Kidney-specific expression of a novel mouse organic cation transporter-like protein

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Abstract Using the signal sequence trap method, we have cloned a novel 12-membrane-spanning transporter-like protein, termed renal-specific transporter (RST), from the mouse kidney. RST is a 553-amino-acid protein highly homologous to recently cloned organic cation transporters, e.g. it is 30% identical to rat organic cation transporter 1 at the amino acid level. Northern blot analysis has revealed that the RST gene is expressed abundantly and specifically in the kidney. In situ hybridization analysis has shown that RST gene expression is restricted to the renal proximal tubule, where various organic cations such as endogenous catecholamines and choline or clinically used cationic drugs are known to be actively excreted.

Key words: Organic cation transporter; Nutrient transporter; Signal sequence trap; In situ hybridization; Mouse kidney

1. Introduction

The eukaryotic and bacterial nutrient transporters include a diverse group of proteins such as the mammalian glucose transporters (GLUT1 to GLUT7), the yeast transporters for maltose, lactose, and glucose, and the proton-driven bacterial transporters for arabinose, xylose, and citrates [1,2]. Recently, organic cation transporter 1 (OCT1) was cloned, which has a 12-membrane-spanning structure similar to those of nutrient transporters [3]. This protein is presumed to be responsible for the elimination of cationic drugs such as antibiotics, antihistaminics, antiarrhythmics, and opiates from the kidney and liver [3]. Subsequently, several proteins highly homologous to OCT1 (OCT2, NLT, and NKT) have been cloned [4–6]; they are called the organic cation transporter family [6]. The genes are all expressed in the kidney, but their distributions in other tissues differ, implying functional differences.

To identify novel soluble and membrane-bound proteins which are involved in the physiologic functions of the kidney, we have screened a mouse kidney cDNA library by the signal sequence trap method [7–9]. We here report the molecular cloning of a cDNA encoding a novel organic cation transporter-like protein, and its complete primary structure, tissue distribution, and intra-renal localization.

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Abbreviations: cDNA, complementary DNA; cRNA, complementary RNA; RACE, rapid amplification of cDNA ends

2. Materials and methods

2.1. Tissue preparation and RNA extraction

The whole kidney and other tissues were obtained from 8-week-old male BALB/c mice. Total RNA extraction was carried out as described [10], and poly(A)$^+$ RNA was purified using PolyATtract (Promega).

2.2. Signal sequence trap

Signal sequence trap was performed as described [8,9]. 2 μg of poly(A)$^+$ RNA from the mouse kidney was reverse transcribed using 80 ng random hexamer and SuperScript II reverse transcriptase (Gibco BRL). The 5′-enriched cDNA was unidirectionally cloned into the expression vector, pcDL-SRα-Tac(3′) [11,12]. The plasmid library was transfected to COS-7 cells with Transfectam (Sepracor), and screened with immunostaining with anti-Tac antibody [13].

2.3. Rapid amplification of 5′- and 3′-cDNA ends (5′- and 3′-RACE)

A cDNA library was constructed using poly(A)$^+$ RNA from the mouse kidney and Marathon cDNA amplification kit (Clontech). Gene-specific primers used were 5′-agcagcaggctgcctgtcttcacc-3′ (nucleotides 1173–1196) for 5′-RACE, and 5′-gcgccttcacctgcatcaccatc-3′ (nucleotides 1340–1362) for 3′-RACE (Fig. 1). A 1.5-kbp and a 0.6-kbp fragment were obtained by 5′- and 3′-RACE, respectively, subcloned into pGEM-T Easy Vector (Promega), and sequenced.

2.4. DNA sequencing

Nucleotide sequences were determined on both strands by Dye Terminator Cycle Sequencing Kit, FS and 373B DNA sequencer (Applied Biosystems).

2.5. Northern blot analysis

Northern blot analysis was performed as described [9]. A [^{32}P]dCTP-labeled cDNA fragment (insert of clone K14D2, nucleotides 1119–1377) was used as a probe. 50 μg of total RNA was loaded in each lane. The blot was used to expose BAS-III imaging plate (Fuji) for 18 h.

2.6. In situ hybridization analysis

The subcloned 5′-RACE product (see above) was used as a template for antisense and sense [^{35}S]CTP-labeled complementary RNA (cRNA) probes. In situ hybridization analysis was performed as described [9]. In brief, 5 μm cryosections from 13-week-old male BALB/c mouse kidneys were fixed with 4% paraformaldehyde, and incubated at 57°C for 8 h with 1.2×10^8 cpm/ml of cRNA probe in the solution previously described [9]. After treatment with RNase A, slides were washed in 0.1×SSC at 60°C, dehydrated, and apposed to Hyperfilm β-max (Amersham) for 16 h, or dipped into NTB-2 (Kodak) for 24 days and counterstained with hematoxylin and eosin.

3. Results and discussion

3.1. Isolation and sequence analysis of a full-length mouse RST cDNA

Five thousand plasmid clones were screened by the signal

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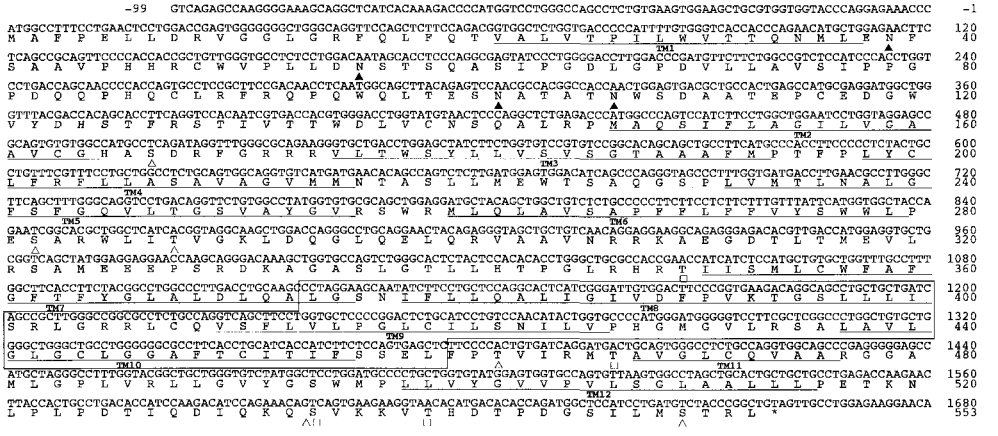

Fig. 1. Nucleotide and deduced amino acid sequences of mouse RST cDNA. The cDNA fragment (clone K14D2) obtained by signal sequence trap is boxed. Putative transmembrane domains (TM1–TM12) are underlined. ▲, potential *N*-glycosylation sites; △/□, potential phosphorylation sites by protein kinase C/protein kinase A.

sequence trap method, 25 positive clones were isolated, and the nucleotide sequences of their inserts were determined. A positive clone (K14D2) encoded an 86-amino-acid polypeptide containing three clusters of hydrophobic amino acids (Fig. 1, TM8–TM10). To determine the full-length cDNA nucleotide sequence, 5′- and 3′-RACE were performed. The longest open reading frame predicted a novel 553-amino-acid protein, termed renal-specific transporter (RST) (Fig. 1). The translation initiation codon at position 1 was reasonably consistent with Kozak's rule [14]. The hydropathy analysis of RST by the method of Kyte and Doolittle predicted the presence of 12 transmembrane domains (TM1–TM12) [15].

3.2. Sequence comparison among organic cation transporters

Fig. 2 shows the amino acid sequence comparison of RST with previously cloned organic cation transporters. RST was 44%, 36%, 30%, and 28% identical to NKT, NLT, OCT1, and OCT2, respectively [3–6]. Based upon the analogy with known organic cation transporters, both the amino- and carboxy-termini of RST were predicted to be located intracellularly. RST exhibited several structural features shared among organic cation transporters and nutrient transporters as follows. Between putative TM6 and TM7, RST possessed a large intracellular loop with several potential phosphorylation sites by protein kinase C and protein kinase A (Fig. 1) [16]. In intracellular portions, RST also contained two sets of three stretches of conserved amino acid sequences. They fulfil, though not completely, three kinds of motifs: D/E-R-X-G-R-R/K at amino acid positions 168–173 and 401–406, E-X-X-X-X-X-X-R at positions 223–230 and 458–465, and P-E-S/T at positions 280–282 and 516–518 [2,6,17]. Furthermore, between putative TM1 and TM2, RST had a characteristic region unique to organic cation transporters, which is a large extracellular loop with several *N*-glycosylation motifs and four conserved cysteine residues [6]. These observations, taken together, suggest that RST belongs to the organic cation transporter family.

3.3. Northern blot analysis

Northern blot analysis with the RST cDNA probe revealed a 2.3-kbp single transcript expressed intensely in the kidney (Fig. 3). The expression level of RST in the kidney seems comparable to those of OCT1, OCT2, and NKT, and much higher than that of NLT [3–6]. No detectable amounts of RST gene expression were observed in the brain, heart, lung, liver, spleen, and intestine. This tissue distribution is apparently

Fig. 2. Amino acid sequence comparison among organic cation transporters. RST and NKT have been cloned from mouse, and NLT, OCT1, and OCT2 from rat. Gaps are introduced for maximal alignment. Putative transmembrane domains (TM1–TM12) are underlined. Identical amino acids are indicated by asterisks. *N*, potential *N*-glycosylation sites; circle, four conserved cysteine residues unique to organic cation transporters; box, six motifs of amino acid sequences in intracellular portions conserved among organic cation transporters and nutrient transporters.

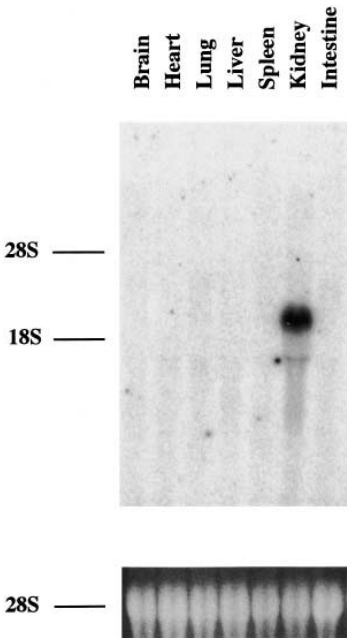

Fig. 3. Northern blot analysis of mouse RST gene expression. In the lower part, 28S ribosomal RNA bands visualized with ethidium bromide are shown.

different from those of known organic cation transporters; outside the kidney, rat OCT1 gene is expressed in the liver and intestine, rat NLT in the liver, mouse NKT in the brain [3,5,6]. Rat OCT2 gene expression is detected in discrete brain regions [18]. The abundant and specific expression of RST in the kidney suggests the physiologic significance of RST in the renal function.

3.4. *In situ hybridization analysis*

To determine the intra-renal localization of RST gene expression, in situ hybridization analysis with the antisense or sense cRNA probe was performed (Fig. 4). At autoradiography, strong hybridizing signals were observed in the cortex and the outer stripe of the outer medulla. No specific signals were seen in sections hybridized with the sense probe. At photomicrography, the intense labeling was confined to the proximal tubule. In contrast, no signals were detected in the glomerulus, distal tubule, and collecting duct. This intra-renal localization of RST gene expression is consistent with previous studies which showed that organic cation transport is performed selectively in the renal proximal tubule [19,20].

3.5. *Conclusion*

We have succeeded in isolating and characterizing a novel member of the organic cation transporter family. The structural and distributional varieties in the family may imply substrate specificities for each transporter, and further studies are required to distinguish their proper functions. Organic cations, not only cationic drugs but also endogenous amines such as catecholamines and choline, are actively excreted through the renal proximal tubule [19,20]. The malfunctions in organic cation transporters may cause dysregulated circulating levels of amines or may affect individual sensitivity for cationic drugs. This study will lead to a better understanding of the molecular mechanisms underlying the physiologic function of the kidney.

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References

[1] Silverman, M. (1991) Annu. Rev. Biochem. 60, 757–794.
[2] Baldwin, S.A. (1993) Biochim. Biophys. Acta 1154, 17–49.
[3] Gründemann, D., Gorboulev, V., Gambaryan, S., Veyhl, M. and Koepsell, H. (1994) Nature 372, 549–552.
[4] Okuda, M., Saito, H., Urakami, Y., Takano, M. and Inui, K. (1996) Biochem. Biophys. Res. Commun. 224, 500–507.
[5] Simonson, G.D., Vincent, A.C., Roberg, K.J., Huang, Y. and Iwanji, V. (1994) J. Cell Sci. 107, 1065–1072.
[6] Lopez-Nieto, C.E., You, G., Bush, K.T., Barros, E.J.G., Beier, D.R. and Nigam, S.K. (1997) J. Biol. Chem. 272, 6471–6478.
[7] Tashiro, K., Tada, H., Heilker, R., Shirozu, M., Nakano, T. and Honjo, T. (1993) Science 261, 600–603.
[8] Tashiro, K., Nakano, T. and Honjo, T. (1996) Methods Mol. Biol. 69, 203–219.
[9] Mori, K., Ogawa, Y., Tamura, N., Ebihara, K., Aoki, T., Muro, S., Ozaki, S., Tanaka, I., Tashiro, K. and Nakao, K. (1997) FEBS Lett. 401, 218–222.
[10] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[11] Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M. and Arai, N. (1988) Mol. Cell. Biol. 8, 466–472.
[12] Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. and Honjo, T. (1984) Nature 311, 631–635.

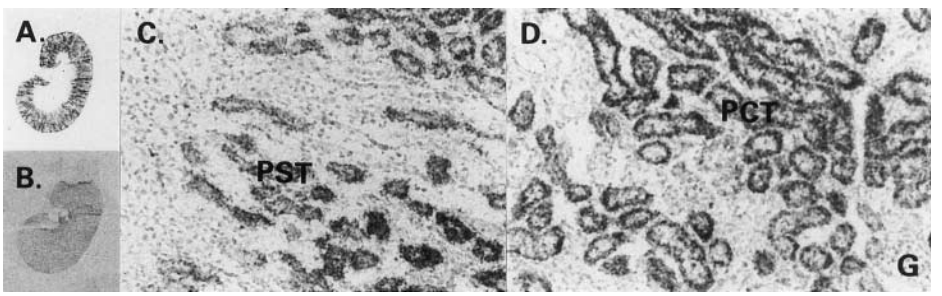

Fig. 4. In situ hybridization analysis of mouse RST gene expression in the kidney. A: Autoradiograph with the antisense probe. B: Control experiment with the sense probe. C, D: Bright-field photomicrographs of the outer medulla (C) and the cortex (D) (magnification ×70). PST, proximal straight tubule; PCT, proximal convoluted tubule; G, glomerulus.

[13] Uchiyama, T., Broder, S. and Waldmann, T.A. (1981) J. Immunol. 126, 1393–1397.
[14] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8132.
[15] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[16] Kennelly, P.J. and Krebs, E.G. (1991) J. Biol. Chem. 266, 15555–15558.
[17] Gingrich, J.A., Andersen, P.H., Tiberi, M., Mestikawy, S.E., Jorgensen, P.N., Fremeau Jr., R.T. and Caron, M.G. (1992) FEBS Lett. 312, 115–122.
[18] Gründemann, D., Babin-Ebell, J., Martel, F., Örding, N., Schmidt, A. and Schömig, E. (1997) J. Biol. Chem. 272, 10408–10413.
[19] Sica, D.A. and Schoolwerth, A.C. (1996) in: Renal Handling of Organic Anions and Cations and Renal Excretion of Uric Acid, Brenner and Rector's the Kidney (Brenner, B.M., Ed.), 5th edn., Vol. 1, pp. 607–626, W.B. Saunders, Philadelphia, PA.
[20] Rennick, B.R. (1981) Am. J. Physiol. 9, F83–F89.