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Received for publication, June 16, 2004, and in revised form, August 2, 2004 Published, JBC Papers in Press, August 10, 2004, DOI 10.1074/jbc.M406724200

Naohiko Anzai‡, Hiroki Miyazaki‡, Rie Noshiro‡, Suparat Khamdang‡, Arthit Chairoungdua‡, Ho-Jung Shin‡, Atsushi Enomoto‡, Shinichi Sakamoto‡, Taku Hirata‡, Kimio Tomita‡, Yoshikatsu Kanai‡, and Hitoshi Endou‡

From the ‡Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-2, Shinkawa, Mitaka-shi, Tokyo 181-8611 and the and ‡Department of Nephrology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1, Honjo, Kumamoto-shi, Kumamoto 860-8556, Japan

The urate-anion exchanger URAT1 is a member of the organic anion transporter (OAT) family that regulates blood urate level in humans and is targeted by uricosuric and antiuricosuric agents (Enomoto, A., Kimura, H., Chaireungdaua, A., Shigeta, Y., Jutabha, P., Cha, S. H., Hosoyamada, M., Takeda, M., Sekine, T., Igarashi, T., Matsuo, H., Kikuchi, Y., Oda, T., Ichida, K., Hosoya, T., Shimotaka, K., Niwa, T., Kanai, Y., and Endou, H. (2002) *Nature* 417, 447–452). URAT1 is expressed only in the kidney, where it is thought to participate in tubular urate reabsorption. We found that the multivalent PDZ (PSD-95, *Drosophila* discs-large protein, *Zonula occludens* protein 1) domain-containing protein, PDZK1 interacts with URAT1 in a yeast two-hybrid screen. Such an interaction requires the PDZ motif of URAT1 in its extreme intracellular C-terminal region and the first, second, and fourth PDZ domains of PDZK1 as identified by yeast two-hybrid assay, in vitro binding assay and surface plasmon resonance analysis (K_d = 1.97–514 nM). Immunoprecipitation studies revealed that the wild-type URAT1, but not its mutant lacking the PDZ-motif, interacts with PDZK1. Colocalization of URAT1 and PDZK1 was observed at the apical membrane of renal proximal tubular cells. The association of URAT1 with PDZK1 enhanced urate transport activities in HEK293 cells (1.4-fold), and the deletion of the URAT1 C-terminal PDZ motif abolished this effect. The augmentation of the transport activity was accompanied by a significant increase in the V_max of urate transport via URAT1 and was associated with the increased surface expression level of URAT1 protein from HEK293 cells stably expressing URAT1 transfected with PDZK1. Taken together, the present study indicates the novel role of PDZK1 in regulating the functional activity of URAT1-mediated urate transport in the apical membrane of renal proximal tubules.

Urates are the major inert end product of purine degradation in humans and higher primates in contrast to most other mammals because of the genetic silencing of hepatic oxidative enzyme uricase (1, 2). The kidney plays a dominant role in urate elimination; it excretes ~70% of the daily urate production. Urate exists primarily as a weak acid at physiological pH (pK_a 7.5), and most of it is dissociated in blood and is freely filtered through the glomerulus. Thus, urate enters the proximal tubule in its anionic form, but it hardly permeates the tubular cells in the absence of facilitated mechanisms owing to its hydrophilicity. The transport mechanisms for urate are localized in the proximal tubule. In humans, urate is almost completely reabsorbed, which results in the excretion of ~10% of its filtered load. The absence of uricase and the presence of an effective renal urate reabsorption system contribute to higher blood urate levels in humans. Therefore, it was postulated that defects in tubular urate transport cause hypouricemia and decreased renal urate clearance leads to hyperuricemia in most hyperuricemic patients (3).

Recently, we have identified the long hypothesized urate transporter in the human kidney (URAT1, encoded by *SLC22A12*), a urate-anion exchanger localized on the apical side of the proximal tubule (4). URAT1 is targeted by uricosuric and antiuricosuric agents that affect urate excretion (e.g., benzamorine, probenecid, and pyrazinamide). The pharmacological properties manifested by URAT1 cRNA-injected *Xenopus* oocytes are consistent with those of the previously described urate transport activities in human brush-border membrane vesicles. We also found that defects in *SLC22A12* lead to idiopathic renal hypouricemia (Mendelian Inheritance in Man number 220150), and patients with such defects show a high fractional urate excretion such as 95 ± 10% (normally <10%). These results indicate that URAT1 regulates blood urate level and *vice versa*, that is, to control blood urate levels, the URAT1 transport function should be regulated. A newly found genetic alteration in *SLC22A12* from a patient of an idiopathic renal hypouricemia has prompted us to consider the importance of the URAT1 extreme intracellular C-terminal region for its function (5). A 5-bp deletion near the URAT1 C-terminal end (1639–1643del) causes frameshift, and the seven amino acids

* This work was supported in part by grants from the Japanese Ministry of Education Science, Sports, Culture and Technology, grants-in-aid for Scientific Research and Bioventure Project from the Science Research Promotion Fund of the Japan Private School Promotion Foundation, and grants-in-aid from the Tokyo Biochemical Research Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§ Both authors contributed equally to this work.

† To whom correspondence should be addressed. Tel.: 81-422-47-5511 (ext. 3451); Fax: 81-422-79-1321; E-mail: endouh@kyorin-u.ac.jp.

1 The abbreviations used are: URAT1, urate-anion exchanger 1; OAT, organic anion transporter; GST, glutathione S-transferase; PDZ, PSD-95/Discs-large homology domain; NHERF, Na+/H+-exchanger regulatory factor; CT, C terminus; wt, wild type; PBS, phosphate-buffered saline; GFP, green fluorescent protein; HEK, human embryonic kidney; MBP, maltose binding protein; SPR, surface plasmon resonance; IKEPP, intestinal and kidney-enriched PDZ protein.
in the terminal sequences have changed into eight different amino acids. The URAT1 transport activity of this mutation is low in the *Xenopus* oocyte expression system. Interestingly, the PDZ binding motif at the C-terminal end of URAT1, which is known to participate in protein-protein interaction, disappears by this amino acid sequence modification.

PDZ (PSD-95,DlgA, and ZO-1)-binding domains have been identified in various proteins and are known to be modular protein-protein recognition domains that play a role in protein targeting and protein complex assembly (6–8). These domains range from 80 to 90 amino acids in length and bind typically to proteins containing the tripeptide motif (S/T/XΩ (X = any amino acid and Ω = a hydrophobic residue) at their C termini (9, 10). These multidomain molecules not only target and provide scaffolds for protein-protein interactions but also modulate the function of receptors and ion channels, by which they associate (11–16). The disruption of the association between PDZ proteins and their targets contributes to the pathogenesis of a number of human diseases, most probably because of the failure of PDZ proteins to appropriately target and modulate the actions of associated proteins (6).

In this study, we use the yeast two-hybrid approach to investigate the putative URAT1-associated proteins that modulate its transport function. We identify the multivalent PDZ domain-containing protein PDZK1 as an apparent partner of URAT1 in the human kidney. Moreover, we show a functional consequence of PDZK1-URAT1 interaction in transfected HEK293 cells, where URAT1 transport activities were increased by 1.4-fold by coexpression of PDZK1. We speculate that PDZK1 is a scaffolding protein that may be a physiological regulator of the function of URAT1.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The C-terminal fragments of human URAT1 cDNA, comprising three mutants (designated as d3, F555A, and T556A), were generated by PCR (antisense primers, 5′-CTC TCG AGC TAA AAC TGT TGG TTT CTT GGT TAT TTG AGG ACA GAG TTC-3′; 5′-CTC TCG AGC TAA AAC TGT TGG TAG TCT GGA AGG GAT TTT-3′; 5′-CTC TCG AGC TAA AAC TGT TGG TAG TCT GGA AGG GAT TTT-3′; antisense primer, 5′-CTC TCG AGC TAA AAC TGT TGG TAG TCT GGA AGG GAT TTT-3′), and inserted into the EcoRI and XhoI sites of the pEG202 plasmid, a LexA DNA-binding domain fusion vector. The same human URAT1 C-terminal fragments were also inserted into the pGEX-6P-1 plasmid (Amer sham Biosciences) for GST fusion protein production. The full-length coding sequences of human URAT1 (wt) as well as its C-terminal deletion constructs or of MBP fusion proteins was applied together with 50 μl of GST-glutathione-Sepharose resin to Handee Spin Cup columns using the ProFound Pull-Down GST Protein:Protein Interaction kit (Pierce), and protein complexes were eluted according to the manufacturer’s instructions. The eluted samples were resuspended in Laemmli buffer, heated for 5 min at 95 °C, and electrophoresed in 10% SDS-PAGE gels; the fractionated proteins were transferred onto polyvinylidene difluoride membranes. The precipitated proteins were detected with the Transcend Chemiluminescent Translation Detection System (Promega) or immunoblotting using anti-MBP antiserum (New England Biolabs) developed by enhanced chemiluminescence.

**Surface Plasmon Resonance**—The interaction of URAT1 C terminus with individual PDZ domains of PDZK1 was investigated by the use of a BIAcore 3000 analyser (BIAcore AB) based on sensor chip samples described previously (17). URAT1 C-terminal fragment, referred to as the ligand, was immobilized on a sensor chip, and the interaction with PDZK1 individual PDZ domains fused with MBP, referred as the analyte, was detected through the mass changes of the reflective index on the sensor surface. All of the reagents such as an amine coupling kit, washing buffer HBS-EP, pH 7.4 (20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% Surfactant P20), and the CM5 sensor chip were obtained from BIAcore AB. Using an amine coupling kit, GST-fused URAT1 CT wild-type or GST protein alone was attached to a CM5 sensor chip according to the manufacturer’s instructions, giving a gain of 10,673 resonance units for GST-URAT1-CT or 8,566 resonance units for GST alone. Binding experiments were performed with the PDZK1 single one PDZ domains (PDZ1 to PDZ4) fused with MBP as described above. The analyte was injected at a flow rate of 30 μl/min in HBS-EP buffer at 25 °C, and the association and dissociation phases (upon switching back to buffer) were monitored for 120 and 180 s, respectively. For data acquisition, five different concentrations of each protein were used. At least two replicate experiments were performed for each fusion protein. The data were analyzed with the BIAevaluation program 3.2 (BIAcore).
AGC GCA CGG GTT GG-3' (reverse primer for hURAT1), 5'-GAG GAG AAC TGG GAT GTG TT-3' (forward primer for hPDZK1), 5'-TTT GTA GCC TGG TGA TGA CT-3' (reverse primer for hPDZK1), 5'-GAG GAG AAC TGG GAT GTG TT-3' (forward primer for human (β-actin), and 5’-TTT GTA GCC TGG TGA TGA CT-3’ (reverse primer for human β-actin).

**Immunohistochemical Analysis**—The antibodies against human URAT1 used in this study have been shown to be specific for its synthetic peptide, as previously described (4). We used human single-tissue slides (Biochain) for light microscopic immunohistochemical analysis using the streptavidin-biotin-peroxidase complex technique (LSAB kit; DAKO, Carpinteria, CA). Sections were deparaffinized, rehydrated, and incubated with 3% H2O2 for 10 min to abrogate endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween 20 (TBST), the sections were treated with 10 µg/ml primary rabbit polyclonal antibodies against hURAT1 and hPDZK1 (4°C overnight). Then, the sections were incubated with the secondary antibody, biotinylated goat polyclonal antibody against rabbit immunoglobulin (DAKO), diluted 1:400 for 30 min with horseradish peroxidase-labeled streptavidin. This step was followed by incubation with diaminobenzidine and hydrogen peroxide. The sections were counterstained with hematoxylin and examined by light microscopy.

**Cell Culture and Transfection**—Human embryonic kidney 293 (HEK293) cells maintained in modified RPMI medium (DMEM) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 µg/ml streptomycin (Invitrogen) at 37°C and 5% CO2. Transient transfection with LipofectAMINE 2000 (Invitrogen) was performed according to the manufacturer’s instructions. After transfection, the cells were grown 36–48 h before the experiments. For the establishment of URAT1-expressing cells, stable transfectants were selected for 2 weeks by adding 0.5 µg/ml G418 to the medium.

**Immunoprecipitation and Immunoblotting**—Twenty-four hours after cotransfection, HEK293 cells in 100-mm plates were lysed with a buffer containing 20 mM Tris (pH 7.4), 250 mM NaCl, 1% Triton X-100, 5 mM EDTA, 0.25% deoxycholate, and protease inhibitor mixture (PIC, Sigma). Lysates were centrifuged at 15,000 rpm for 5 min, and the supernatant was collected. 1 µl of the anti-GFP antibody (Full-Length A.v. Polyclonal Antibody, Clontech) was added to the supernatant, and the mixture was incubated overnight at 4°C with continuous gentle shaking. For the immunoprecipitation of endogenous URAT1 and PDZK1, we used human kidney membrane fractions (Biochain) and added anti-PDZK1 antibody or control IgG to this solution. Then URAT1 and associated proteins were immunoprecipitated using the Seize Protein A ImmunoPrecipitation kit (Pierce), and protein complexes were eluted according to the manufacturer’s instructions. The elution samples were resuspended in SDS sample buffer and heated for 5 min at 100°C, and proteins were resolved on 10% SDS-PAGE gels. The resolved proteins were then electrophoretically transferred to polyvinylidene difluoride membranes. The affinity-purified rabbit PDZK1 antibody against the carboxyl terminus of URAT1, have been replaced by alanine, which was expected to abolish or strongly suppress the binding of PDZ proteins (10). These three baits did not interact with PDZK1 (Fig. 1B). Thus, binding through the C terminus of URAT1 suggests that the PDZ motif of URAT1 is the site of interaction with PDZK1.

**Domain Analysis of PDZK1 Protein-Protein Interaction with URAT1 C Terminus—**PDZK1 possesses four PDZ domains that assemble target proteins by binding to a C-terminal motif with a consensus sequence such as (S/T)Xoo. To determine the possible interactions of the URAT1 C-terminal region with the

\[
V = \frac{V_{\text{max}} \times S}{K_m + S} \quad \text{(Eq. 1)}
\]

where \(V\) is the uptake rate of the substrate (picomoles/min/mg of protein), \(S\) is the substrate concentration in the medium (µM), \(K_m\) is the Michaelis-Menten constant (µM), and \(V_{\text{max}}\) is the maximum uptake rate (picomoles/min/mg of protein). These values were determined with the Eadie-Hofstee equation.
PDZ domains of PDZK1, we produced prey vectors each containing individual PDZ domain (PDZ1, PDZ2, PDZ3, and PDZ4). The interaction with URAT1 C terminus was observed for PDZ1, PDZ2, and PDZ4 but not for PDZ3 of PDZK1 (Fig. 1C).

**In Vitro Binding of URAT1 and PDZK1**—To confirm *in vitro* the ability of the C terminus of URAT1 to bind with PDZK1, we used GST pull-down assay to validate the protein-protein interaction (Fig. 2A). GST fusion proteins bearing the wild-type C-terminal region (URAT1-CT-wt) or C-terminal region mutants (URAT1-CT-d3, F555A, and T553A) of URAT1 were used to pull down the full-length PDZK1 from *in vitro* translation experiments. The data showed the same specificity of the interaction of PDZK1 and URAT1 as exhibited in the yeast two-hybrid assays (Fig. 1B). As expected, we did not observe the binding of PDZK1 to the URAT1 in which the C-terminal PDZ recognition motif was removed (URAT1-CT-d3) or mutated (URAT1-CT-F555A or URAT1-CT-T553A) (Fig. 2A).

To confirm the binding specificity between URAT1 C terminus and PDZK1 PDZ domains, we generated MBP-fused proteins consisting of PDZK1 individual PDZ domain (PDZ1, PDZ2, PDZ3, or PDZ4) and tested the interaction with GST-fused URAT1-CT-wt. Fig. 2B shows that MBP-PDZ1, MBP-PDZ2, and MBP-PDZ4 could bind with URAT1 C terminus same as the results obtained from the yeast two-hybrid assays (Fig. 1C).

These interaction specificities were further confirmed by surface plasmon resonance (SPR) method. We observed dose-responsive bindings of the PDZ1, PDZ2, and PDZ4 domains of PDZK1 fused with MBP to an immobilized URAT1 C terminus (Fig. 3, A, B, and D). In contrast, no significant binding was detected in the PDZ3 domain of PDZK1 (Fig. 3C).

The results of kinetic analysis performed on all four single PDZ domains of PDZK1 are summarized in Table I. The dissociation constant ($K_D$) values are as follows: PDZK1 PDZ1 domain, 1.97 nM; PDZ2 domain, 514 nM; and PDZ4 domain, 296 nM for URAT1 C terminus. These binding affinities are similar to the value reported for the interaction between CAP70 and CFTR ($K_D = 8–220$ nM) (14).

**Coimmunoprecipitation of URAT1 and PDZK1 from Heterologous Cells and from Human Kidney Membrane Fraction**—To demonstrate that URAT1 and PDZK1 can also interact in mammalian cells, first we generated a rabbit anti-PDZK1 polyclonal antibody directed against the N-terminal 14 amino acids of human PDZK1. This antibody was tested by Western blot analysis using human normal adult kidney protein extracts and crude membrane fractions from HEK293 cells transfected with pcDNA3.1-PDZK1. Western blot analysis showed that the anti-PDZK1 antibody reacted with a strong band of $-63$ kDa, which is consistent with PDZK1 on a human kidney protein extract and with a strong band of $-70$ kDa and a weak band of $-63$ kDa on crude membrane fractions from PDZK1-transfected HEK293 cells (Fig. 4A). Because the weak 63-kDa band was also identified in cells transfected with vector alone (pcDNA3.1) and we detected the PDZK1 mRNA expression in HEK293 cells by RT-PCR (data not shown), these weak bands may be explained by the endogenous expression of PDZK1 in these cells rather than an artifact. All of these bands disappeared after the incubation of synthetic peptides (200 µg/ml, data not shown). The different molecular sizes detected in the kidney and in HEK293 cells may be due to the different post-translational modifications in the native tissues and the cells.
FIG. 3. Surface plasmon resonance analyses of the interaction between C terminus of URAT1 and the PDZK1 single PDZ domains. A, the binding of the PDZK1 PDZ domain 1 fused with MBP, at different concentrations, to the URAT1 C terminus. B, the binding of the PDZK1 PDZ domain 2 fused with MBP, at different concentrations, to the URAT1 C terminus. C, the binding of the PDZK1 PDZ domain 3 fused with MBP, at different concentrations, to the URAT1 C terminus. D, the binding of the PDZK1 PDZ domain 4 fused with MBP, at different concentrations, to the URAT1 C terminus.
The kinetic characteristics of the interaction of immobilized GST-fused URAT1 C terminus with single one-PDZ domains of PDZK1 (PDZ1–4) fused with MBP are summarized (see Fig. 3). Association rate constants ($k_a$), dissociation rate constants ($k_d$), and equilibrium dissociation constants ($K_d = k_d/k_a$) are given.

| Construct | $k_a$ (1/min) | $k_d$ (1/min) | $K_d$ (nM) |
|-----------|---------------|---------------|------------|
| PDZK1-PDZ1 | 3.45 x 10^3  | 6.79 x 10^-4  | 1.97 |
| PDZK1-PDZ2 | 4.02 x 10^3  | 2.07 x 10^-3  | 514 |
| PDZK1-PDZ3 | —   | —   | — |
| PDZK1-PDZ4 | 1.89 x 10^3  | 5.61 x 10^-4  | 296 |

Note: —, not binding detectable.

To determine whether URAT1 and PDZK1 colocalize at the apical membrane of the renal proximal tubules, we performed immunostaining of serial kidney sections with the anti-URAT1 and anti-PDZK1 antibodies. Consistent with the previous reports, in the renal cortex, the overlapping expressions of URAT1 and PDZK1 was detected in most of the proximal tubular cells (Fig. 5, B and C). The specificities of each antibody were confirmed by the reduced immunoreactivities caused by the preincubation of the antibodies with the corresponding synthetic peptides (200 µg/ml, data not shown).

**Table I**

| Construct | $k_a$ (1/min) | $k_d$ (1/min) | $K_d$ (nM) |
|-----------|---------------|---------------|------------|
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| PDZK1-PDZ3 | —   | —   | — |
| PDZK1-PDZ4 | 1.89 x 10^3  | 5.61 x 10^-4  | 296 |

* —, not binding detectable.

**Fig. 4. Coimmunoprecipitation of URAT1 and PDZK1 proteins in HEK293 cell lysates and from human kidney membrane fractions.** A, Western blot analysis of normal human kidney protein extract (lane 1), and crude membranes from HEK293 transfected with PDZK1 (lane 2) and from HEK293 transfected with mock (lane 3). A single strong band of ~63 kDa, which is consistent with PDZK1, was observed in lane 1, a strong band of ~70 kDa and a weak band of 63 kDa were observed in lane 2, and a weak band of 63 kDa was observed in lane 3. B, HEK293 cells were transfected with pEGFP-C2 vectors alone (lane 1) or vectors encoding wild-type URAT1 (lane 2) or URAT1-d3 (lane 3) with pcDNA3.1-PDZK1 and coimmunoprecipitated with the anti-GFP antibody. Then, the coimmunoprecipitates were resolved by SDS-PAGE and probed with anti-PDZK1 antibodies. C, human kidney membrane fractions were immunoprecipitated with control IgG and anti-PDZK1 antibody. The presence of URAT1 in the immunoprecipitates was determined by Western blotting with the anti-URAT1 C-terminal antibody used in previous report (4).

Then, we expressed pEGFP-C2 vector alone and coexpressed GFP-fused full-length human URAT1 wt and d3 with PDZK1 in HEK293 cells. Wild-type EGFP-URAT1 was coimmunoprecipitated with a GFP-specific antibody with a strong 70-kDa band (and a very faint 63-kDa band after long exposure), but EGFP-URAT1 lacking the last three amino acids and vector alone was not (Fig. 4B). These results also confirm the interaction between PDZK1 and URAT1 detected in the yeast two-hybrid system, in addition to other in vivo binding assays.

Furthermore, we demonstrated an association between endogenous PDZK1 and URAT1 in tissue by coimmunoprecipitating URAT1 from human kidney membrane fractions using PDZK1 antibody, but not control IgG (Fig. 4C). This result provided us evidence that the observed interaction occurs between protein partners expressed from the endogenous genes in relevant tissue.

**Tissue Distribution of URAT1 and PDZK1 mRNA in Human Tissues**—In humans, URAT1 has been detected exclusively in the kidney (4), and PDZK1 has been detected mainly in the liver, kidney, pancreas, gastrointestinal tract, and adrenal cortex (19), but their expressions in other tissues has not yet been analyzed. Using human multiple-cDNA panels, we examined the mRNA distributions of both URAT1 and PDZK1. The URAT1 transcript was detected strongly in the kidney, whereas the PDZK1 transcripts were detected in most of the tissues analyzed in a various levels of intensity, confirming and expanding the previously described distribution in humans (Fig. 5A).

**Coexpression of URAT1 and PDZK1 in the Human Kidney**—URAT1 is present at the luminal (apical) membrane of proximal tubules (4), and PDZK1 is reported to be expressed at the brush border (apical) of the proximal tubular cells (19). To determine whether URAT1 and PDZK1 colocalize at the apical membrane of the renal proximal tubules, we performed immunostaining of serial kidney sections with the anti-URAT1 and anti-PDZK1 antibodies. Consistent with the previous reports, in the renal cortex, the overlapping expressions of URAT1 and PDZK1 was detected in most of the proximal tubular cells (Fig. 5, B and C). The specificities of each antibody were confirmed by the reduced immunoreactivities caused by the preincubation of the antibodies with the corresponding synthetic peptides (200 µg/ml, data not shown).

**URAT1 Transport Activity Increases in the Presence of PDZK1**—To determine whether URAT1/PDZK1 interactions change URAT1 activity, we transfected transiently HEK293 cells with the pcDNA3.1(+) construct containing full-length URAT1 or URAT1 lacking the last three amino acids of its C-terminal. At an incubation time of 1 min, we demonstrated that the uptake of [14C]urate by the wild-type URAT1 and URAT1 deletion mutant were from 3- to 4-fold higher than that by the mock (Fig. 6A). When full-length URAT1 coexpressed with pcDNA3.1(+) containing PDZK1, the coexpression significantly increased urate transport activity by 1.4-fold (Fig. 6A). This effect was abolished when the C-terminal deletion mutant of URAT1 (URAT1-d3) was coexpressed with PDZK1. These results indicate that an interaction between the URAT1 C terminus and PDZK1 is necessary for the functional increase of urate transport.

Next, we examined the effect of PDZK1 on the kinetics of [14C]urate transport via URAT1 stably expressed in HEK293 cells (HEK-URAT1) that had been transfected with pcDNA3.1-PDZK1 or vector (pcDNA3.1) alone. Kinetic data showed that PDZK1 increased the $V_{max}$ significantly ($p < 0.05$) from 2.78 ± 0.34 to 3.34 ± 0.25 nanomoles/mg of protein/min but did not change the $K_m$ (from 203.8 ± 25.6 to 198.7 ± 33.3 µM) ($p = 0.78$) (Fig. 6B).

To determine changes in the cell surface expression level of URAT1, we used a cell membrane-impermeant biotinylation reagent to label cell surface proteins selectively. After the treatment, cell lysates from HEK-URAT1 cells transfected with pcDNA3.1-PDZK1 or pcDNA3.1 alone were collected. The amount of plasma-biotinylated URAT1 expression on plasma membranes increased 2.1-fold (mock-transfected: 25.6 to 198.7 versus PDZK1-transfected: 60.1 ± 18.9 arbitrary units, $n = 3$) when PDZK1 was coexpressed (Fig. 6, C and D). This change seems close to the one in $V_{max}$ of URAT1-mediated transport observed in Fig. 6B.
DISCUSSION

URAT1 is of primary importance in regulating blood urate level in humans. Through a yeast two-hybrid screen of a human kidney cDNA library, we identified PDZK1 as a binding partner of URAT1. PDZK1 is a PDZ domain-containing protein that was originally identified as a protein that interacts with MAP17, a membrane-associated protein (19). In addition to MAP17, PDZK1 has also been reported to interact with several membrane proteins through its PDZ domain; these proteins include the type IIa Na/Pi cotransporter (26), scavenger receptor class B type I (SR-BI) (27), CFTR (14), and multidrug resistance-associated protein MRP2 (cMOAT) (28).

Here we report the specific interaction between URAT1 and PDZK1 as demonstrated by yeast two-hybrid assays, pull-down assays, surface plasmon resonance (SPR) analysis, coimmunoprecipitation, and colocalization experiments. The C-terminal of URAT1 (STQF) falls into class I ((S/T)XØ, where Ø indicates a hydrophobic residue) of the PDZ-binding motif (9, 10). All the proteins that interact with PDZK1 except SR-BI (EAKL, class II) (27) have the PDZ-binding motif belonging to class I: type IIa Na/Pi, ATRL (26); CFTR, DTRL (14); and MRP2, STKF (28). The results obtained using the various mutants of the URAT1 C-terminal in yeast two-hybrid and GST pull-down assays (Figs. 1 and 2) confirmed the importance of the 0 and /H positions of the PDZ motif (9, 10). Furthermore, the interaction profiles of the C termini of the PDZK1 partner against each PDZ domain of PDZK1, which were confirmed by GST pull-down assay and SPR analysis, were different, although they

FIG. 5. Colocalization of URAT1 and PDZK1. A, distributions of URAT1 and PDZK1 mRNA in human multiple cDNA panels. URAT1 and PDZK1 expression overlaps in kidney, which is the sole site of URAT1 expression. Control amplification with β-actin was performed in parallel (bottom panel). B, immunohistochemical analysis of URAT1 and PDZK1 in serial sections of human kidney, URAT1 was detected in the apical membrane of renal proximal tubules (upper panel) and no staining was observed in the basolateral membrane and glomeruli (G). PDZK1 was also detected in the apical membrane of proximal tubules (lower panel).
have similar C-terminal amino acid sequences. SR-BI and MRP2 bind only to PDZ1 (27, 28), CFTR binds to PDZ1, PDZ3, and PDZ4 (14), and type IIa Na/Pi binds only to PDZ3 (26), whereas URAT1 binds to PDZ1, PDZ2, and PDZ4 (Figs. 1 and 2). Therefore, the observed interaction profile of the URAT1 C-terminal against each PDZ domain is unique and specific among PDZK1 partners.

The urate transport study revealed that the coexpression of PDZK1 with URAT1 in HEK293 cells leads to a significant enhancement of URAT1-mediated [14C]urate transport. It was previously shown that PDZK1 potentiates CFTR chloride channel activity (13, 14). Recently, IKePP, a PDZ domain protein closely related to PDZK1, was reported to alter the function of intestinal receptor guanylyl cyclase C (29). Therefore, the association of PDZK1 with URAT1 is an additional new example of the role of a PDZ-containing protein in modulating the function of their associated proteins (6–8). But the mechanism regarding PDZK1 modulation in URAT1 transport activity seems to be different from those two cases. Kinetic studies of urate transport showed a significant increase (1.4-fold) in $V_{\text{max}}$ between PDZK1- and mock-transfected HEK293 cells (Fig. 6B). Therefore, PDZK1 may enhance urate transport either through an increase in the catalytic rate of the transporter or through an increase in cell surface availability. Cell surface biotinylation experiment revealed that the augmentation of the transport activity was associated with the increased surface expression level of URAT1 protein from HEK293 cells stably expressing URAT1 transfected with PDZK1 (Fig. 6C). These results suggested that PDZK1 stabilize and/or anchor URAT1 at the cell membrane, making it less likely to be internalized and subsequently degraded. However, we could not rule out the possibility derived from the studies of CFTR channel interactions with multi-PDZ domain protein CAP70 (mouse homologue of PDZK1) and NHERF (30). In two previous studies, it was concluded that PDZ domains in NHERF and CAP70 play a modulatory function by directly affecting CFTR channel gating: by linking the C termini of CFTR monomers within an NHERF PDZ1–2 or CAP70 PDZ3–4 tandem construct (dimerization) causes a further increase in open channel probability (13, 14).

In the case of NHERF, no observable change in single-channel conductance or the number of channels is reported (13). As shown in Fig. 1C, because URAT1 can bind to PDZ1, PDZ2, and PDZ4 domains of PDZK1, it seems possible that the tandem PDZK1 PDZ1–2 domains form the dimer of URAT1 and the complex formation between URAT1 and the two PDZ domains of PDZK1 exerts its effect via allosteric modulation, as observed in the interaction of CFTR with both NHERF and PDZK1.

Kocher et al. (31) have recently reported the targeted disruption of the PDZK1 gene. PDZK1-deficient mice displayed no gross phenotypic abnormalities, and no significant redistribution of proteins known to interact with PDZK1, such as MAP17, MRP2, and the type IIa Na/Pi cotransporter, was observed. They concluded that the absence of a more significant phenotype may be due to functional compensation by other PDZ-domain proteins. However, they did not perform the reverse transcription-PCR experiment against renal-specific transporter, mouse homologue of URAT1 (32, 33), and did not check the urate levels in both blood and urine. Further studies are required to evaluate the importance of PDZK1 for the urate transport system.

Very recently, Gisler et al. (34) performed the yeast two-hybrid screens using single PDZ domains derived from mouse PDZK1 (NaPi-Cap1) as bait and reported that, besides NaPi-IIa, mouse PDZK1 interacts with many other membrane transporters including RST (mouse URAT1) in the brush border of proximal tubular cells. Although they did not show the functional consequence of its interaction, these studies, combined with our results, indicate the physiological significance of the interaction between PDZK1 and URAT1 in the kidney.

In summary, we have identified that the PDZ domain protein PDZK1 is a binding partner of URAT1, which augments URAT1-mediated urate transport activity. A further study of transporter-interacting proteins, such as PDZ domain proteins, will better our understanding of the different regulation mechanisms of other transporters.
Acknowledgments—We thank Akie Toki for technical assistance. The anti-PDZK1 and anti-URAT1 polyclonal antibodies were supplied by Transgenic Inc., Kumamoto, Japan.

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