Urea Transporter UT3 Functions as an Efficient Water Channel

DIRECT EVIDENCE FOR A COMMON WATER/UREA PATHWAY

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Baoxue Yang and A. S. Verkman‡
From the Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521

A family of molecular urea transporters (UTs) has been identified whose members appear to have an exceptionally high transport turnover rate. To test the hypothesis that urea transport involves passage through an aqueous channel, osmotic water permeability was measured in Xenopus oocytes expressing UTs. The UT3 class of urea transporters functioned as efficient water channels. Quantitative measurement of single channel water permeability (p) using epitope-tagged rat UTs gave (in cm/s × 10⁻¹⁴) of 0.14 ± 0.11 (UT2) and 1.4 ± 0.2 (UT3), compared with 6.0 and 2.3 for water channels AQP1 and AQP3, respectively. Relative single channel urea permeabilities (P_water) were 1.0 (UT2), 0.44 (UT3), and 0.0 (AQP1). UT3-mediated water and urea transport were weakly temperature-dependent (activation energy <4 kcal/mol), inhibited >75% by the urea transport inhibitor 1,3-dimethylthiourea, but gave response to different urea gradients (induced osmosis) pressing oocytes, the time course of oocyte volume in response to different urea gradients (induced osmosis) gave (for the UT3 pathway, in agreement with ) = 1. These results indicate that UT3 functions as a urea/water channel utilizing a common aqueous pathway. The water transporting function and low urea reflection coefficient of UT3 in vasa recta may be important for the formation of a concentrated urine by countercurrent exchange in the kidney.

Several related urea transporters (UTs) have been cloned recently. The UT2 transporter (rat form referred to as rUT2, Ref. 1) was first identified in rabbit by expression cloning (2). The UT2 cDNA encodes a 397-amino acid glycoprotein that is expressed in the medullary portion of descending limbs of Henle in kidney (3, 4). UT1 is larger protein (929 amino acids) that contains the UT2 sequence at its C terminus fused to a 67% identical amino acid sequence at its N terminus. UT1 functions a CAMP-regulated urea transporter that is expressed at the apical membrane of inner medullary collecting duct cells. UT1 and UT2 are thought to be derived from a single gene by alternative splicing. A 391-amino acid urea transporter expressed in erythrocyte was initially cloned from human bone marrow (HUT11, Ref. 6) and subsequently from rat kidney (named UT3, Ref. 7). Rat UT3 has 62% amino acid identity to UT2 and is expressed strongly in kidney in descending vasa recta in the inner stripe of outer medulla, as well as in testis and brain (7, 8). It is thought that these urea transporters have an important role in the urinary concentrating mechanism to establish a hypertonic renal medullary interstitium (9).

UT3 was initially recognized as the Kidd antigen (Jk) in erythrocytes (10–12). Humans lacking the Kidd antigen (Jk—/—) have low erythrocyte urea permeability. These individuals are phenotypically grossly normal, but have a defect in their ability to produce a maximally concentrated urine (13). From the number of Kidd antigen proteins per erythrocyte and the erythrocyte urea permeability, it was estimated that the UT3 turnover rate is 2–15 × 10⁶ urea molecules/s (9, 14). This turnover rate is substantially higher than that of usual solute carriers, suggesting a channel mechanism for urea transport. Additional functional data involving urea concentration dependence and trans-inhibition (15) support a channel-type mechanism.

The purpose of this study was to test whether urea transporters of the UT3-type (and possibly the UT2-type) transport urea by a channel mechanism involving urea passage through an aqueous pore. It was found that UT3 functions as an efficient water channel with water transport rates comparable with those of aquaporin-type water channels. Water movement through UT3 was weakly temperature-dependent and inhibited by urea transport inhibitors but not by water transport inhibitors. A key finding was that the reflection coefficient for urea was remarkably less than unity, providing strong evidence for a common water and urea pathway through UT3. These results have important implications regarding urea transporter structure and transporting mechanism and suggest a novel role for UT3 in the urinary concentrating mechanism as a facilitator of urea solvent drag in renal vasa recta.

MATERIALS AND METHODS
cDNA Constructs—Full-length cDNAs encoding rat AQP1 (GenBank™ accession number L07268), AQP3 (GenBank™ accession number D17695), UT2 (GenBank™ accession number U09957), and UT3 (GenBank™ accession number U81518) were polymerase chain reaction-amplified using rat kidney cDNA as template and primers (BamHI or EcoRI and Xba1 engineered restriction sites underlined): AQP1 sense, 5′-CGGATCCATGGCCACGGATTTAACAGAAGAAG-3′; AQP1-antisense, 5′-GCTTGAAGATGCCTGCCTGGTGTCGACCGCTT-3′; UT2 sense, 5′-GCGAATTCTAGATCGCTGCTCGCT-3′; UT2 antisense, 5′-GCTTGAAGATGCCTGCCTGGTGTCGACCGCTT-3′; UT3 sense, 5′-GCTTGAAGATGCCTGCCTGGTGTCGACCGCTT-3′; UT3 antisense, 5′-GCTTGAAGATGCCTGCCTGGTGTCGACCGCTT-3′. Amplified DNA fragments were confirmed by sequence analysis and
subcloned into oocyte expression plasmid (pSP64-T-N-c-Myc) (16). The constructs encoded fusion proteins consisting of each full-length aquaporin or urea transporter fused downstream and in-frame from a 10-amino acid human c-Myc epitope (EQKLLISEEDL).

**RNA Transcription and Oocyte Expression**—Complementary RNA was transcribed in vitro using SP6 polymerase (Life Technologies, Inc.) and 5 μg of plasmid DNA in a 100-μl volume at 37 °C for 1 h in the presence of digoxigenin triphosphate (1 A 2000 unit, Amersham Pharmacia Biotech). Plasmid DNA was digested with RNase-free DNase (In-vitrogen), extracted with phenol-chloroform, precipitated twice in ethanol, and suspended in distilled water. Stage V and VI oocytes from Xenopus laevis were isolated and defolliculated with collagenase in Barth’s buffer (200 mosM). Oocytes were microinjected with 50-nl samples of specified cRNAs (0–200 ng/μl) and incubated at 18 °C for 24 or 48 h.

**Transport Measurements**—Osmotic water permeability (Pₜ) was measured from the time course of oocyte swelling at 10 °C in response to a 2- or 5-fold dilution of the extracellular Barth’s buffer with distilled water. Oocyte Pₜ was calculated from the initial rate of swelling as described previously (17). In some experiments, oocytes were incubated with 1,3-dimethylthiourea or phloretin for 30 min or HgCl₂ for 5 min at 20 °C prior to and during permeability measurements. To determine urea reflection coefficient, oocytes were partially swelled in 0.2 ml of 1:1 water or hyperosmolar (400 mosM) by addition of sucrose. To determine water permeability, oocytes were incubated in 0.2 ml of Barth’s (100 mosM) for 30 s, and then 0.2 ml of water containing urea uptake, groups of 10 oocytes were incubated in 0.2 ml of different concentrations of urea (0–1600 mM) was added (see below). To determine urea reflection coefficient, oocytes were partially swelled in 0.2 ml of 1:1 water or hyperosmolar by addition of sucrose.

* RESULTS AND DISCUSSION*

**Fig. 1A** shows the time course of osmotically induced swelling in control (water-injected) oocytes, oocytes expressing aquaporin water channels AQP1 and AQP3, and oocytes expressing urea transporters UT2 and UT3. Averaged water permeability coefficients (Pₜ) were summarized in Fig. 1B. The large increase in Pₜ in oocytes expressing UT3 was an unexpected finding. Fig. 1C shows the 90-s uptake of [¹⁴C]urea for the same groups of oocytes. Urea permeability in oocytes expressing UT2 or UT3 was >12-fold increased over that in control and AQP1-expressing oocytes.

To determine the intrinsic (per channel) water and urea permeabilities of the expressed AQPs and UTs, permeability values from Fig. 1, B and C, were normalized to account for differences in transporter expression at the plasma membrane. Oocytes expressing c-Myc epitope-tagged AQPs and UTs were metabolically labeled with [³⁵S]methionine, and c-Myc-tagged proteins were immunoprecipitated from microdissected oocyte plasma membranes. Fig. 1D shows a representative autoradiogram of immunoprecipitated proteins. The protein sizes are in agreement with cDNA sequence and published immunoblot data. Absolute single channel water permeability coefficients (ₚᵢₜ) were computed as described previously (16) using a reference Pᵢₜ of 6 × 10⁻¹⁴ cm/s for AQP1. Pᵢₜ (in cm/s × 10⁻¹⁴, S.E., n = 3) were: 0.14 ± 0.11 (UT2), 1.4 ± 0.2 (UT3), and 2.3 ± 0.1 AQP3. Single channel urea permeabilities (ᵢₜ) relative to that of UT2 (defined as 1.0) were: 0.44 (UT3), 0.0 (AQPI) and 0.02 (AQP3).

The significant intrinsic water permeability of UT3 suggests the existence of a continuous aqueous channel through the UT3 protein that passes both water and urea. Studies of temperature dependence, inhibitor specificity, and urea reflection coefficient were done to test this possibility. Fig. 2A shows a weak temperature dependence for UT3-mediated transport of both

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**Fig. 1. Water and urea permeability in oocytes expressing aquaporins and urea transporters.** A, time course of oocyte swelling at 10 °C in response to a 5-fold dilution of extracellular Barth’s buffer with distilled water. Oocytes were injected with 50 nl of water (control) or cRNAs (5 ng) encoding AQP1, AQP3, UT2, or UT3. B, averaged water permeability coefficients (Pₜ). Data are mean ± S.E. for measurements on 9–12 oocytes from three separate sets of experiments. C, percent 90-s uptake of [¹⁴C]urea (expressed as percentage of uptake at 2 h, mean ± S.E.) at 23 °C in oocytes from the same batches as used in B. D, autoradiogram of proteins from oocyte plasma membranes that were immunoprecipitated by c-Myc antibody.
water and urea. The low Arrhenius activation energy (<4 kcal/mol) is consistent with an aqueous pore pathway and is in agreement with the low activation energies found for several of the aquaporin-type water channels. Fig. 2B indicates that UT3-mediated water and urea transport were each strongly inhibited by the urea analog 1,3-dimethylthiourea and by phloretin. Neither water nor urea transport were inhibited by HgCl₂, a potent inhibitor of most aquaporin-type water channels. HgCl₂, but not the urea transport inhibitors, strongly inhibited water permeability in oocytes expressing AQP1 (Fig. 2C). Together these results support a common aqueous route for water and urea transport through UT3.

The most direct evidence for a common water/solute pathway is the finding of a low solute reflection coefficient (19, 20). Reflection coefficient measurements have in general been challenging because of the tight coupling between volume and solute transport as described by Equations 1 and 2. Of note, different labs have reported substantially different reflection coefficients for urea transport across erythrocytes (21, 22) and NaCl transport across proximal tubule (23–25). Our strategy to determine the urea reflection coefficient (σ_u) in oocytes involved independent measurements of induced osmosis and solvent drag, and quantitative comparison of experimental results with numerical solution of the Kedem-Katchalsky equations.

In the induced osmosis method, oocytes were briefly swelled in 100 mM Barth’s buffer (because oocytes do not shrink well below their normal volume, Ref. 26) and then the external solution was switched to 50 mM Barth’s buffer containing different concentrations of urea. As seen in Fig. 3A (top), oocytes expressing UT3 initially swelled for external [urea] of 200 and 400 mM and shrunk for [urea] of 600 and 800 mM, suggesting σ_u = 0.3. The same measurements were simulated numerically using Equations 1 and 2 for σ_u = 0.3 versus 1.0. An important control study was done to validate the above approach for determination of σ_u and to show that the low σ_u is not a consequence of apparent solvent/solute coupling (“pseudo-solvent drag”) due to unstirred layers. An identical set of measurements (as in Fig. 3A, top) was done in oocytes coexpressing AQP1, which is permeable to water but not urea, and UT2, which is permeable to urea and not water. The amounts of injected cRNAs encoding AQP1 and UT2 were adjusted to give oocyte water and urea permeabilities comparable with those for UT3-expressing oocytes. Fig. 3A (bottom) shows little initial oocyte swelling or shrinking for external [urea] ~200...
mₚ, suggesting that $\sigma_{\text{urea}} \sim 1$. This result was confirmed by the simulated curves in Fig. 3B (bottom).

As an independent method to measure $\sigma_{\text{urea}}$ utilizing solvent drag, the 90-s uptake of [14C]urea into oocytes was measured for different external solution osmolalities. For $\sigma_{\text{urea}}$ less than 1, the osmotic water influx induced by a low external osmolality would result in increased [14C]urea uptake (compared with no osmotic gradient) because of additive urea diffusion and solvent drag mechanisms (Equation 2). Fig. 3C shows that [14C]urea uptake was significantly enhanced by hypoorosmolar external solutions and depressed by a hyperosmolar solution. The model simulations for $\sigma_{\text{urea}} = 0.3$ determined above were in reasonable agreement with the [14C]urea uptake data.

Our results provide strong evidence that the UT3 protein is associated with an aqueous channel that transports water and urea in a coupled manner. The high UT3-mediated urea turnover and the trans-stimulation data cited in the Introduction are explainable by a common water/urea channel. Although electron crystallography data suggest the location of a putative aqueous pore through aquaporin-type water channels (27, 28), there is no structural information about the UT proteins to suggest the existence or location of an aqueous channel. Of note, the UT and AQP proteins share no homology. Structure and mutagenesis studies are needed to resolve the mechanism of UT3-mediated water and urea transport.

Although not directly addressed in this study, we speculate about the physiological implications of a common water/urea channel in UT3. UT3 is expressed in descending vasa recta in kidney (7, 8), where it confers high transmural urea permeability (29). The countercurrent exchange mechanism in the renal medulla requires the delivery of large quantities of urea and water from the vasa recta to the inner medullary interstitium (30–32). The UT3-mediated solvent drag of urea could provide a simple and elegant solution to the problem of insuring an adequate rate of urea exit from vasa recta to balance osmotically driven water exit.

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REFERENCES