Molecular Cloning and Functional Reconstitution of a Urate Transporter/Channel*

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Maintenance of urate homeostasis requires urate efflux from urate-producing cells with subsequent renal and gastrointestinal excretion. The molecular basis for urate transport, however, has not been identified. A novel full-length cDNA encoding a 322-amino acid protein, designated UAT (urate transporter), has been cloned from a rat renal cDNA library by antibody screening. UAT mRNA transcripts that approximate 1.55 kilobases are present, but differentially expressed in various rat tissues. Recombinant UAT protein that was expressed from the cloned cDNA in *Escherichia coli* and purified via immobilized metal affinity chromatography has been functionally reconstituted as a highly selective urate transporter/channel in planar lipid bilayers. The IgG fraction of the polyclonal antibody that was used to select the UAT clone from the cDNA library, but not nonimmune IgG, blocked urate channel activity. Based on the wide tissue distribution of the mRNA for UAT we propose that UAT provides the molecular basis for urate flux across cell membranes, allowing urate that is formed during purine metabolism to efflux from cells and serving as an electrogenic transporter that plays an important role in renal and gastrointestinal urate excretion.

The purine bases adenine and guanine, which are essential components of DNA, RNA, and high energy phosphorylated compounds such as ATP and GTP, are either salvaged and reutilized in the production of ribo- or deoxyribonucleotides or degraded by the enzyme xanthine oxidase to a relatively insoluble product, uric acid (1). As a first step in eliminating this intracellularly formed urate from the body, urate must exit cells. Thereafter, in most mammals, a large fraction of the extracellular urate enters the peroxisomes of hepatocytes, where it is oxidized by the enzyme uricase to a water-soluble product, allantoin (2–4), which is then excreted by the kidneys (5). In other vertebrates, notably humans, some non-human primates, birds, and reptiles, uricase is not expressed, and therefore, uric acid is the end product of purine metabolism (6). In all species, uric acid is ultimately cleared from the extracellular compartment via both the kidneys and intestine, with the former being the predominant excretory route (7, 8).

Although all cells of the body that contain xanthine oxidase have the capacity to generate and accumulate urate intracellularly during the process of purine metabolism, there is no information on the mechanism(s) by which urate is transported out of cells into the extracellular compartment. Since the solubility of urate is quite low (9), an efficient mechanism(s) must exist to prevent intracellular urate accumulation during periods of normal as well as accelerated nucleic acid turnover. Similarly, despite the important contribution of the intestine in the clearance of extracellular urate, with as much as one-third eliminated via this route in humans (7, 8), there is minimal information on the mechanism(s) by which urate is transported by intestinal cells. In contrast, the participation of the kidney in disposing of urate has been extensively examined in multiple species (6). It is now generally accepted that urinary urate excretion occurs by a complex process that includes filtration at the glomerulus and tubular reabsorption and secretion that take place primarily within the convoluted portion and pars recta of the proximal tubule (6). Two modalities of transport have been described in renal cortical cell membranes, an electroneutral anion exchanger that transports urate in exchange for a variety of organic and inorganic anions (10–15) and an electrogenic urate transporter, a uniporter (16–18). Although mechanistically well described, neither of these transporters has been identified and characterized at the molecular level.

As a strategy to clone the electrogenic urate transporter we took advantage of our prior observation that the voltage-sensitive urate uniporter in rat and rabbit kidney has a number of characteristics that suggest that this transport protein has some homology with the hepatic peroxisomal enzyme uricase (16–19). Although solely an oxidative enzyme within peroxisomes, hepatic uricase appeared to function as a urate transporter when incorporated into liposomes (20) and as a highly selective urate channel when inserted in lipid bilayers (21). Moreover, urate-binding proteins that were affinity-purified from rat renal cortical cell membranes were shown to be highly immunoreactive to a polyclonal antibody to affinity-purified porcine hepatic uricase (19). Importantly, this antibody also specifically inhibited electrogenic urate transport in rat renal cortical membrane vesicles and, in immunocytochemical studies, localized to the proximal tubule, the site of urate transport (19). Based on these cumulative data, we used the polyclonal antibody to hepatic uricase as a tool to clone a urate transporter cDNA. Recombinant protein that was expressed from the full-length cDNA was used to demonstrate the functional role of the encoded protein: when fused with lipid bilayers, a selective urate uniporter in rat and rabbit kidney has a number of characteristics that suggest that this transport protein has some homology with the hepatic peroxisomal enzyme uricase (16–19). Although solely an oxidative enzyme within peroxisomes, hepatic uricase appeared to function as a urate transporter when incorporated into liposomes (20) and as a highly selective urate channel when inserted in lipid bilayers (21). Moreover, urate-binding proteins that were affinity-purified from rat renal cortical cell membranes were shown to be highly immunoreactive to a polyclonal antibody to affinity-purified porcine hepatic uricase (19). Importantly, this antibody also specifically inhibited electrogenic urate transport in rat renal cortical membrane vesicles and, in immunocytochemical studies, localized to the proximal tubule, the site of urate transport (19). Based on these cumulative data, we used the polyclonal antibody to hepatic uricase as a tool to clone a urate transporter cDNA. Recombinant protein that was expressed from the full-length cDNA was used to demonstrate the functional role of the encoded protein: when fused with lipid bilayers, a highly selective urate transporter/channel was reconstituted from the encoded protein. In view of the wide tissue distribution of the mRNA for this transporter/channel, it is proposed that this protein may serve an essential “housekeeping” function for urate efflux from cells of many tissues in addition to potentially serving an important role in renal and intestinal urate excretion.

**EXPERIMENTAL PROCEDURES**

**Expression Cloning**

A rat whole kidney cDNA library (approximately 1.2 × 10⁶ plaques) that was unidirectionally cloned in Uni-ZAP® XR Vector using EcoRI and XhoI at the 5’ and 3’ ends, respectively (Stratagene, La Jolla, CA)

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was screened according to the Stratagene protocol with an affinity-purified IgG fraction of a polyclonal antibody to pig liver uricase. This antibody was raised in rabbits to partially purified uricase (Sigma) that was further enriched by affinity chromatography (19). The IgG fraction of the antibody was obtained using a protein A antibody purification kit (Repligen, Cambridge, MA), concentrated to 10 mg/ml with Centricon 30 concentrators (Amicon, Beverly, MA), and then affinity-purified on an ImmunoPure® Ag/Ab column (Pierce) to which pig liver uricase had been coupled. Western blots were performed using the picoBlue™ Immunoassay Kit (Stratagene) with a 1:5000 dilution of the antibody in Tris-buffered saline containing 1% albumin. The library screen yielded one immunoreactive plaque, which has been designated UAT. The cDNA by PCR using the 5′-reversetranscription (RT) and then rapid amplification of the 5′ RNA was selected by affinity-purification of cDNA ends; bp, base pair(s); kb, kilobase(s); PE, phosphatidylethanolamine; PS, phosphatidylserine; HIV-1, human immunodeficiency virus, type 1.

Determination of Sequence of Full-length cDNA

Both strands of UAT were completely sequenced by automated sequence analysis using an Applied Biosystem Sequencer (ABI 373A) using dye terminator chemistry. To obtain the sequence of the full length of the mRNA for UAT, rat renal poly(A)⁺ RNA was subjected to reverse transcription (RT) and then the amplification of the 3′ end of the cDNA by PCR using the 5′-AmpliFINDER™ RACE kit (Clontech Laboratories, Inc., Palo Alto, CA). Poly(A)⁺ RNA was selected by affinity chromatography on oligo(dT)-cellulose (22) from RNA that was harvested from rat renal cortex (23). RT was performed using nucleotides 598–579 of the antisense strand of UAT as primer (Fig. 1). After linking the AmpliFINDER anchor to the 3′ end of the first-strand cDNA, PCR was performed using the AmpliFINDER anchor primer (complementary to the anchor) and two different nested primers, nucleotides 267–249 and 234–215 of the antisense strand of UAT (Fig. 1). PCR was performed with Ampli-Taq™ DNA polymerase (Perkin-Elmer, Roche) according to the Clontech protocol. PCR products were purified using the Wizard™ PCR Prep DNA purification system (Promega, Madison, WI), subcloned into pCR™ II vector using the TA Cloning® kit (Invitrogen, San Diego, CA), and sequenced by automated sequence analysis as described above. Nucleotide sequences were analyzed using the Genetics Computer Group (GGC; Madison, WI) sequence analysis package on a VAX mainframe computer to identify open reading frames. Data base searches were performed with the BLAST algorithms (24).

RT-PCR of UAT from Rat Kidney

Three separate preparations of poly(A)⁺ RNA that were harvested from rat kidneys were reverse-transcribed using random primers. Controls reactions were performed in the absence of reverse transcriptase to assess the presence of contaminating DNA. PCR was carried out using nucleotides 249–267 of UAT as the sense primer and either nucleotides 1015–998 or 1272–1253 as the antisense primer (Fig. 1). PCR was performed using an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. After a final cycle that prolonged extension at 72 °C to 7 min, the samples were maintained at 4 °C. To determine size, the PCR products were electrophoresed on 1% agarose gels. PCR products were purified, subcloned into pCR™ II vector, evaluated by restriction digestion with EcoRI, and sequenced using the methods described above.

Northern Blot Analysis

Three cDNA clones were made for use in Northern blots. Since UAT has an internal EcoRI site, one probe was made by restriction digestion of pBluescript-UAT with EcoRI. This 349-bp EcoRI fragment contains 5 bp of the 5′ EcoRI site, 8 bp of the EcoRI linker (used in making the cDNA library), 14 bp of 5′ noncoding sequence, and the initial 226 bp of the open reading frame of UAT (Fig. 1). The second probe, prepared by restriction digestion of pBluescript-UAT with StI, provided a 902-bp probe encompassing nucleotides 147-1048 (Fig. 1). The third probe, the linker probe, was made by PCR using pBluescript-UAT as template.

Preparation of Recombinant Protein

The full length of the coding sequence of pBluescript-UAT was amplified by PCR. The sense primer was constructed with a BamHI site immediately 5′ to the start codon (5′-GGCGATCTACGTTGCTTCT-GCGACCCAG-3′) and encompassed nucleotides 84–104 of UAT (Fig. 1). The antisense primer, constructed with a PstI site (5′-GCTG-CACGCTAGGTCGAGGTGACTGAC-3′) encompassed nucleotides 1052–1031 of UAT, including the stop codon (1050–1052) (Fig. 1). PCR was performed under the following conditions: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 55 °C for 1 min, 54 °C and 72 °C for 2 min, followed by one additional cycle in which extension was prolonged to 7 min at 72 °C, after which the sample was maintained at 4 °C. The PCR product was purified and subcloned into pRSET A (Invitrogen Corp., San Diego, CA) to allow the production of a fusion protein with a six-histidine metal chelating domain 5′ to the coding region of UAT. SURE cells (Stratagene) were transformed with pRSET A-UAT, plated colonies were grown in culture, a maxiprep was performed (Qiagen Plasmid Maxi kit), and double restriction digests were done with combinations of EcoRI, BamHI, and PstI. Plasmids with inserts, as determined on agarose gel electrophoresis, were used to transform BL21(DE3)pLysE cells (Novagen, Inc., Madison, WI). Colonies of BL21(DE3)pLysE cells with pRSET A-UAT were grown overnight at 37 °C in 10 ml of Super media (Qiagen) with ampicillin (100 μg/ml). Following cell lysis, immobilized metal affinity chromatography was performed on a nickel chelating resin, Ni-NTA, (Qiagen) according to the Qiagen protocol for insoluble proteins. Eluate fractions containing peak protein concentrations (determined with the Bio-Rad assay) were pooled, diluted to 0.1 mg/ml, and dialyzed slowly at 4 °C to remove the recombiant protein: eluate to dialysed fluid volumes approximated 1.5. Dialysis solution urea concentration was progressively decreased from 8 to 0 mM as NaCl and glycerol were progressively added to 0.8 and 10%, respectively; dialysis solutions were buffered to pH 7.5 with 0.1 M Tris-HCl. The DPPC-polyacrylamide gel electrophoresis with Coomassie Blue staining and Western blots using a 1:5000 dilution of the affinity-purified IgG fraction of anti-pig liver uricase were performed pre- and postdialysis.

Functional Assessment of Recombinant Protein

Preparation of Proteoliposomes—A mixture of bovine phosphatidylethanolamine (PE, 10 mg/ml) and phosphatidylserine (PS, 10 mg/ml) (Avanti Polar Lipids, Birmingham, AL) in a ratio of 1:1 (w/w) was evaporated to dryness under nitrogen. The resultant phospholipid pellet was suspended in 48 μl of a solution containing 220 mM KCl, CaCl₂, and Serine was suspended in 48 μl of a solution containing 220 mM KCl, CaCl₂, and Serine was suspended in 48 μl of a solution containing 220 mM KCl, CaCl₂, and
or 2 mM HEPES-NaOH at pH 7.4. Following the addition of 2 µl of recombinant UAT protein (50–100 µg/µl), proteoliposomes were formed by sonicating the mixture for 30 s at 80 kHz in a bath sonicator (Laboratory Supplies, Hicksville, NY). In some experiments, proteoliposomes were prepared with the same lipids but with 10 µl of recombinant HIV-1 Tat protein (20) at 380 µg/ml and 40 µl of 2.5 mM Cs2SO4, 10 mM HEPES-NaOH at pH 7.4. Fresh proteoliposomes were prepared for each experiment.

Lipid Bilayer Chamber and Formation of Lipid Bilayer—The lipid bilayer chamber was identical to that previously described (21). The two cups of the plexiglass bilayer chamber were each initially filled with 1 ml of a solution whose electrolyte composition was identical to that in the brain PE (10 mM HEPES-NaOH, and 0.25 mM CaCl2 at pH 7.4 before activity was compared in symmetrical solutions of 2.5 mM urate, 220 mM KCl, CsCl or Cs2SO4, 10 mM HEPES-NaOH, and 0.25 mM CaCl2 at pH 7.4. In some experiments, channel activity was compared in the absence and presence of 5 mM Ba2+, which the proteoliposomes were replaced with 1 ml of an identical solution, without proteoliposomes, to limit further channel incorporation. In all experiments, channel activity was initially evaluated in the presence of symmetrical solutions of 2.5 mM urate in either 220 mM KCl, CsCl or Cs2SO4, 10 mM HEPES-NaOH, and 0.25 mM CaCl2 at pH 7.4. In some experiments, channel activity was compared in the absence and presence of 5 mM Ba2+. To evaluate channel selectivity, experiments were performed in symmetrical solutions of 2.5 mM urate, but with infinite gradients of K+, Ca2+, Cl−, and/or SO42−, established by using the following combination of buffered solutions in the cis and trans chambers, respectively: (a) CaCl or KCl, (b) Cs2SO4 and CsCl, and (c) Cs2SO4 with Ca2+ and SO42−. Selectivity was also evaluated in symmetrical solutions of 220 mM Cs2SO4, 10 mM HEPES-NaOH, and 0.25 mM CaCl2 at pH 7.4 in the presence of a 1:10 urate gradient; the 2.5 mM urate, buffered Cs2SO4 solution in the trans chamber was replaced with a Cs2SO4 solution containing 0.25 mM urate. In a final group of experiments, channel activity was compared in symmetrical solutions of 2.5 mM urate, 220 mM Cs2SO4, 10 mM HEPES-NaOH, and 0.25 mM CaCl2 at pH 7.4 before and after an increasing amount of nonimmune IgG (up to 200 µg/ml), or the IgG fraction of antiserum uricicure (up to 15 µg/ml) was sequentially added to each side of the lipid bilayer.

Data Collection and Analysis—Current output of the patch clamp amplifier was filtered at 1 kHz through an eight-pole filter (Bessel filter model TL-1, Axon Instruments, Burlingame, CA). Data were analyzed with commercial software (pCLAMP, version 6.1, Axon Instruments) on a microcomputer after additional digitizing filtering at not less than 100 Hz.

RESULTS

Nucleotide and Amino Acid Sequence of Cloned cDNA—A single plaque that expressed protein immunoreactive to anti-pig liver uricase was detected in a rat renal cDNA library. The cloned cDNA, which has been designated UAT, is 1476 bp. 5′ extension of cDNA was obtained by RT-PCR of renal cortical poly(A)+ RNA extended the sequence 69 bp; there was no further extension of the cDNA when RT-PCR was repeated using a different, more 5′ primer. The nucleotide sequence of the full-length cDNA is depicted in Fig. 1 with the putative translation start site (ATG) at nucleotides 84–86, the stop codon (TAG) at 1050–1052, and the polyadenylation signal (AATAAA) at 1434; the poly(A)+ tail consists of 86 bp. The presence of guanine at positions −3, −6, and +4 and the absence of thymine from −12 to −1, both relative to ATG at 84–86, as well as the fact that the ATG at this position is the first in the sequence support the assumption that the ATG at 84–86 is the actual initiation codon (26). Utilizing this ATG as the start codon, the 1545-nucleotide sequence contains a 966-bp open reading frame that encodes a 322-amino acid protein with an estimated molecular mass of 36,341 daltons (GenBank™ accession number U67958).

Data base searches with the deduced amino acid sequence of UAT indicate that this sequence is novel, with no linear sequence homology to uricase but with homology to a family of
galactoside-binding proteins, the galectins (27–34). UAT has the greatest degree of homology with galectin 5, a 145-amino acid protein of rat reticulocytes (32); 119 of 125 of the identical amino acids are localized in the carboxyl terminus of UAT, between amino acids 186 and 322 (Fig. 2A). Less homology is present between UAT and the other galectins (Fig. 2A). The percentages of amino acid identity between UAT and the galectins, as determined with the GCG program BestFit, are 24.2, 21.2, 21.2, 43.5, 43.5, 85.2, 85.2, 41.9, and 36.6 for galectin 1, 2, 3, 4, 5, 7, and 8, respectively. As is the case with galectins 4 and 8 (30, 34), the sequence of UAT can be subdivided into three regions, a 124-amino acid NH2 terminus, a 61-amino acid linker region, and a COOH terminus of 137 amino acids (Fig. 2B). Although the amino and carboxyl termini of UAT have homology with other galactoside-binding proteins (Fig. 2A) and the termini have 41% identity with each other (GCG BestFit), the linker region is unique, revealing no significant homology to any protein listed in the data bases.

The deduced amino acid sequence of UAT (Fig. 2A) has a number of possibly important sites and signatures (Fig. 2B): two potential N-glycosylation sites (amino acids 209 and 251), five potential myristoylation sites within the amino terminus (amino acids 19, 23, 24, 28, and 54), three potential phosphorylation sites including casein kinase II, tyrosine kinase, and protein kinase C (at amino acids 53, 64, and 122, respectively), and two vertebrate galactoside-binding lectin signatures (amino acids 81 and 254) (GCG program Motifs). Based on the method of Garnier et al. (35) that includes the assumption that at least 20% of the protein assumes a secondary structure, UAT contains a stretch of 21 amino acids (97–117) that is predicted to assume the conformation of an α-helix (Fig. 2B). Alternative methods of analysis of the amino acid sequence do not predict an α-helix that is long enough to span a membrane.

**RT-PCR of UAT from Rat Kidney—**To confirm the authenticity of the cDNA isolated by library screening, RT-PCR of poly(A)+ RNA from rat kidney was performed with two sets of PCR primers that encompassed nucleotides 249-1015 and 249-1272 of the UAT cDNA. This resulted in the generation of PCR products that approximated those predicted on the basis of the number of nucleotides between the sense and antisense primers, 767 and 1024 nucleotides (Fig. 3, lanes 2 and 3). Amplification products were not detected in the absence of reverse transcriptase, confirming the absence of contaminating DNA. Because EcoRI sites are present 5′ and 3′ to the PCR products that are subcloned into pCR™ II and UAT contains an internal EcoRI site, the sequences were evaluated by restriction digestion with EcoRI. Each PCR product yielded two fragments whose sizes were consistent with those predicted on the basis of an internal EcoRI site at nucleotide 405 of UAT (plus 10 bp of the vector 5′ to the PCR product and 6 bp 3′ of the product): 167- and 616-bp fragments for the smaller PCR product and 167- and 873-bp fragments for the larger product (Fig. 3, lanes 7 and 9). In view of the very high degree of homology between the carboxyl terminus of UAT and galectin 5 (Fig. 2A), the PCR products were also sequenced to assess the possibility that the cDNA that was cloned from the rat renal cDNA library might be chimeric. The nucleotide sequences of both PCR products were 100% identical to the sequence of UAT (not depicted). Since both PCR products included the expected amino terminus, the linker region, and the carboxyl terminus of UAT, this finding indicates that the cDNA for UAT is not chimeric and that an mRNA for UAT is transcribed within renal tissue.

**Northern Blot Analysis—**As demonstrated in Fig. 4A, a cDNA probe representing the unique, linker region of UAT primarily recognizes a 1.55-kb mRNA in a variety of organs. mRNAs at approximately 6.0 and 7.5 kb are also recognized by this probe but at much lower intensity (Fig. 4A). The detection of an mRNA that approximates 1.55 kb supports the conclusion that the 1545-bp sequence of UAT, which represents the sum of the nucleotide sequence of the clone from the cDNA library plus the 5′ extension obtained by RT-PCR of rat renal poly(A)+ RNA, is the full-length sequence of UAT (Fig. 1). It remains to be determined whether the larger mRNAs represent heteronuclear mRNA for UAT or alternate messages with significant homology to UAT. Although UAT mRNA has a wide tissue distribution, it is evident that there is differential expression of the mRNA with expression high in liver and very low in testes (Fig. 4, A and B). Of interest, in a multitissue Northern blot in which total (rather than poly(A)+) RNA and the same probe were used, the 1.55-kb mRNA was severalfold more abundant in duodenum than in other tissue, including liver (Fig. 4C). Identical patterns were obtained with two other cDNA probes on two multitissue poly(A)+ RNA blots; however, these alternate probes also recognized mRNAs that approximated 1.0 kb in heart and spleen (not depicted). Since one probe (Styl probe) covered both the amino and carboxyl termini of UAT (nucleotides 147-1048), while the second (EcoRI probe) was confined to the amino terminus (through nucleotide 406), this smaller mRNA would appear to have a region of homology within the amino terminus of UAT. Thus, it is unlikely that the 1.0-kb mRNA in heart and spleen is galectin 5, since the high degree of homology between UAT and galectin 5 is located within the carboxyl terminus of UAT (Fig. 2A).

**Production of Recombinant UAT—**SDS-polyacrylamide gel electrophoresis analysis of induced bacterial lysates (BL21(DE3)pLysE cells that had been transformed with pRSET A-UAT and stimulated with isopropyl-1-thio-β-D-galacto-pyranoside to produce recombinant UAT protein) revealed a 36–37-kDa band that was absent in BL21(DE3)pLysE cells that were transformed with the same vector (pRSET A) without UAT (Fig. 5A). Western blots demonstrated that the 36–37-kDa protein was reactive to the affinity-purified IgG fraction of rabbit anti-pig liver uricase (Fig. 5B). Following affinity chromatography on a Ni-NTA resin, the purified protein, which was eluted in 8 M urea at pH 4.5, was extensively dialyzed to both renature the protein and change the solute content and pH. SDS-polyacrylamide gel electrophoresis with Coomassie Blue staining indicated that the dialyzed, affinity-purified protein was identical in size to that identified in lysates of cells transformed with pRSET A-UAT, and Western blots demonstrated that the protein remained immunoreactive to anti-pig liver uricase (not depicted).

**Functional Assessment of Recombinant Protein—**As depicted in Fig. 6A, single channel activity (evidenced by clear transitions between the open and closed states) was detected in symmetrical urate solutions following fusion of recombinant UAT containing proteoliposomes with the lipid bilayer. Both the closed and open time histograms fit single exponential curves (Figs. 6B), suggesting that only one type of channel was present and that this channel has single open and closed states. In contrast to the channel activity that was evident within minutes of addition of UAT containing proteoliposomes to the chamber, no channel activity was detectable when proteolipo- somes containing an unrelated protein (recombinant HIV-1 Tat protein) was utilized. Since recombinant UAT and HIV-1 Tat proteins were both generated in BL21(DE3)pLysE cells and both proteins were purified and renatured by the same methodology (25), we conclude that the channel is specific to UAT and not a channel that derives from a bacterial protein that might have co-purified with UAT.

The mean current/voltage relationship in 11 experiments performed in symmetrical urate solutions is depicted in Fig.
The shaded residues indicate identity between amino acids in UAT and the individual galectins. Alignment was produced using the GCG program BestFit. The sequences for galectins 1, 3, 4, 5, and 8 were reported in rats; those for galectins 2 and 7 were reported in humans. The following are the accession numbers for the galectins: galectin 1, M19036 (27); galectin 2, M87842 (28); galectin 3, J02962 (29); galectin 4, M73553 (30); galectin 5, L36862 (32); galectin 7, L07769 (33); and galectin 8, U09824 (34). B, model of amino acid (AA) sequence of UAT with potentially important sites (myristoylation, phosphorylation, N-glycosylation, and α-helix) depicted.
A. Linear regression analysis of the mean currents at positive and negative voltages yielded a slope conductance of 9.5 ± 0.47 pS (r = 0.99). The reversal potential was not significantly different from 0 (3.0 ± 2.8 mV). To assess the channel’s selectivity, the current/voltage relationship was assessed in symmetrical solutions of 2.5 mM urate and 220 mM KCl, with and without 5 mM Ba2+.

B. Ba2+ failed to alter channel activity, suggesting that the reconstituted channels are not K+ channels. This conclusion was supported by the finding that the current/voltage relationship was not altered when urate was dissolved in symmetrical solutions of 220 mM CsCl or Cs2SO4 rather than KCl or when an infinite gradient for K+ was created (not depicted). The current/voltage relationship that was observed with symmetrical urate and salt solutions was also not significantly changed in the presence of infinite gradients of Cs+, Ca2+, Cl−, or SO42− (not depicted). Since these observations indicate that the channel is minimally permeant to K+, Cs+, Ca2+, Cl−, and SO42−, it seemed likely that the current was carried by urate. To more directly assess the ability of the channel to conduct urate, the current/voltage relationship was determined in the presence of a 10:1 urate gradient.
ent (cis to trans). Creation of a urate gradient resulted in a significant shift ($p < 0.0001$) in the equilibrium potential to $38.3 \pm 3.3$ mV (Fig. 7B), a value that approaches the equilibrium potential for urate. This change in equilibrium potential occurred without a significant change in slope conductance ($10.8 \pm 0.63$ versus $9.5 \pm 0.47$ picoisiemens in the presence and absence of the urate gradient). This finding confirms that the channel conducts urate and indicates that it is highly selective for this organic anion. Finally, the polyclonal antibody that was used to select UAT from the cDNA library (the IgG fraction of nonimmune serum) failed to alter channel activity. In contrast, the IgG fraction of nonimmune serum failed to alter channel activity.

**DISCUSSION**

A novel full-length cDNA, UAT (Fig. 1), that encodes a 322-amino acid protein was cloned by screening a rat renal cDNA library with a polyclonal antibody to pig liver uricase. UAT mRNA for this sequence was present but differentially expressed in multiple tissues of the rat (Fig. 4). Recombinant protein that was produced from the cloned cDNA approximated the size (36–37 kDa) that was estimated from the deduced amino acid sequence. Surprisingly, the cDNA sequence revealed no homology to uricase, but the recombinant protein was immunoreactive to anti-pig liver uricase (Fig. 5). Functional assessment indicated that a voltage-sensitive ion channel was reconstituted when this recombinant protein was fused with planar lipid bilayers (Fig. 6). In contrast, no channel activity was detected in the presence of an alternate recombinant protein, HIV-1 Tat. Of note, the ion channel that was encoded by the cloned cDNA was highly selective to the organic anion urate (Fig. 7) relative to the inorganic ions potassium, cesium, calcium, chloride, and sulfate, and activity of this channel was blocked by the same antibody that identified the cDNA clone in the rat renal library (Fig. 8).

The polyclonal antibody to pig liver uricase that was used in the present studies is immunoreactive with renal proximal tubule brush-border membranes and with urate-binding proteins affinity-purified from renal cortical membrane vesicles (19). Additionally, this anti-urate antibody specifically blocks electrogenic urate transport in renal membrane vesicles (19) and blocks urate channel activity that is reconstituted upon fusion of pig liver uricase with planar lipid bilayers (21). Based on these findings, it was assumed that this antibody would at least recognize the tertiary structure of the unipporter and thus be valuable in cloning a urate transporter. Utilization of this antibody did provide a means of selecting the UAT clone from the rat renal cDNA library. Moreover, the antibody was immunoreactive to recombinant UAT protein (Fig. 5) and it blocked channel activity of the UAT that was reconstituted in planar lipid bilayers (Fig. 8). The basis for the immunologic and functional reactivity of the antibody with both the cloned UAT and the renal membrane urate transporter, however, is currently unknown, since no homology was evident between the linear amino acid sequences of UAT and uricase. Since noncontiguous sequences of a linear amino acid sequence may bind to antibodies, it can be speculated that epitopes on UAT and the renal membrane transporter do have homology with uricase but that the homology is only evident after noncontiguous portions of the epitopes are brought into contact with each other when the proteins assume their tertiary structures. Alternatively, although the affinity-purified pig liver uricase appeared to be a single antigen, and the polyclonal antibody that was employed appeared to be nonspecific to uricase (19), a non-urate urate transport protein may have co-purified with hepatic uricase. As a consequence, polyclonal antibodies may have been simultaneously generated against both the urate transporter and uricase. If such were the case, interactions between the antibody and renal membrane or recombinant proteins may represent reactivity to anti-urate transport protein rather than to anti-urate. Regardless of the basis of the antigen-antibody interactions, the antibody functioned as an essential tool in cloning and characterizing the urate transporter/channel.

The deduced amino acid sequence of UAT exhibits homology with the galectins (Fig. 2A), a family of $\beta$-galactoside-binding proteins previously referred to as S-Lac lectins (soluble lactase-binding vertebrate lectins). Seven members of the galectin family have been identified to date, galectins 1–5, 7, and 8 (27–34). The tissue distribution of each type of galectin differs considerably, however; within tissues they have generally been localized to the cell cytoplasm, although some, despite the absence of a classical signal sequence, are also externalized to...
the extracellular compartment (31). It is of note that none have been reported to be localized to cell membranes. Since the antibody that was used to clone and characterize UAT recognizes an identical protein in renal tissue, our prior observations imply that UAT, unlike the galectins, resides within renal cell membranes; urate-binding proteins that were affinity-purified from renal cell membranes were immunoreactive to this antibody, urate transport across renal membrane vesicles was specifically inhibited by anti-pig liver uricase, and immunoreactivity to this antibody was localized to brush-border membranes of renal proximal tubules (19). In addition to UAT having a novel cellular localization for a protein that is a member of the galectin family, none of the previously described galectins have been considered to function as membrane transporter/channels. In fact, the biologic function(s) of the reported galectins remains to be established. Rather, galectins have been postulated to play a role in cell migration and adhesion, in the regulation of cell proliferation, in immune function, and in neoplasia (31). The demonstration that UAT operates as a highly selective urate transporter/channel (Figs. 6–8) thus suggests that some galectins may have biologic functions not previously considered.

Northern analysis has revealed that the mRNA for UAT has a wide tissue distribution with considerable variability in the abundance of the mRNA among the organs examined (Fig. 4). While prior immunocytochemical and functional studies strongly suggest that UAT protein is expressed in the kidney (19), we have not as yet determined whether UAT is expressed in other tissues in which its mRNA has been detected. Based on the demonstrated avidity of UAT for lipid bilayers and its activity as a urate channel (Figs. 6–8), however, it seems reasonable to propose that, if translated, UAT is likely to be directed to and inserted as functional urate transporter/channels in cell membranes of the various organs. In this view, it is proposed that the urate transporter/channel that has been cloned provides the molecular basis for urate excretion via polarized epithelial cells of both kidney and intestine.

Within the kidneys, urate is both reabsorbed and secreted in proximal tubules with little if any net transport beyond the pars recta (6). As a consequence, excreted urate derives primarily from proximal tubule urate secretion and, to a lesser extent, from filtered, but nonreabsorbed urate (6). In view of the electronegativity of renal cells relative to their extracellular environment and in view of the negative charge carried by the movement of urate through the channel, the functional role of the channel in the kidney is likely to be in urate secretion (efflux of urate into the tubular lumen across the brush-border membrane). This suggestion is consistent with our previous demonstration of an electrogenic urate uniporter in rat and rabbit renal cortical membrane vesicles (16–19). In contrast to the extensive studies relative to the physiologic mechanisms of renal urate transport (6), there is virtually no information on the mechanism(s) of urate transport across polarized intestinal epithelial cells. Since approximately one-third of the daily urate that is produced in humans is eliminated via the gastrointestinal tract (7, 8) and since the mRNA for UAT is most abundant in intestinal tissue (Fig. 4), it seems likely that UAT plays an important role in the intestinal disposal of urate. Insofar as a negative membrane potential exists in intestinal cells, as it does in all mammalian cells, the intestinal urate transporter/channel may serve a secretory function similar to that in the kidney, allowing urate efflux into the lumen of the gastrointestinal tract. Since the kidneys and intestine provide the sole routes for eliminating urate from the body and since secretion is the mechanism that results in urate excretion, the renal and intestinal UAT channels may play an essential role in modulating systemic urate homeostasis.

The urate transporter/channel may also maintain intracellular urate concentration within the limits of its solubility. If not salvaged and reutilized in the formation of RNA and DNA, the purine bases adenine and guanine are degraded to xanthine and hypoxanthine; the latter are subsequently oxidized to urate by xanthine oxidase (1). Urate represents the intracellular end product of purine metabolism (1) in all cells except hepatic cells of species in which urate is oxidized to allantoin by uricase within peroxisomes of hepatocytes (2–4). Because of its limited solubility (9), it is essential that urate efflux from cells to obviate intracellular crystallization. Since urate does not move through lipid bilayers by nonionic diffusion (36, 37), a specific transporter must exist to serve this function; however, to date none have been described. The urate transporter/channel that has been cloned (Figs. 6–8) may serve this essential housekeeping function in the multiple tissues in which its mRNA has been detected. Whether the abundance of the mRNA for UAT correlates with the abundance of xanthine oxidase in the various tissues and/or with the relative rates of reutilization versus degradation of purine bases remains to be determined.

Although the majority of ion channels that have been described to date are predicted to have a number of transmembrane-spanning domains (38), UAT contains only one stretch of 21 amino acids, 97–117, that is predicted to form an a-helix. While uncommon, the presence of a single transmembrane-spanning domain is not unprecedented, having previously been described in a mini-K+ channel (39) and in channel-forming peptides (40). These ion channels are believed to be formed by a bundle of a-helices that surround a central pore through which the ions move (40). In this context, the functional form of the UAT channel, like the mini-K+ channel and channel-forming peptides, would also be multimeric, with each a-helix functionally amphipathic such that the more hydrophilic face of the helix participates in forming the channel pore while its more hydrophobic amino acids interact with the lipid bilayer. As has been previously postulated for the cysteine residue in the carboxyl terminus of the mini K+ channel (39), the five cysteine residues within the long carboxyl terminus of UAT may participate in oligomerizing and thereby bundling its monomers into the multimer that is needed for formation of the urate channel.

Five potential myristoylation sites are predicted to be present within the first 54 amino acids of UAT (Fig. 2B). Posttranslational modification of UAT with covalent binding of myristate and insertion of one or more of the myristoyl chains into the cytoplasmic monolayer of the cell membrane lipid bilayer would anchor UAT to the membrane (41, 42) and localize its amino terminus to the cytoplasmic face of the membrane. Insofar as the amino terminus of UAT is cytoplasmic, the presence of a single transmembrane-spanning domain would result in an extracellular carboxyl terminus. In this postulated model, the two potential glycosylation sites at amino acids 209 and 251 would reside in the extracellular domain while two of the three potential phosphorylation sites (casein kinase II and tyrosine kinase at amino acids 53 and 64, respectively) would be located on the cytoplasmic side of the cell membrane (Fig. 2B). Since renal and intestinal urate excretion can be modified (8, 43) and since cellular efflux of urate must increase when urate production is accelerated, the phosphorylation sites may play a role in regulating urate transport in response to various stimuli. In this model, the galactoside binding sites that reside in the amino and carboxyl termini of UAT would be located on the cytoplasmic and extracellular sides, respectively, of the cell membrane; however, there is
currently no information available that would suggest a physiologic role for either of these galactoside binding domains within UAT.

In summary, the present studies have provided evidence that a novel recombinant protein that was prepared from a cloned cDNA functions as a selective urate channel. A number of structural characteristics of this protein have been predicted on the basis of its linear structure; however, as with other structural models of ion channels, validation will require mutagenesis studies and ultimately direct determination of the channel's three-dimensional structure.

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