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Identification of Essential Histidine and Cysteine Residues of the H^+/Organic Cation Antiporter Multidrug and Toxin Extrusion (MATE)[S]

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ABSTRACT

Multidrug and toxin extrusion 1 (MATE1) has been isolated as an H^+/organic cation antiporter located at the renal brush-border membranes. Previous studies using rat renal brush-border membrane vesicles indicated that cysteine and histidine residues played critical roles in H^+/organic cation antiport activity. In the present study, essential histidine and cysteine residues of MATE1 family were elucidated. When 7 histidine and 12 cysteine residues of rat (r)MATE1 conserved among species were mutated, substitution of His-385, Cys-62, and Cys-126 led to a significant loss of tetraethylammonium (TEA) transport activity. Cell surface biotinylation and immunofluorescence analyses with confocal microscopy indicated that rMATE1 mutant proteins were localized at plasma membranes. Mutation of the corresponding residues in human (h)MATE1 and hMATE2-K also diminished the transport activity. The transport of TEA via rMATE1 was inhibited by the sulfhydryl reagent *p*-chloromercuribenzenesulfonate (PCMBS) and the histidine residue modifier diethyl pyrocarbonate (DEPC) in a concentration-dependent manner. The PCMBS-caused inhibition of the transport via rMATE1 was protected by an excess of various organic cations such as TEA, suggesting that cysteine residues act as substrate-binding sites. In the case of DEPC, no such protective effects were observed. These results suggest that histidine and cysteine residues are required for MATE1 to function and that cysteine residues may serve as substrate-recognition sites.

Proximal tubules play important roles in the renal elimination of drugs. Cationic drugs are secreted from blood to urine by the combined efforts of two distinct classes of organic cation transporters: one driven by the transmembrane electrical potential difference in the basolateral membranes, and the other driven by the transmembrane H^+ gradient in the brush-border membranes (Inui and Okuda, 1998). To date, three kinds of membrane potential-dependent organic cation transporters (OCT1~3) have been identified and characterized (Burckhardt and Wolff, 2000; Inui et al., 2000; Wright, 2005).

In contrast to OCTs, the molecular nature of H^+/organic cation antiport system has not been characterized, but recently, orthologs of the multidrug and toxin extrusion (MATE) family have been identified in various species (Otsuka et al., 2005a; Hiasa et al., 2006; Masuda et al., 2006; Terada et al., 2006). We have cloned rat (r)MATE1 cDNA and demonstrated that rMATE1 mRNA is mainly expressed in the kidney (proximal convoluted and straight tubules), and rMATE1 can transport not only organic cations such as tetraethylammonium (TEA), cimetidine, and metformin but also the zwitterionic compound cephalexin (Terada et al., 2006). Furthermore, we revealed recently that an oppositely directed H^+ gradient serves as a driving force for the transport of TEA via rMATE1 (Tsuda et al., 2007). We also cloned human (h)MATE2-K cDNA and revealed that hMATE2-K and the hMATE1 was localized at the brush-border membranes of renal proximal tubules (Masuda et al., 2006). hMATE2-K can also transport organic cations such as TEA, procainamide, metformin, and creatinine and works as an H^+/organic cation antiporter (Masuda et al., 2006). These studies suggested that the mammalian MATE family showed similar characteristics to the renal H^+/organic cation antiport system (Inui and Okuda, 1998; Wright, 2005). During

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ABBREVIATIONS: MATE, multidrug and toxin extrusion; TEA, tetraethylammonium; DEPC, diethyl pyrocarbonate; PCMBS, *p*-chloromercuribenzenesulfonate; OCT, organic cation transporter; PAH, *p*-aminohippurate; TBS, Tris-buffered saline; HEK, human embryonic kidney.

the course of studying the transport of TEA using rat renal brush-border membrane vesicles, we found that treatment of membrane vesicles with sulfhydryl reagents such as *p*-chloromercuribenzenesulfonate (PCMBS) (Hori et al., 1987) and the histidine residue modifier diethyl pyrocarbonate (DEPC) (Hori et al., 1989) significantly inhibited [^{14}C]TEA transport by H^+/organic cation antiport system. Furthermore, very recently, Ohta et al. (2006) demonstrated that PCMBS inhibited the uptake of TEA by rMATE1. Based on these findings, it was speculated that histidine and cysteine residues play important roles in the transport activity of MATE family. The present study was undertaken to identify the essential histidine and cysteine residues of the MATE family (especially rMATE1) using site-directed mutagenesis and to examine their functional roles using chemical modifiers.

Materials and Methods

Materials. Cephalexin was donated by Shionogi Co. (Osaka, Japan). [^{14}C]TEA (2.035 GBq/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). DEPC, cimetidine, and TEA were obtained from Nacalai Tesque (Kyoto, Japan). PCMBS, metformin, and *p*-aminohippurate (PAH) were purchased from Sigma (St. Louis, MO). All other chemicals used were of the highest purity available.

Plasmids and Site-Directed Mutagenesis. The rMATE1 cDNA was excised from rMATE1/pcDNA3.1 (Terada et al., 2006), and was subcloned into pFLAG-CMV-6 (Sigma) to yield FLAG-rMATE1. The site-directed mutations of histidine or cysteine residues were introduced into FLAG-rMATE1, hMATE1/pcDNA3.1 (Yonezawa et al., 2006), or hMATE2-K/pcDNA3.1 (Masuda et al., 2006) with a QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the primers listed in Supplemental Table S1. The nucleotide sequences of these constructs were confirmed using multicapillary DNA sequencer RISA384 system (Shimadzu, Kyoto, Japan).

Cell Culture, Transfection, and Transport Measurements. HEK293 cells (CRL-1573; American Type Culture Collection, Manassas, VA) were cultured as described previously (Urakami et al., 2002; Terada et al., 2006). Various constructs were transfected into HEK293 cells using LipofectAMINE 2000 Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. At 48 h after the transfection, the cells were used for uptake experiments. Cellular uptake of [^{14}C]TEA was measured with monolayers grown on poly(D-lysine)-coated 24-well plates. To manipulate the intracellular pH, intracellular acidification was performed by pretreatment with ammonium chloride (30 mM, 20 min at 37°C, pH 7.4) (Masuda et al., 2006; Terada et al., 2006). The medium was then removed, and 0.2 ml of incubation medium, pH 7.4, containing [^{14}C]TEA was added. The medium was aspirated off at the end of the incubation, and monolayers were rapidly rinsed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. The protein content of the solubilized cells was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

Pretreatment with DEPC and PCMBS. HEK293 cells were washed twice with incubation medium, pH 7.4. Monolayers were then incubated with DEPC, pH 6.0, in phosphate-buffered saline for 10 min at 25°C or PCMBS, pH 7.4, in incubation medium for 10 min at 25°C. Then cells were washed twice before intracellular acidification.

Cell Surface Biotinylation. Cell surface biotinylation was performed according to the methods of Hong et al. (2004) with some modification. HEK293 cells were grown on poly(D-lysine)-coated six-well plates and transfected with the rMATE1cDNAs. At 48 h after the transfection, cells were washed with ice-cold phosphate-buffered saline calcium/magnesium (138 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 9.6 mM Na_2HPO_4, 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$, pH 7.3) and then treated with 1 ml of membrane-impermeable biotinylating agent, sulfo-NHS-SS-biotin (Pierce, Rockford, IL) (1.5 mg/ml) at 4°C for 1 h. Subsequently, the cells were washed three times with ice-cold phosphate-buffered saline calcium/magnesium containing 100 mM glycine and then incubated for 20 min at 4°C with the same buffer to remove the remaining labeling agent. After washing with phosphate-buffered saline calcium/magnesium, cells were disrupted with 700 μl of lysis buffer (10 mM Tris-base, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, and 1% Triton X-100, pH 7.4) containing protease inhibitors at 4°C for 1 h with constant agitation. After centrifugation, 140 μl of streptavidin agarose beads (Pierce) were added to 600 μl of cell lysate and incubated for 1 h at room temperature to isolate the plasma membrane protein.

Western Blot Analysis. The procedures for Western blot analysis were described previously (Terada et al., 1996). Monoclonal anti-FLAG-M2 antibody (1:4000 dilution; Sigma) or Na^+/K^+-ATPase antibody (1:2000 dilution; Upstate Biotechnology, Lake Placid, NY) was used as the primary antibody. A peroxidase-conjugated anti-mouse IgG antibody was used for detection of bound antibodies, and strips of the blots were visualized by chemiluminescence on X-ray film.

Immunofluorescence of Transfected Cells. HEK293 cells were seeded onto poly(D-lysine)-coated cover glasses (BD Biosciences, San Jose, CA), and then transfection was performed. Cells were washed twice in Tris-buffered saline (TBS), fixed for 1 min at room temperature in a mixture of methanol/acetone (1:1), and rewashed in TBS. The cells were incubated for 1 h at room temperature in TBS containing monoclonal anti-FLAG M2-FITC antibody (Sigma) (1:1000). Cells were thoroughly washed, and coverslips were mounted. These samples were examined with Eclipse E800 fluores-

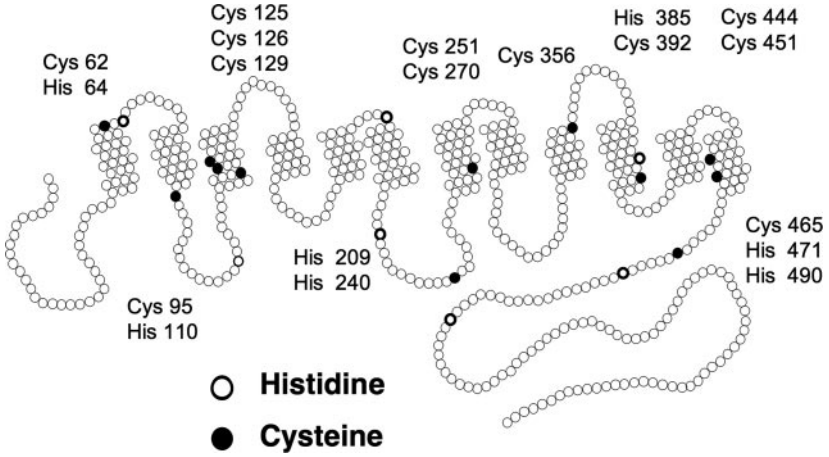

Fig. 1. Putative secondary structure of rMATE1 protein. The histidine (○) and cysteine (●) residues conserved among species are shown.

cence microscope (Nikon, Tokyo, Japan) equipped with MRC-1024 laser confocal system (Bio-Rad Laboratories).

Statistical Analysis. The significance of differences between the wild-type and mutant were analyzed using Dunnett's post hoc analysis. Two or three experiments were conducted, and representative results are shown. Other analyses were conducted with Student's *t* test.

Results

Transport Analysis of rMATE1 Histidine and Cysteine Mutants. When amino acid sequences of rat, human, and mouse MATE1 were compared, 7 histidine and 12 cysteine residues were found to be conserved. Figure 1 shows the location of these amino acids in the predicted secondary structure of rMATE1. To determine which residues are essential for the transport activity of rMATE1, conserved histidine and cysteine residues were changed to glutamine and glycine residues, respectively. As shown in Fig. 2A, [^{14}C]TEA uptake was significantly reduced only in the rMATE1 H385Q among histidine mutants. As for cysteine mutants, the transport activity of C62G and C126G mutants was remarkably reduced, and [^{14}C]TEA uptake by C129G, C356G, C392G, and C451G mutants was significantly but modestly inhibited (Fig. 2B). We then focused on the amino acid residues His-385, Cys-62, and Cys-126 and further evaluated their functional importance. The substitution of these three amino acid residues in rMATE1 for other amino acid residue also abolished [^{14}C]TEA uptake (Fig. 3). These three amino acid residues are also conserved in hMATE1 (His-386, Cys-63, and Cys-127) and hMATE2-K (His-382, Cys-59, and Cys-123), and therefore, histidine and cysteine mutants of hMATE1 and hMATE2-K were prepared. As shown in Fig. 4, the transport of [^{14}C]TEA via the hMATE1 and hMATE2-K mutants was also diminished, suggesting that these histidine and cysteine amino acid residues are essential to the MATE families.

Protein Expression of rMATE1 Mutants. One possible reason for the defective transport activity of these mutants is a decreased level of the mutant protein in the plasma membranes of HEK293 cells, which could be caused by reduced stability and/or impaired insertion into the membranes of the mutants. To examine this possibility, Western blot analyses of plasma membranes prepared from each rMATE1 mutant and the immunolocalization of rMATE1 mutants were performed. Cell surface biotinylation was performed to specifically capture plasma membranes, and biotinylation techniques were confirmed to detect Na^+/K^+-ATPase for all samples (Goel et al., 2005). As shown in Fig. 5, all MATE1

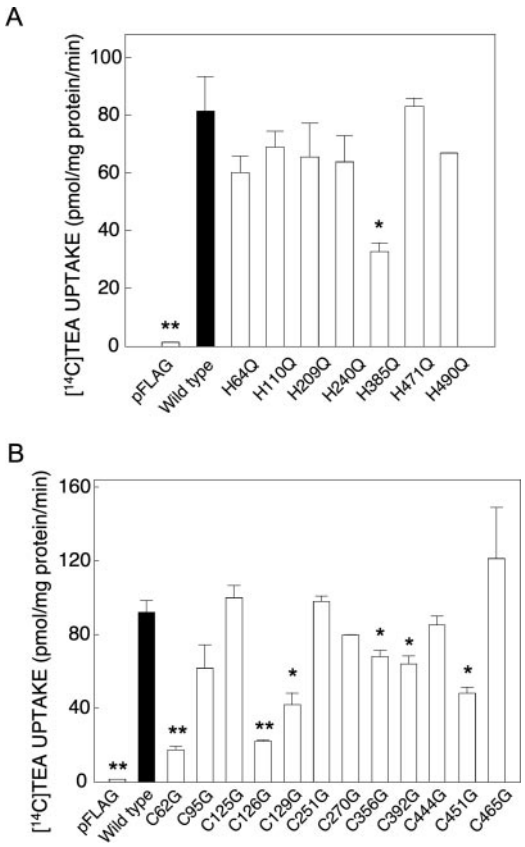

Fig. 2. Uptake of [^{14}C]TEA by HEK293 cells expressing wild-type and histidine (A) or cysteine (B) mutants of rMATE1. The cells were preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA (10.36 kBq/ml, pH 7.4) for 1 min at 37°C. Each column represents the mean ± S.E. of three monolayers. This figure is a representative one from three separate experiments. *, $p < 0.05$, **, $p < 0.01$, significantly different from the wild type.

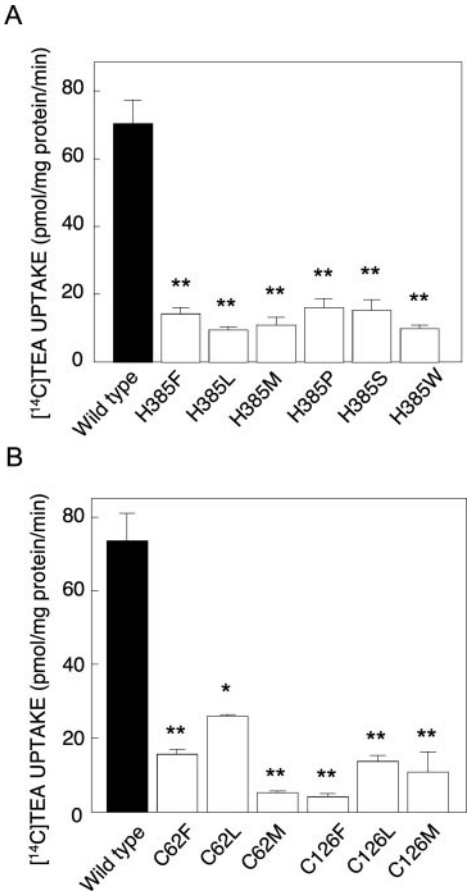

Fig. 3. Effect of various amino acid substitutions of His-385 (A), Cys-62, and Cys-126 (B) of rMATE1 on the uptake of [^{14}C]TEA by HEK293 cells expressing each rMATE1 mutant. The cells were preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA, pH 7.4, for 1 min at 37°C. Each column represents the mean ± S.E. for three monolayers. This figure is a representative one from three separate experiments. *, $p < 0.05$, **, $p < 0.01$, significantly different from the wild type.

mutant proteins were expressed at plasma membranes and wild-type MATE1. Furthermore, immunofluorescence analyses with confocal microscopy revealed that the rMATE1 mutant proteins with H385Q, C62G, and C126G were localized at the plasma membranes (Fig. 6). Although intracellular staining in addition to membrane labeling was observed, this might be caused by the overexpression of rMATE1. This issue of expression pattern could not be ruled out in the transient expression system. These findings suggested that the low levels of transport activity of rMATE1 mutants with H385G, C62G, and C126G were not caused by the alteration of protein expression in plasma membranes.

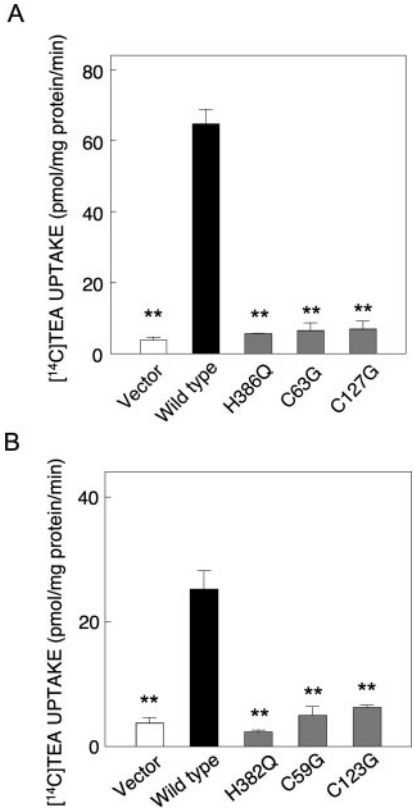

Fig. 4. Uptake of [^{14}C]TEA by HEK293 cells expressing wild-type and histidine and cysteine mutants of hMATE1 (A) or hMATE2-K (B). The cells were preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA, pH 7.4, for 1 min at 37°C. Each column represents the mean ± S.E. of three monolayers. This figure is a representative one from two separate experiments. **, $p < 0.01$, significantly different from wild type.

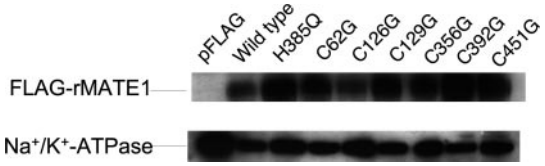

Fig. 5. Western blot analysis of plasma membranes obtained from HEK293 cells transiently expressing wild-type rMATE1 or histidine or cysteine mutants of rMATE1. Plasma membrane fractions prepared by cell surface biotinylation were separated by SDS-polyacrylamide gel electrophoresis (10%) and blotted onto polyvinylidene difluoride membranes. Monoclonal anti-FLAG-M2 antibody or Na^+/K^+-ATPase antibody was used as a primary antibody. A horseradish peroxidase-conjugated anti-mouse IgG antibody was used for detection of the bound antibody, and the strips of blots were visualized by chemiluminescence on X-ray film.

Effects of DEPC or PCMBS Treatment on rMATE1 Function. We next examined the functional roles of the cysteine and histidine residues by using chemical modifiers such as the sulfhydryl reagent PCMBS and the histidine residue modifier DEPC. As shown in Fig. 7, pretreatment of rMATE1-expressing cells with PCMBS or DEPC led to a concentration-dependent decrease in the transport of [^{14}C]TEA. The half-maximal inhibition for [^{14}C]TEA transport via rMATE1 was calculated as 1.13 ± 0.91 mM for DEPC and 37.2 ± 10.2 μM for PCMBS.

Effect of DEPC or PCMBS in the Presence of Unlabeled TEA on rMATE1. We examined the effect of DEPC or PCMBS pretreatment in the presence of unlabeled TEA on rMATE1 function. As shown in Fig. 8A, unlabeled TEA had no effect on the inhibition of [^{14}C]TEA uptake by DEPC. On the other hand, unlabeled TEA protected against the inhibition of [^{14}C]TEA uptake caused by PCMBS pretreatment (Fig. 8B). The PCMBS-caused inhibition of [^{14}C]TEA transport via rMATE1 was also blocked by an excess of other MATE1 substrates such as cephalexin, cimetidine, and metformin but not by a typical organic anion, PAH. The results suggest that cysteine residues in rMATE1 interact with the substrates.

We further examined the protective effect of unlabeled TEA on [^{14}C]TEA uptake by C62G and C126G mutants treated with PCMBS (0.1 mM). [^{14}C]TEA uptake by C62G or C126G mutant was significantly decreased by the pretreatment of PCMBS (C62G: 7.52 ± 1.68 to 1.68 ± 0.13 pmol/mg

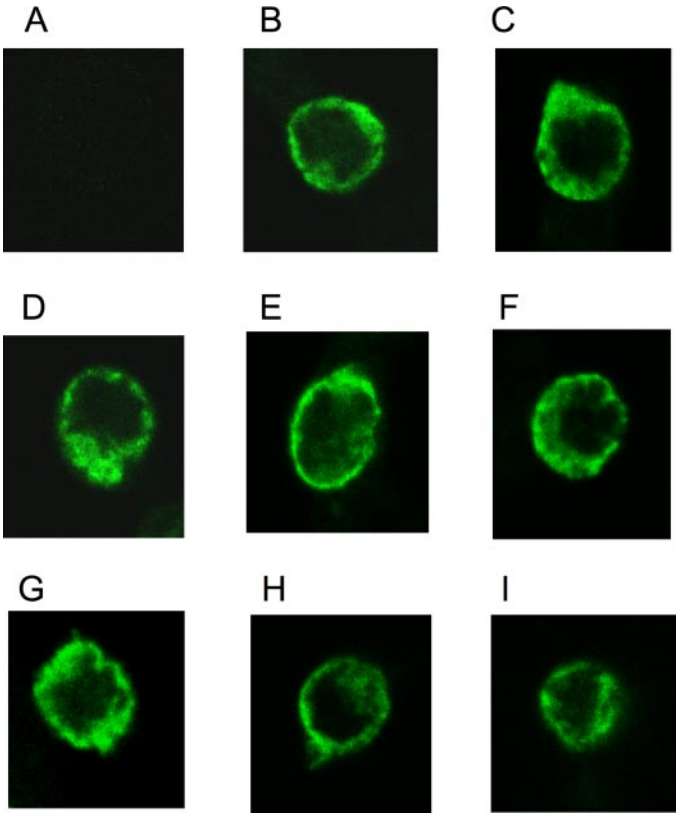

Fig. 6. Localization of FLAG-rMATE1 protein in HEK293 cells transiently transfected with vector alone (A), wild-type rMATE1 (B), or the H385Q (C), C62G (D), C126G (E), C129G (F), C356G (G), C392G (H), or C451G (I) mutant observed by confocal microscopy. HEK293 cells transfected with vector alone, wild-type cDNA, and mutant rMATE1 cDNA were fixed and stained with monoclonal anti-FLAG M2-FITC antibody.

of protein/min, $p < 0.05$; C126G: 8.68 ± 0.17 to 2.72 ± 0.09 pmol/mg of protein/min, $p < 0.05$, mean ± S.E., $n = 3$), indicating that cysteine residues other than Cys-62 and Cys-126 were sensitive to PCMBS. In contrast to wild-type rMATE1, the PCMBS-caused inhibition of [^{14}C]TEA uptake in these cysteine mutants was not protected by unlabeled TEA (C62G, 1.70 ± 0.19 pmol/mg of protein/min; C126G, 2.55 ± 0.41 pmol/mg of protein/min; mean ± S.E., $n = 3$). These results raised the possibility that at least both Cys-62 and Cys-126 contributed to the substrate binding.

pH Profile of TEA Uptake by rMATE1 H385Q. To investigate the role of the histidine residues, the pH profile of the uptake of [^{14}C]TEA by the rMATE1 H385Q mutant was examined. When the extracellular pH was changed from 6.0 to 8.5, the transport of [^{14}C]TEA by wild-type rMATE1 showed a bell-shaped curve with the greatest uptake value at pH 7.5. On the other hand, in the case of rMATE1 H385Q, no peak of the uptake was observed (Fig. 9).

Discussion

Recent molecular biological approaches have revealed that the mammalian MATE family functions as the renal H^+/organic cation antiport system. MATE1 can transport not only a typical organic cation TEA (Otsuka et al., 2005a; Ohta et al., 2006; Terada et al., 2006) but also various cationic drugs such as cimetidine and metformin and zwitter ion cephalexin (Terada et al., 2006). Furthermore, we recently found that hMATE1 also transports platinum agents (Yonezawa et al., 2006). To understand the molecular mechanisms behind the multispecificity of MATE1, it is necessary to define substrate-binding and/or recognition sites located in the transporter protein. Among amino acid residues, cysteine and histidine are of interest because we found previously that sulfhydryl groups and histidine residues are essential for the transport activity of H^+/organic cation antiport system using rat renal brush-border membrane vesicles (Hori et al., 1987, 1989) and a pig kidney epithelial cell line, LLC-PK$_1$ (Inui et al., 1985; Saito et al., 1992). Based on these backgrounds, in the present study, functional roles of cysteine and histidine residues of the MATE family, especially rMATE1, were examined.

By mutational analysis, we found that Cys-62 and Cys-126 of rMATE1, which are located in the first and the third transmembrane domain, respectively, played critical roles in the transport activity of TEA (Figs. 1 and 2). It is interesting that the corresponding cysteine residues of hMATE1 and hMATE2-K also function as essential amino acid residues (Fig. 4), suggesting that these cysteine residues play critical roles in the MATE family. Furthermore, protection by the substrate against PCMBS-caused inhibition of the transport of TEA via rMATE1 (Fig. 8) suggested that cysteine residues of rMATE1 function as substrate-binding sites. Protection assay using rMATE1 C62G and C126G mutants suggested that both Cys-62 and Cys-126 are involved in the substrate binding, although we cannot rule out the possibility that other cysteine residues participated in the substrate recognition. Pelis et al. (2006) have recently found that Cys-474 of hOCT2, which is suggested to be located in the 11th transmembrane helix that participates in the formation of the hydrophilic cleft, contributes to substrate-protein interaction. Because OCTs and

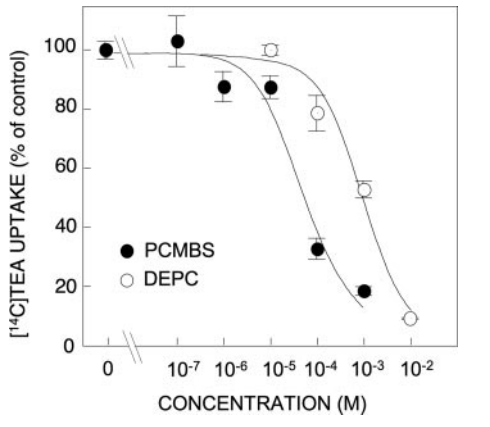

Fig. 7. Effects of DEPC (○) and PCMBS (●) on the uptake of TEA by rMATE1-expressing HEK293 cells. FLAG-rMATE1 cDNA was transfected into the HEK293 cells. The cells were preincubated at 25°C for 10 min with various concentrations of DEPC, pH 6.0, or PCMBS, pH 7.4. After incubation, the cells were rinsed twice with incubation medium and preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA, pH 7.4, for 1 min at 37°C. Each point represents the mean ± S.E. of three monolayers. This figure is a representative one from three separate experiments.

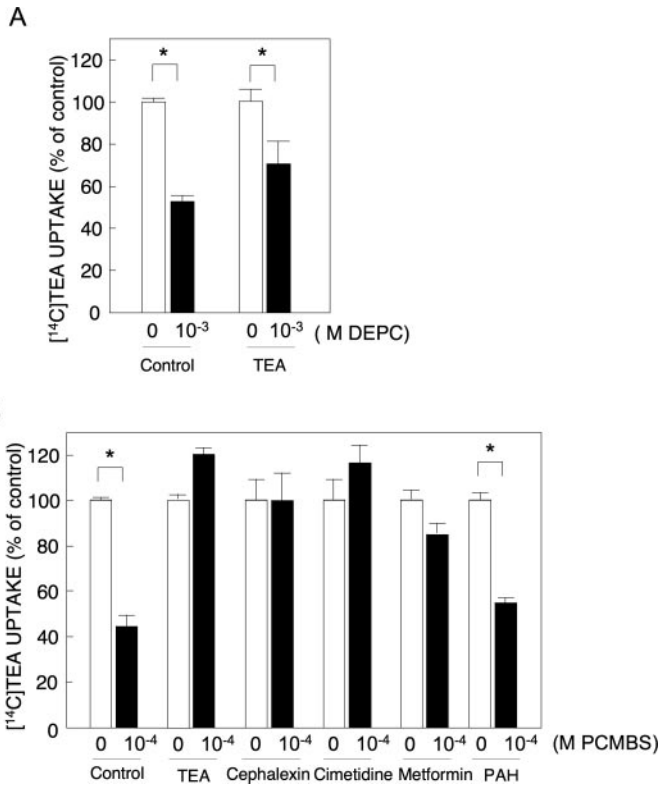

Fig. 8. Effects of unlabeled compounds on the inhibition of [^{14}C]TEA uptake by DEPC (A) or PCMBS (B)-pretreated rMATE1-expressing HEK293 cells. The cells were preincubated at 25°C for 10 min with or without unlabeled TEA (10 mM) and DEPC (1 mM), pH 6.0 (A) or were preincubated at 25°C for 10 min with or without unlabeled compounds (10 mM TEA, cephalexin, metformin, and PAH or 10 μM cimetidine) and PCMBS (0.1 mM) pH 7.4 (B). After the incubation, the cells were rinsed twice with incubation medium and preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA, pH 7.4, for 1 min at 37°C. Each column represents the mean ± S.E. of three monolayers. This figure is a representative one from two separate experiments. *, $p < 0.05$, significantly different from wild type.

MATEs have similar substrate specificity, although their driving forces are quite different, it is reasonable that the same amino acid cysteine is involved in the substrate recognition. The present results strongly suggest that Cys-62 and Cys-126 of rMATE1 play an important role for substrate-interaction sites.

Most of the His-385 mutants of rMATE1 (Figs. 2 and 3) and corresponding histidine mutants of hMATE1 and hMATE2-K (Fig. 4) did not have the TEA transport activity. Furthermore, the histidine modifier reagent DEPC also inhibited the transport of TEA via rMATE1 (Fig. 7). In contrast to the effect of PCMBS, the DEPC-caused inhibition of TEA transport was not blocked in the presence of excess TEA (Fig. 8), suggesting that histidine residue of rMATE1 does not serve as substrate-binding site. In other H^+-coupled transporters such as H^+/peptide cotransporter 1 (Uchiyama et al., 2003) and Na^+/H^+ exchanger (Cha et al., 2003), histidine residues function as an H^+-binding site. It is, therefore, suggested that histidine residue of the MATE family acts as a H^+-binding site for driving force.

The H^+/organic cation antiport system is very sensitive to pH. The uptake of TEA was optimal at pH 7.0, and the uptake was markedly decreased at either an acidic or alkaline pH in renal brush-border membrane vesicle (Maegawa et al., 1988). No peak in the uptake of TEA by the rMATE1 H385Q mutant was observed when the pH of the medium changed gradually (Fig. 9). His-385 of rMATE1 may be important to the bell-shaped transport activity and function not only in making the driving force but as a regulator of substrate transport. Further studies such as detailed kinetic analyses may be needed. To our knowledge, there has been no report that a histidine residue is involved in the transport of a substrate by the MATE family.

Site-directed mutagenesis revealed that Asp-32, Glu-251, and Asp-367 of NorM protein, which is a member of the *Vibrio parahaemolyticus* MATE family, are essential for the Na^+-driven organic cation export (Otsuka et al., 2005b). Mutated hMATE1 with Glu-273 replaced with glutamine, the counterpart of Glu-251 of NorM protein, lacked TEA transport activity (Otsuka et al., 2005a). This glutamate residue is also conserved among rMATE1, mMATE1, and hMATE2-K. Previous studies using renal brush-border membrane vesicles treated with chemical modifiers revealed that carboxylate groups are critical for transport activity but are not involved in the substrate binding (Sokol et al., 1987). Alternatively, it was speculated that carboxylate groups are responsible for H^+ translocation process. It is therefore suggested that cysteine residues in the mammalian MATE family play a more important role for the substrate recognition than other amino acid residues. In MATE1, cysteine residues are located in the first and third transmembrane domains. The development of a three-dimensional model of MATE1 will clarify the molecular interaction of these amino acid residues with cationic substrates, as proposed for rat OCT1 (Popp et al., 2005) and rabbit OCT2 (Zhang et al., 2005).

In conclusion, we demonstrated that His-385, Cys-62, and Cys-126 in rMATE1 and corresponding amino acid residues of hMATE1 and hMATE2-K play an important role for the transport activity of MATE family. Cysteine residues of MATE1 make a key contribution to substrate recognition. This is the first study to identify the histidine and cysteine residues essential to the mammalian MATE family.

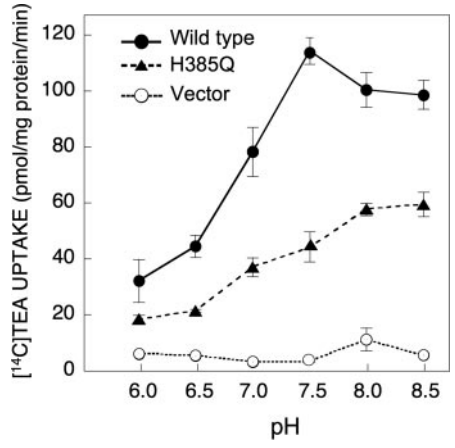

Fig. 9. Uptake of [^{14}C]TEA by HEK293 cells expressing wild-type rMATE1 (●), the H385Q mutant (▲), or vector alone (○). The cells were preincubated with incubation medium, pH 7.4, in the presence of 30 mM ammonium chloride for 20 min. Then the preincubation medium was removed, and the cells were incubated with 5 μM [^{14}C]TEA, (pH 6.0 to 8.5, for 1 min at 37°C. Each point represents the mean ± S.E. of three monolayers. This figure is a representative one from two separate experiments.

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