Antigenic and Functional Properties of the Human Red Blood Cell Urea Transporter hUT-B1*

Received for publication, May 23, 2002, and in revised form, June 28, 2002
Published, JBC Papers in Press, July 1, 2002, DOI 10.1074/jbc.M205073200

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The Kidd (JK) blood group locus encodes the urea transporter hUT-B1, which is expressed on human red blood cells and other tissues. The common JK*A/JK*B blood group polymorphism is caused by a single nucleotide transition G838A changing Asp-280 to Asn-280 on the polypeptide, and transfection of erythroleukemic K562 cells with hUT-B1 cDNAs carrying either the G838 or the A838 nucleotide substitutions resulted in the isolation of stable clones that expressed the Jk* or Jk polysaccharide antigens, respectively, thus providing the first direct demonstration that the hUT-B1 gene encodes the Kidd blood group antigens. In addition, immunochemical analysis of red blood cells demonstrated that hUT-B1 also exhibits ABO determinants attached to the single N-linked sugar chain at Asn-211. Moreover, immunoadsorption studies, using inside-out and right-side-out red cell membrane vesicles as competing antigens, demonstrated that the C- and N-terminal ends of hUT-B1 are oriented intracellularly. Mutagenesis and functional studies by expression in Xenopus oocytes revealed that both cysteines Cys-25 and Cys-30 (but not alone) are essential for plasma membrane addressing. Conversely, the transport function was not affected by the JK*A/JK*B polymorphism, C-terminal deletion (residues 368–389), or mutation of the extracellular N-glycosylation consensus site and remains poorly para-chloromercuribenzene sulfonate (pCMBS)-sensitive. However, transport studies by stopped flow light scattering using Jk+ and Jk− K562 transfectants demonstrated that the hUT-B1-mediated urea transport is pCMBS-sensitive in an erythroid context, as reported previously for the transporter of human red blood cells. Mutagenesis analysis also indicated that Cys-151 and Cys-236, at least alone, are not involved in pCMBS inhibition. Altogether, these antigenic, topologic, and functional properties might have implications into the physiology of hUT-B1 and other members of the urea transporter family.

In the last 10 years, facilitated urea transporters (UT), which play a major role in urinary concentration mechanism, have been molecularly characterized in different animal species (1) following the cloning by functional expression in Xenopus oocytes of the rabbit urea transporter (2). Currently, two types of mammalian UT can be distinguished, those that are encoded by the Slc14a2 gene (type UT-A) and those that are encoded by the Slc14a1 gene (type UT-B) (3–6). In humans and mice, these two UT genes occur in tandem on chromosome 18q12 (for humans, see GenBankTM accession number AC023421 (7–9). The Slc14a2 gene encodes five alternative spliced isoforms named UT-A1 to -AS mainly expressed in the renal tubules, except for UT-A5, which is expressed only in testis (6, 10). The Slc14a1 gene only encodes the UT-B1 protein expressed on the red blood cells (RBCs) (8, 11) and in endothelium of the descending vasa recta irrigating renal medulla (12, 13). UT-B1 is also expressed in various organs, as shown in the rat model (14–16).

Recently, it was reported that the urea transport function of human RBCs and the Kidd (JK) blood group are carried by the same protein, hUT-B1/Jk (8). The two major codominant alleles of the JK gene, JK*A and JK*B, have a similar frequency in Caucasian populations (0.51 and 0.49, respectively) and define the three common phenotypes Jk(a+b−), Jk(a−b+), and Jk(a+b+) (17, 18). The genetic basis of the JK*A/JK*B blood group polymorphism is a single nucleotide transition G838A changing Asp-280 to Asn-280 in the JK* and JK* polysaccharide, respectively (19). Because of hUT-B1, RBCs do not undergo excessive cell volume changes during their transit in the vasa recta irrigating the hypertonic renal medulla. In addition, they participate in medulla urea sequestration by taking up urea when flowing in the descending vasa recta and subsequently releasing urea when flowing in the ascending vasa recta (20).

The presence of hUT-B1 in the endothelium descending vasa recta enables a countercurrent exchange of urea with the ascending vasa recta, a process that also contributes to the urea medulla gradient required for water reabsorption. More recently, renal rUT-B1 has been shown to be regulated by anti-diuretic hormone independently from medulla hypertonicity (16). Despite these functional properties, hUT-B1 deficiency, which occurs in the rare human phenotype called Jknull (21), is not associated with any obvious clinical syndrome, except for mild urinary concentration defect, which is, however, more severe in transgenic UT-B null mice (22, 23). The absence of UT-B1 prevents Jknull RBCs from releasing urea as they traverse the ascending vasa recta, thus decreasing the efficiency of the countercurrent exchange and the renal concentration ability. Although a very rare form of Jknull may be inherited as a dominant character, most result from homozygous inheritance of a silent allele at the JK locus (18) and may arise by at least four distinct molecular mechanisms: (i) splice site mutations causing the skipping of either exon 6 or exon 7 (5), (ii) missense mutation resulting in a S291P substitution (24), (iii) nonsense
An erythroid context by expressing the protein in the K562 erythroleukemic cell line, and (iv) to examine the structure-function relationship with respect to plasma membrane targeting and PCMBs sensitivity of the hUT-B1 protein.

MATERIALS AND METHODS

Blood Samples and Reagents—RBC samples from individuals of common and rare Jk phenotypes were obtained from the Centre National de R éférence sur les Groupes Sanguins (CNRGS, Paris, France). Restriction endonucleases and modifying enzymes were from New England B (Hertfordshire, UK). The [14C]urea (1.96 GBq/mmol) and the [3H]raffinose (188.7 GBq/mmol) came from Amersham Biosciences and PerkinElmer Life Sciences, respectively. Two DNA polymerase from Roche Molecular Biochemicals was used for PCR amplification. Nucleotide sequences were determined on both strands with ThermoSequenase fluorescently labeled primer cycle sequencing kit from Amersham Biosciences using 5' (Cys5) primers (Genset, Paris, France) and an automated AlF-Express sequencer (Amersham Biosciences). The human antisera containing an alloanti-Jk3 was obtained from an immunized Jk(a-b-) individual also called Jknull. The human monoclonal antibodies (mAbs) anti-Jkα (IgM, MS15) or anti-Jkβ (IgM, MS8) and the human polyclonal antisera anti-Jkα or anti-Jkβ were from Biostest AG (Dreieich, Germany). Rabbit polyclonal antisera and affinity purified antibodies directed against the N-terminal region (anti-Nter, residues 8–22) or the C-terminal region (anti-Cter, residues 377–389) of the hUT-B1 protein were described previously (8, 12).

Cell Culture, Transfection, and Flow Cytometry Analysis—Human erythroleukemic K562 cells were obtained from the American Type Culture Collection (Manassas, VA) and were grown in Iscove’s modified Dulbecco’s medium with Glutamax-1 (Invitrogen) supplemented with penicillin-streptomycin and 10% fetal calf serum. To establish a stable cell line expressing the Kidd/urea transporter, hUT-B1 cDNA’s (nucleotides 9–1182) carrying either a G838 (JK*A allele) or an A838 (JK*B allele) were amplified by PCR from the Jk(Jk)/p7TTS constructs (19) using SP-1 and AS-1 primers (see Table 1), subcloned into the pcEP4 episomal expression vector (Invitrogen), and transfected into K562 cells using the Lipofectin reagent according to the manufacturer’s instructions (Invitrogen). Stable transfectants resistant to hygromycin (300 µg/ml) were selected for Jk antigen expression by immunomagnetic separation using a human anti-Jk3 antisera and Biogam Goat anti-human-IgG (PerSeptive Biosystems, Connecticut, MA). Stable clones were isolated, and Jkα, Jkβ, and Jk3 antigens expression was analyzed by flow cytometry. Briefly, K562 transfectants (3–5 × 106) were incubated for 60 min at 4°C with appropriate antibodies used at saturating concentration and then washed and stained with 100 µl of phycoerythrin-conjugated F(ab')2 fragments of goat anti-human IgG diluted 1:40 (Coulter/Immunotech, Marseille, France). After another washing step, 20 nM TO-PRO-1, Molecular Probes (Interchim, Mingen) to exclude dead cells (TO-PRO positive-cells).

Kidd Urea Transporter Immunoprecipitation and ABO Immunotyping—RBC membranes of known ABO phenotypes were prepared by hypotonic lysis (30) and solubilized in 10 mM sodium phosphate, pH 7.4, 150 mM NaCl containing 5% (w/v) Triton X-100 and 1 mM (2-aminoethyl)-benzenesulfonylfluoride (AEBSF, Interchim). The clear supernatant (22,000 × g for 30 min) was incubated overnight at 4°C with affinity-purified antibody anti-Nter of hUT-B1 (see above), and immunoprecipitates were purified on Protein A-Sepharose CL-4B (Amersham Biosciences), washed, and eluted by heating as described (31). The immunoprecipitates were separated by SDS-PAGE (15% separating gel) and immunoblotted by reaction with the murine mAbs anti-A (clone 26W2, CNRGS, Paris, France) (1:10 dilution), anti-B (Ortho Diagnostic System, Raritan, NJ) (1:5 dilution), or the affinity-purified antibody anti-Cter of hUT-B1 (10 µg/ml). Bound antibodies were detected with alkaline phosphatase-labeled goat anti-mouse or anti-rabbit IgH(+/L) (1:800 dilution) and CDP-Star chemiluminescence reagent (PerkinElmer Life Sciences).

Immunoadsorption Assays—Right-side-out (ROVs) and inside-out (IOVs) vesicles were prepared from Jk-positive RBCs and purified through a barrier of 8% (w/v) dextran T70, as described previously (32). One volume of each rabbit polyclonal serum against hUTB1 was mixed for 2h at 37°C with 4 volumes of sealed vesicles at the protein concentration of 2.5 mg/ml and subsequently centrifuged at 20,000 × g for 15 min. Free antibodies in the supernatants and the bound antibodies eluted from the goat polyclonal by the digitonin method (33) were analyzed by Western blot on Jk-positive RBC membrane proteins separated by SDS-PAGE and transferred to nitrocellulose sheets.
(Schleicher and Schuell, Keene, NH; 0.1 μm). Bound antibodies were detected with alkaline phosphatase-labeled goat anti-rabbit IgG (1:800 dilution) and the alkaline phosphatase substrate kit (Bio-Rad).

**Site-directed Mutagenesis and Truncation of the hUT-B1/Jk cDNA**—All primers used are given in Table I. Single point mutations C25S, C30S, C15S, N211I, and C236S were introduced into the Jk⁺ allele cDNA using a two-step PCR approach with the common SP-1 and AS-1 primers, in addition to sequence-specific mutagenic primers overlapping each other, AS-2 to 6 and SP-2 to 6, respectively. The second PCR was performed with 1/100 of each two first PCR reactions. The double mutation C25S,C30S was generated similarly using the Jk⁺ cDNA carrying the single mutation C25S as initial PCR matrix. Deletions of nucleotide sequences encoding the first 59 (ΔN) and the last 30 (ΔC) amino acids of the Jk⁺ polypeptide were generated by PCR amplification between SP-ΔN and AS-1 or SP-1 and AS-ΔC primers. For efficient translation, artificial ATG codon and Stop codon were inserted at the 5’ end of the SP-ΔN and AS-ΔC primers, respectively. All PCR amplifications were done under stringent conditions: 94 °C for 0.5 min (1 cycle); 94 °C for 1 min (30 cycles); 72 °C for 1 min (1 cycle). All mutant Jk⁺ cDNAs were subcloned into the EcoRV-digested pTT7S plasmid (kindly provided by P. Krieg, Austin, TX) except for the C15S and C236S mutants, which were subcloned into Kpn I-linearized K562 cells as described above. All constructs were sequenced on both strands using an automated AlfaExpress sequencer (Amersham Biosciences) or an ABI-Prism 310 Genetic Analyser (Applied Biosystems, Foster city, CA) to confirm that the correct junctions/mutations were obtained.

**Oocyte Expression, Flux Measurements, and Immunocytochemistry**—Capped sense RNAs were transcribed *in vitro* from the pTT7S-cDNA constructs linearized with Smal restriction enzyme using T7 polymerase and the mCAP mRNA capping kit from Stratagene (La Jolla, CA). Expression studies were carried out by microinjection of each cDNA (0.1 ng/oocyte in 50 nl) in collagenase-treated K562 cells resuspended at the density of 0.5–1.0 × 10⁷ cells/ml to mix an equal volume of hypertonic solution containing solutes (sorbitol or urea) to create an inwardly directed osmotic gradient of 150 mosM/kg of H₂O, and the scattered light intensity variation was followed.

The first part of the curve corresponded to a water efflux to osmotically equilibrate cells with the external medium and allowed to calculate the osmotic permeability coefficient (Pₒ). Data averaged from 5 to 10 time courses were fitted to a double exponential function by using the simplex procedure of the Biokine software (Biologic), and the apparent Pₒ was calculated according to the following equation: 

\[ Pₒ = \text{Permout} \times \frac{Cₒ \times Vₒ}{Vₒ - Vᵢ} \]

where \( Cₒ \) is the final concentration of total solute inside the cells; \( Vₒ \) and \( Vᵢ \) are the relative volumes of the K562 cells at time, \( t \), and the cell volume, respectively. Impout and Permout were the external concentrations of impermeant and permeable solutes, respectively. In some experiments, pCMBS (0.1–3.0 mM) was added to the cells and hypertonic media 15 min before the shrinkage swelling measurement. Reversibility was carried out after addition of 5 mM β-mercaptoethanol (Sigma).

**RESULTS**

The hUT-B1 Polypeptide Carries Jk and ABO Blood Group Specificities—As Northern blot analysis revealed that the K562 erythroleukemic cell line lacked hUT-B1 transcripts (not shown), stable transfectants expressing the hUT-B1/Jka⁺ and hUT-B1/JkB⁺ allelic cDNAs were established in these cells. Flow cytometry analysis showed that polyclonal antiseraum anti-Jk⁺ strongly reacted with K562-Jk⁺ but not K562-JkB⁺ transfectants, as indicative of an invariant erythroid marker profile.
tants, whereas antiserum anti-Jk\textsuperscript{b} strongly reacted with K562-Jk\textsuperscript{b} but not K562-Jk\textsuperscript{a} transfectants (Fig. 2). Identical results were obtained with human mAbs directed against Jk\textsuperscript{a} and Jk\textsuperscript{b} antigens (not shown). The geometric mean of fluorescence was higher with Jk\textsuperscript{b} as compared with Jk\textsuperscript{a} transfectants (Fig. 2), presumably because of a better transfection efficiency. As expected, both transfectants reacted with the human alloantibody anti-Jk3, indicating that both the JK*A and JK*B alleles also encode the Jk3 antigenic specificity. In contrast, parental K562 cells did not react with antibodies directed against Jk\textsuperscript{a}, Jk\textsuperscript{b}, or Jk3 antigens (Fig. 2).

Although hUT-B1 is known to carry a single N-glycan attached to Asn-211 (Fig. 1) (24), it has not been determined whether this oligosaccharide chain may carry some blood group determinants. To address this issue, the hUT-B1 protein was immunopurified from RBCs of known ABO blood group phenotypes with the affinity-purified anti-Nter antibody to hUT-B1, and the immunoprecipitate was submitted to SDS-PAGE and immunoblotted with murine mAbs anti-A or anti-B. hUT-B1 from blood group A individuals was detected with mAb anti-A only, and hUT-B1 from B individuals was detected with mAB anti-B only, whereas hUTB1 from AB individuals was detected with both mAbs anti-A or anti-B (Fig. 3). In contrast, hUT-B1 prepared from group O individuals were unreactive with anti-A or anti-B antibodies (Fig. 3). As a control, all preparations, except that prepared from a Jk\textsuperscript{null} individual, gave a strong signal with the affinity-purified anti-Cter antibody to hUT-B1. Altogether, these results clearly demonstrated that the hUT-B1 polypeptide carries Jk blood group antigens, and in addition, exhibits ABO antigens attached to the N-glycan at Asn-211 (Fig. 1).

**Topology of the N- and C-terminal Ends of hUT-B1**—To provide experimental evidence that the N and C terminus of hUT-B1 are intracytoplasmic, immunoadsorption assays of anti-Nter or anti-Cter antibodies to hUT-B1 onto ROVs and IOVs were performed. Antibodies remaining unabsorbed and those acid-eluted from each type of vesicles were immunoblotted on RBC membrane proteins from Jk-positive individuals as compared with the starting serum (anti-Nter or anti-Cter). These studies revealed that the anti-Nter or anti-Cter antibodies could not be recovered from acid eluate from ROVs (Fig. 4) and remained in the supernatant. In contrast, the antibodies were recovered in the acid eluate but not in the supernatant from IOVs. As a control, both starting sera reacted with hUT-B1 as a diffuse band of 46–69 kDa, as expected. These data are in agreement with the hydrophobicity...
profile of hUT-B1 predicting an intracellular orientation of the N and C terminus of the polypeptide (Fig. 1).

Membrane Expression and Urea Transport of hUT-B1 in Oocytes—Membrane expression and urea transport activity of wild type and selected mutants of hUT-B1 were investigated in the Xenopus expression system. Urea uptake at 90 s was performed from oocytes injected with the corresponding cRNAs, and membrane expression was analyzed by immunohistochemistry of oocyte sections stained with affinity-purified anti-N-ter or anti-C-ter antibodies to hUT-B1.

First, immunocytochemical analysis revealed that the Jkα and Jkβ allelic forms of hUT-B1 were expressed at the plasma membrane of cRNA-injected oocytes (Fig. 5, B and C), whereas water-injected oocytes remain unstained with both the anti-N-ter or anti-C-ter antibodies (Fig. 5A). Transport studies showed that both proteins mediated a urea flux of the same rate inhibited strongly by phloretin as reported previously (Fig. 6) (28), but not both proteins mediated a urea flux of the same rate inhibited very poorly (Fig. 6).

Water and Urea Permeabilities of K562 Transfectants—To understand why urea transport mediated by the cloned hUT-B1 expressed in Xenopus oocytes and the physiological UT of human RBCs differ in their sensitivity to pCMBS, the urea transport function of hUT-B1 was analyzed in an erythroid context following expression in the erythroleukemic cell line K562. Accordingly, cell suspensions of K562-Jkα or K562-Jkβ transfectants were rapidly mixed with a hyperosmolar solution to drive osmotic water efflux and cell shrinking. The rate of increase in scattered light intensity corresponding to the cell shrinking was measured in K562 cells with (0.15 M) sorbitol as osmolyte (Fig. 7A). The apparent P critiques values in cm s⁻¹ × S.D. calculated were 1.50 ± 0.47 × 10⁻², 1.20 ± 0.65 × 10⁻², and 1.48 ± 0.28 × 10⁻² for wild type K562 cells, K562-Jkα, and K562-Jkβ transfectants, respectively (Table II). When the hyperosmolarity was performed with (0.15 M) urea, the light scattering increase in wild type K562 cells (P critiques = 1.71 ± 0.53 × 10⁻² cm s⁻¹, n = 5) was identical to the rate increase observed with sorbitol excluding an endogenous urea transport activity (Fig. 7A). However, only the K562-Jkα and -Jkβ transfectants presented, after a brief scattered light intensity increase due to the efflux of water, a significant decrease of light scattering due to the cell swelling directly related to the osmotic influx of water accompanying urea uptake to ensure osmotic equilibration (Fig. 7B). The P critiques values calculated were 1.78 ± 0.9 × 10⁻² and 3.35 ± 0.9 × 10⁻⁵ cm s⁻¹ for K562-Jkα and K562-Jkβ, respectively (Table II).

The urea permeability of K562-Jkα was measured in the presence of 0.3 mM pCMBS. We found that the urea flux was strongly reduced to 20%, a value close to that reported for RBCs (29). Moreover, pCMBS inhibition was reversed by 5 mM β-mercaptoethanol (Fig. 7B). Identical results were obtained with K562-Jkβ (not shown). Next, to identify the cysteine residues involved in the pCMBS sensitivity, the unique extracellular cysteine Cys-236 and the intramembranous cysteine Cys-151 specific of hUT-B1, near the cell surface, were mutated to
serine (Fig. 1). Thus the pCMBS sensibility of K562-Jka and of two K562 transfectants expressing Jka mutant proteins carrying the C151S and C236S substitutions at the same level were compared. All exhibited comparable \( P_{\text{urea}} \) values found for K562-Jka and Jkb (Table II). Fig. 7C shows that K562-Jk\( ^a \) and K562-C151S had identical dose-response inhibition by pCMBS, whereas the K562-C236S exhibited a higher sensitivity. At 0.3 mM pCMBS, the remaining transport activities were 55% for the former transfectants and only 20% for the latter. At 3 mM pCMBS, the urea transport activity of all samples was completely inhibited (Fig. 7C). Thus, the urea transport mediated by hUT-B1 is pCMBS-sensitive in an erythroid context, but Cys-151 and Cys-236, at least alone, are not involved in pCMBS inhibition.

**Fig. 5. Immunocytochemical analysis.** Sections of oocytes injected with water (A) as negative control or cRNAs encoding the JKA (B and D) or JKBA (C) alleles and the mutant JKVA alleles \( \Delta N \) (E), \( \Delta C \) (F), N211I (G), C30S (H) or C25S,C30S (I). The sections A–C, F–I, and D and E were stained with affinity-purified antibodies against the N or C terminus of hUT-B1, respectively, as described under "Materials and Methods." Negative control for antibodies against the C terminus tested with water injected oocytes was identical to section A (not shown). Images were generated using a Nikon Eclipse TE300 microscope (Nikon, Paris, France) (×40 objective) with epifluorescence illumination and treated with a Biocon informatic system of image integration (Biocom, les Ulis, France).

**Fig. 6. Functional analysis of wild type and hUT-B1 mutant proteins in Xenopus oocytes.** Urea uptake and effect of pCMBS and phloretin on urea transport of oocytes injected with cRNAs encoding the JKVA or JKVB alleles and the following mutant JKVA alleles: \( \Delta N \), \( \Delta C \), \( \Delta N+\Delta C \), N211I, C25S, C30S, and C25S,C30S. For each oocyte, 0.1 ng of cRNA were injected, and at least six oocytes/point were preincubated or not with pCMBS (1 mM) or phloretin (1 mM) containing medium for 20 and 10 min, respectively. Inhibitors were maintained during the whole experiment. The urea transport assay was initiated by suspending individual oocytes in 0.2 ml of Barth's solution containing 8 \( \mu \)Ci/ml \( \left[{^{14}}\text{C}\right] \)urea (145 \( \mu \text{Ci} \)) and 5 \( \mu \text{Ci/ml} \) \( \left[{^{3}}\text{H}\right] \)raffinose as a control of oocyte plasma membrane integrity. The urea uptake was stopped after 90 s of incubation by addition of 3 ml of ice-cold Barth's solution and followed by two fast washes with 5 ml of the same solution. After solubilization, the samples were subjected to liquid scintillation in a counter, and the urea permeability \( (P_{\text{urea}}) \) was calculated from the oocyte-associated amount of \( \left[{^{14}}\text{C}\right] \)urea at 90 s, corrected for the optically determined oocyte surface area. Water-injected oocytes served as negative controls. Data (mean ± S.E.) correspond to one representative experiment of at least three.
DISCUSSION

The hUT-B1 Protein carries Kidd (Jk) as Well as ABO Blood Group Antigens—The formal proof that the hUT-B1 gene encodes the Jka and Jkb blood group antigens was provided by showing that human mAbs and polyclonal antisera anti-Jka or anti-Jkb only react with K562 cells transfected with Jka or Jkb alleles of the cloned hUT-B1 urea transporter, respectively. In addition, these studies showed that the N-glycan chain attached to Asn-211 of hUT-B1 carries ABO blood group determinants as found for the N-glycan chains of Band-3, Band-4.5 (glucose transporter), and AQP-1 (38–40). Knowing that all hUT-B1 molecules appear glycosylated in RBCs (8), less than 1% of RBC ABO determinants is carried by the hUT-B1 protein, which is present at a low copy number (1.4 × 10⁴ molecules/cell), as compared with Band-3 and AQP-1 proteins, which are present at high copy numbers (10⁶ and 2 × 10⁵ molecules/cell, respectively). Thus, the antigenic properties of hUT-B1 and its expression in renal tissues may have biological implications in kidney transplantation.

Topological and Functional Properties of hUT-B1—In accordance with the predicted membrane topology of hUT-B1 (11), expression studies reported here indirectly confirmed the extracellular exposure of the third and fourth loops carrying the N-glycosylation site (at Asn-211) and of the JKA/JKB allelic polymorphism, respectively (Fig. 1). Moreover, immunoadsorption studies provide evidence for the intracellular orientation of the C and N terminus of the protein in RBCs (Fig. 1). Similar membrane topology has been found for other membrane proteins such as the anion exchanger Band-3 (AE1) (41) and the water channel AQP-1 (42). Whether the N- and/or C-terminal domains play some role in the UT function and/or protein-protein interaction with other membrane components presently not known. However, our preliminary results in Xenopus oocytes suggested that cysteines Cys-25 and Cys-30 together (not alone) within the 59 N-terminal amino acid domain are critical for correct addressing and insertion of hUT-B1 in the plasma membrane. Conversely, neither the deletion of the last 30 C-terminal nor the JKB/JKA polymorphism had any affect on the expression level and urea transport activity of hUT-B1. Although N-glycosylation often determines membrane expression level and addressing of polypeptides to the membrane, (43–45), we found that unglycosylated hUT-B1 polypeptide is normally addressed and inserted in the oocyte plasma membrane and exhibited a normal urea transport activity as reported for AQP2 (46, 47).

Another goal of this study was to analyze protein expression and urea transport activity of hUT-B1 into the erythroleukemic K562 cells to investigate the sensitivity to mercurial agents in an erythroid context. Indeed, recombinant hUT-B1 in oocytes confers a urea permeability, which is poorly inhibited by pCMBS, whereas the native UT of human RBCs is strongly inhibited by mercurial agents (28, 29). Accordingly, the rates of volume change of wild type K562 cells and K562-Jka and -Jkb transfectants submitted to an osmotic sorbitol gradient were measured by light scattering stopped flow experiments. Under these conditions, parental and K562 transfectants exhibited relatively high apparent water permeabilities close to published values (48). The absence of a further increase in K562-
Jkα and Jkβ transfectants confirmed that the hUT-B1 is not a water channel as reported previously (28). When an osmotic urea gradient was imposed, the urea transport capacity of hUT-B1 was preserved in K562-Jkα and -Jkβ transfectants, the calculated $P_{\text{urea}}$ values being of 1.78 ± 0.34 × 10−5 and 3.35 ± 0.9 × 10−5 cm s−1, respectively. The difference of $P_{\text{urea}}$ values observed might be related to the difference in the transporter density in K562 transfectants, as suggested by the shift in fluorescence intensity detected by the alloanti-Jk3 antibody (Fig. 2).

We further demonstrated that the urea flux mediated by K562-Jkα and -Jkβ transfectants was strongly inhibited by a low concentration of pCMBS and was reversed by β-mercaptoethanol, as reported for the UT of human RBCs (49). These findings suggest that the low pCMBS sensitivity in Xenopus oocytes reported previously was probably related to membrane environmental factors (lipid and protein) specific to this heterologous expression system (50). Thus, it is possible that hUT-B1 adopts a different conformation in oocyte and red cell membranes, which might lead to the design of novel diuretic drugs. Moreover, our findings provide a basis for further studies regarding hUT-B1 and presumably other members of the UT family for clinical applications.

We thank Pierre Gane (Institut National de Transfusion Sanguine, Paris, France) for flow cytometry analysis.

Acknowledgment—We thank Pierre Gane (Institut National de Transfusion Sanguine, Paris, France) for flow cytometry analysis.

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J. Biol. Chem. 2002, 277:34101-34108.
doi: 10.1074/jbc.M205073200 originally published online July 1, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205073200

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