their ability to

discriminate amongst substrates, it would appear that cotransporters contain a selective aqueous pathway that is shared by water and urea.

Cotransporters also have the unique ability of coupling urea transport to substrate transport. In the presence of substrates, urea transport is remarkably similar to substrate-coupled water transport by cotransporters. Urea uptake by rbSGLT1 increased significantly beyond that of passive urea transport and was inhibited by phlorizin. Furthermore, urea is transported in the ratio of its concentration in water. Given that ~ 260 water molecules are transported for every 2 Na⁺/sugar and that $\sim 2.1 \times 10^{-3}$ urea molecules are transported per sugar molecule, the urea/water coupling coefficient for rbSGLT1 is 8.1×10^{-6} (range, 3×10^{-6} to 40×10^{-6} urea/H₂O). The ratio of urea to water transported is roughly equivalent to the molar concentration ratio in the bulk phase (55 μ M urea/55 M H₂O).

Can the present observations be due to indirect effects of cotransporter expression in oocytes? In our view this is unlikely for the following reasons. (1) SGLT1 and the other cotransporters we have studied in the oocyte expression system are well behaved. For example, in our extensive studies of SGLT1 in oocytes using radioactive tracers, electrophysiological and electron microscopic techniques, we have found that the properties of Na⁺-glucose cotransport are as expected from our studies in other expression systems (Sf9 cells, COS cells) and in the

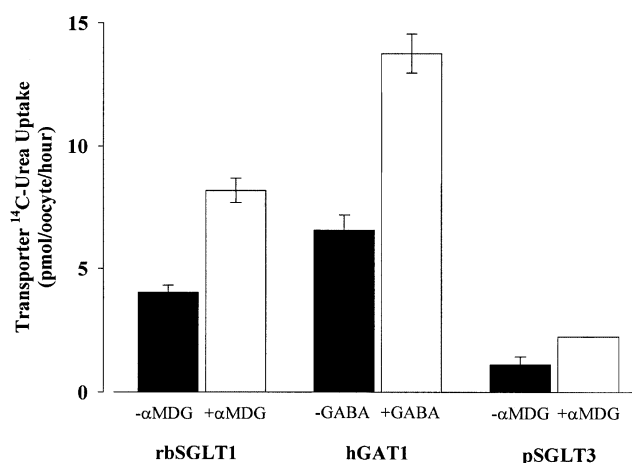


Figure 4. Urea transport in the presence (+) and absence (-) of substrate by oocytes expressing rbSGLT1, hGAT1 or pSGLT3

Urea transport was measured for 1 h in 100 mM NaCl buffer and ± 100 μ M α MDG for rbSGLT1, ± 1 μ M GABA for hGAT1, or ± 1 mM α MDG for pSGLT3 oocytes. Each bar represents the mean urea uptake by 4–6 oocytes after 5 days expression and has been corrected for the uptake by non-injected oocytes. In the presence of the respective substrate, urea transport through rbSGLT1, hGAT1 and pSGLT3 increases by $\sim 50\%$.

native tissue. (2) The kinetics of Na⁺-glucose cotransport in oocytes are independent of the level of expression (e.g. Hirsch *et al.* 1996). (3) Freeze-fracture electron microscopic studies show a homogeneous population of monomeric SGLT1 proteins in the oocyte plasma membrane, even at widely different levels of expression (Zampighi *et al.* 1995; Eskandari *et al.* 1998). (4) Very specific blockers for the cotransporters, phlorizin for SGLT1 and SGLT3, and SKF 89976A for GAT1, inhibit urea uptake. (5) Only urea transport is correlated to cotransporter expression. Similar increases in permeability were not observed for other molecules such as mannitol and L-alanine.

We previously suggested that water is cotransported by a non-osmotic mechanism; specifically, we hypothesized that water is actively transported as a result of the conformational changes the protein undergoes during the transport of Na⁺ and sugar (Zeuthen, 1991, 1994; Zeuthen *et al.* 1996, 1997; Loo *et al.* 1996, 1999; Meinild *et al.* 1998, 2000). We now propose that urea is also cotransported by a similar mechanism. The finding that urea transport is coupled to substrate transport provides strong evidence against an osmotic mechanism for substrate-coupled water flow. In addition, the ratio of urea to water transported by rbSGLT1 is approximately equal to its concentration ratio, a proportion that would be significantly less if urea is transported by solvent drag. Our results suggest that urea and water are both transported as a result of protein conformational changes occurring during the cotransport cycle.

The glomeruli in the human kidney filter approximately 54 g of urea from the blood plasma every day. About 12–14 g of the filtered urea is reabsorbed, 40% of which occurs in the proximal tubule. So far, no urea uniporters or channels have been found in the proximal tubule. We suggest that cotransporters, including the Na⁺-glucose cotransporter, behave as urea channels and urea cotransporters, and may account for urea reabsorption in the proximal tubule. The cotransporters may also have a role in the movement of urea across the plasma membranes in the intestine, thyroid gland and brain.

- BORGNA, M., NIELSEN, S., ENGEL, A. & AGRE, P. (1999). Cellular and molecular biology of the aquaporin water channels. *Annual Review of Biochemistry* **68**, 425–458.
- ESKANDARI, S., LOO, D. D. F., DAI, G., LEVY, O., WRIGHT, E. M. & CARRASCO, N. (1997). Thyroid Na⁺/I⁻ symporter. Mechanism, stoichiometry, and specificity. *Journal of Biological Chemistry* **272**, 27230–27238.
- ESKANDARI, S., WRIGHT, E. M., KREMAN, M., STARACE, D. M. & ZAMPIGHI, G. A. (1998). Structural analysis of cloned plasma membrane proteins by freeze-fracture electron microscopy. *Proceedings of the National Academy of Sciences of the USA* **95**, 11235–11240.
- HEDIGER, M. A., IKEDA, T., COADY, M., GUNDERSEN, C. B. & WRIGHT, E. M. (1987). Expression of size-selected mRNA encoding the intestinal Na⁺/glucose cotransporter in *Xenopus laevis* oocytes. *Proceedings of the National Academy of Sciences of the USA* **84**, 2634–2637.
- HIRSCH, J. R., LOO, D. D. F. & WRIGHT, E. M. (1996). Regulation of Na⁺/glucose cotransporter expression by protein kinases in *Xenopus laevis* oocytes. *Journal of Biological Chemistry* **271**, 14740–14746.
- IKEDA, T. S., HWANG, E.-S., COADY, M. J., HIRAYAMA, B. A., HEDIGER, M. A. & WRIGHT, E. M. (1989). Characterization of a Na⁺/glucose cotransporter cloned from rabbit small intestine. *Journal of Membrane Biology* **110**, 87–95.
- KATO, A. & SANDS, J. M. (1998). Evidence for sodium-dependent active urea secretion in the deepest sub segment of the rat inner medullary collecting duct. *Journal of Clinical Investigation* **101**, 423–428.
- KAWAMURA, S. & KOKKO, J. P. (1976). Urea secretion by the straight segment of the proximal tubule. *Journal of Clinical Investigation* **58**, 604–612.
- LOO, D. D. F., HIRAYAMA, B. A., MEINILD, A.-K., CHANDY, G., ZEUTHEN, T. & WRIGHT, E. M. (1999). Passive water and ion transport by cotransporters. *Journal of Physiology* **518**, 195–202.
- LOO, D. D. F., ZEUTHEN, T., CHANDY, G. & WRIGHT, E. M. (1996). Cotransport of water by the Na⁺/glucose cotransporter. *Proceedings of the National Academy of Sciences of the USA* **93**, 13367–13370.
- MACKENZIE, B., LOO, D. D. F. & WRIGHT, E. M. (1998). Relationships between Na⁺/glucose cotransporter (SGLT1) currents and fluxes. *Journal of Membrane Biology* **162**, 101–106.
- MEINILD, A.-K., KLAERKE, D. A., LOO, D. D. F., WRIGHT, E. M. & ZEUTHEN, T. (1998). The human Na⁺-glucose cotransporter is a molecular water pump. *Journal of Physiology* **508**, 15–21.
- MEINILD, A.-K., LOO, D. D. F., PAJOR, A. M., ZEUTHEN, T. & WRIGHT, E. M. (2000). Water transport by the renal Na⁺-dicarboxylate cotransporter. *American Journal of Physiology* **278**, F777–783.
- PANAYOTOVA-HEIERMANN, M., LOO, D. D. F., LOSTAO, M. P. & WRIGHT, E. M. (1994). Sodium/D-glucose cotransporter charge movements involve polar residues. *Journal of Biological Chemistry* **269**, 21016–21020.
- PRESTON, G. M., CARROLL, T. P., GUGGINO, W. B. & AGRE, P. (1992). Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* **256**, 385–387.
- TSUKAGUCHI, H., SHAYAKUL, C., BERGER, U. V. & HEDIGER, M. A. (1998). Urea transporters in kidney: molecular analysis and contribution to the urinary concentrating process. *American Journal of Physiology* **275**, F319–324.
- ZAMPIGHI, G. A., KREMAN, M., BOORER, K. J., LOO, D. D. F., BEZANILLA, F., CHANDY, G., HALL, J. E. & WRIGHT, E. M. (1995). A method for determining the unitary functional capacity of cloned channels and transporters expressed in *Xenopus laevis* oocytes. *Journal of Membrane Biology* **148**, 65–78.
- ZEUTHEN, T. (1991). Secondary active transport of water across ventricular cell membranes of choroid plexus epithelium of *Necturus maculosus*. *Journal of Physiology* **444**, 153–173.
- ZEUTHEN, T. (1994). Cotransport of K⁺, Cl⁻, and H₂O by membrane proteins from choroid plexus epithelium of *Necturus maculosus*. *Journal of Physiology* **478**, 203–219.
- ZEUTHEN, T., HAMANN, S. & LA COUR, M. (1996). Cotransport of H⁺, lactate and H₂O by membrane proteins in retinal pigment epithelium of bullfrog. *Journal of Physiology* **497**, 3–17.

- ZEUTHEN, T., MEINILD, A.-K., KLAERKE, D. A., LOO, D. D. F., WRIGHT, E. M., BELHAGE, B. & LITMAN, T. (1997). Water transport by the Na⁺/glucose cotransporter under isotonic conditions. *Biology of the Cell* **89**, 307–312.
- ZHANG, R. & VERKMAN, A. S. (1991). Water and urea permeability properties of *Xenopus* oocytes: expression of mRNA from toad urinary bladder. *American Journal of Physiology* **260**, C26–34.

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