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Short Sequence-Paper

Sequence of a putative transporter from rabbit kidney related to the Na^+/glucose cotransporter gene family ☆

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Abstract

A 2 kb cDNA, RK-D, was isolated from a rabbit renal library by hybridization with the Na^+/glucose cotransporter (SGLT1) cDNA. The mRNA for RK-D is also approximately 2 kb and is found predominantly in kidney. The RK-D cDNA encodes a protein of 597 amino acids related in sequence to the SGLT family of sodium-coupled transporters, all of which are related to the Na^+/glucose cotransporter, SGLT1. Because of the high sequence similarity and conservation of 'signature' family features, it is very likely that RK-D encodes a Na^+-dependent cotransporter.

Key words: Cotransport; Sodium ion/glucose cotransporter; Glucose transport; cDNA sequence; (Kidney)

The sodium-coupled transport of sugars and nutrients is mediated by plasma membrane-bound proteins. In recent years, the cDNAs of a number of these transporters have been isolated, and comparison of the deduced amino acid sequences has shown that several families of related transport proteins exist. The SGLT family consists of sodium-coupled transporters that are structurally related to the Na^+/glucose cotransporter, SGLT1, of the small intestine and kidney. All of the members of this family utilize the inwardly-directed electrochemical gradient for sodium to drive the uphill transport of substrates, such as glucose [1], *myo*-inositol [2], nucleosides [3] and neutral amino acids [4], across the plasma membrane.

The proximal tubule of the mammalian kidney contains a number of different sodium-coupled transport systems which function to reabsorb filtered nutrients [5]. In a previous study, a rabbit renal library was screened for cDNAs related to SGLT1, and the cDNA encoding a Na^+/nucleoside cotransporter, SNST1, as well as six related cDNAs were isolated [3]. In this study, the sequence and tissue distribution of one of the SGLT-related cDNAs, RK-D (rabbit kidney clone D) is reported.

The cDNA and deduced amino acid sequence of RK-D are shown in Fig. 1. The cDNA is 1959 nucleotides in length and contains a single open reading frame between nucleotides 28 and 1818. The first ATG lies within a consensus sequence for translation initiation [6], containing A in position −3 and G in position +4. The protein encoded by RK-D cDNA is 597 amino acids, with a molecular mass of 64 649 Da.

There is considerable sequence conservation between RK-D and the other members of the SGLT family. The amino acid sequence of RK-D is 54% identical and 73% similar to the rabbit renal Na^+/nucleoside cotransporter, SNST1 [3], and 53% identical and 73% similar to the rabbit renal and intestinal Na^+/glucose cotransporter, SGLT1 [1,7] (GCG program, GAP). There is approximately 50% identity and 70% similarity between RK-D and the other mammalian members of the SGLT family: human intestinal SGLT1 [8], pig renal SGLT1 [9], rat intestinal SGLT1 (GenBank D16101), pig renal Na^+/neutral amino acid transporter (SAAT1) [4], human renal SGLT1-related sequence (K15/hu14) [10], dog renal Na^+/*myo*-inositol cotransporter (SMIT) [2], and rabbit renal SGLT1-related sequence (rkST1) [11]. Sequence alignments of SGLT family members (not shown) indicate that the greatest number of conserved

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residues is found in two regions, located around transmembrane domains 2 (26 out of 49 amino acids) and 8 (31 out of 51 amino acids). Several bacterial proteins, including the Na^+/proline [12,13] and Na^+/pantothenate [14] cotransporters, are distantly related members of the SGLT family. In addition, a search of the database has revealed two new bacterial sequences related to RK-D and other SGLT family members. YIDK is a putative 62.1 kDa protein from *Escherichia coli* [15], and ipa-31r is a gene product of *Bacillus subtilis* [16], neither of which has a known function yet. Both proteins contain at least twelve hydrophobic do-

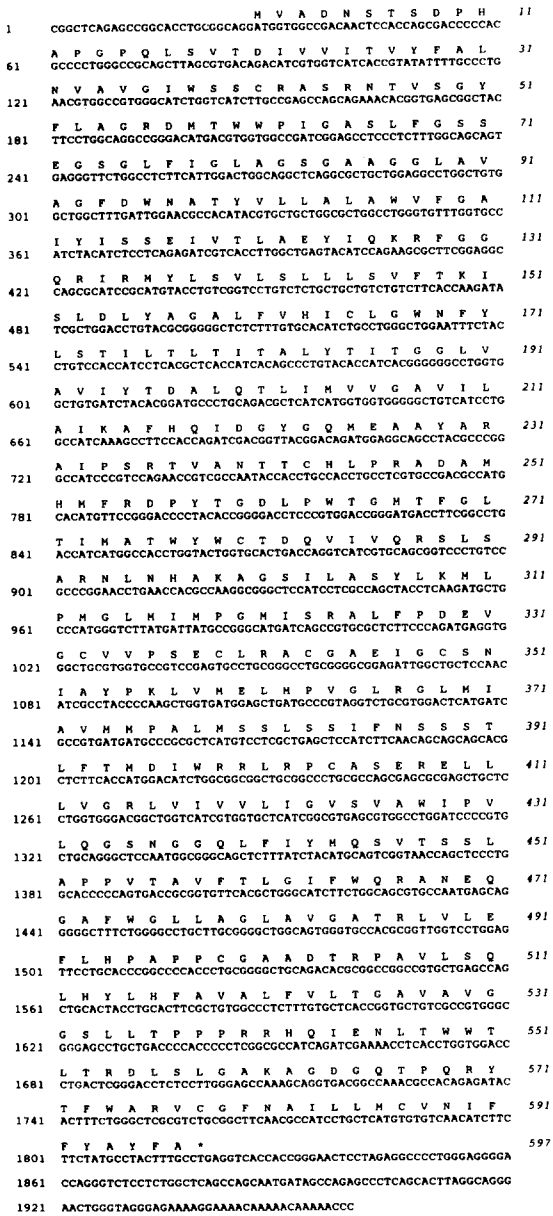

Fig. 1. Nucleotide and deduced amino acid sequence of RK-D. The numbers corresponding to nucleotides are listed in the left margin, the numbers corresponding to amino acids are listed in the right margin. RK-D was sequenced on both strands by a thermal cycler based protocol (CircumVent, New England Biolabs). Sequence analysis was done using the Genetics Computer Group (GCG) programs.

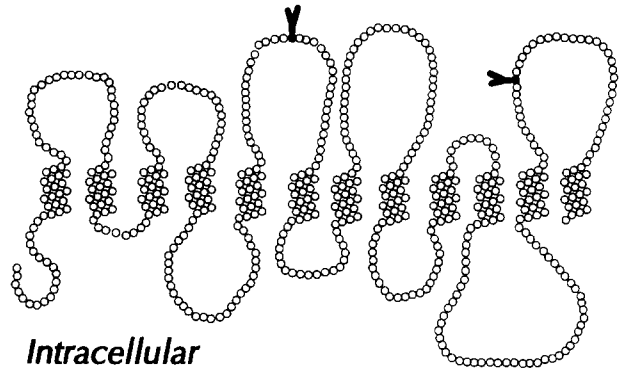

Fig. 2. Predicted secondary structure of RK-D based on hydropathy analysis [17]. There are twelve potential transmembrane domains, and two consensus sites for N-linked glycosylation (shown by Y symbols).

mains that could form transmembrane α-helices. YIDK is more closely related to the mammalian transporters than the other bacterial proteins, and also contains more of the conserved amino acids. YIDK is 24% identical and 55% similar in sequence to RK-D.

The predicted secondary structure model of RK-D protein (Fig. 2) based on hydropathy analysis [17] contains twelve putative transmembrane domains, and intracellular amino and carboxy termini. There are two consensus sequences for N-linked glycosylation located in the third and sixth extracellular loops (Asn^{240} and Asn^{546}). This predicted secondary structure of RK-D is very similar to the secondary structures of other members of the SGLT family, although the size of RK-D (597 amino acids) is considerably smaller than that of the other SGLT family members, which vary from 660 to 718 amino acids. The decrease in size of RK-D is evident primarily in the reduced extracellular loop between transmembrane helices 11 and 12, which contains a consensus sequence for a second N-linked glycosylation site. Aside from this difference, other features seen in SGLT family members are also found in RK-D. There are a number of conserved amino acids that form the 'SGLT family signature'. The aspartic acid at the beginning of the first transmembrane domain appears to be required for function of SGLT1, since a mutation to asparagine is seen in the human disease glucose/galactose malabsorption syndrome [18]. This aspartic acid is found in RK-D in position 21 and is conserved among all mammalian members of the SGLT family. Several mutagenesis studies of the bacterial Na^+/proline transporter have implicated Gly^{22} and Arg^{257} in Na^+ binding or translocation [19,20]. These residues are found in all SGLT family members, including RK-D (Gly^{36} and Arg^{288}). Finally, RK-D also contains a sequence of five amino acids present in many Na^+/coupled transporters, including those not related to SGLT1 [21].

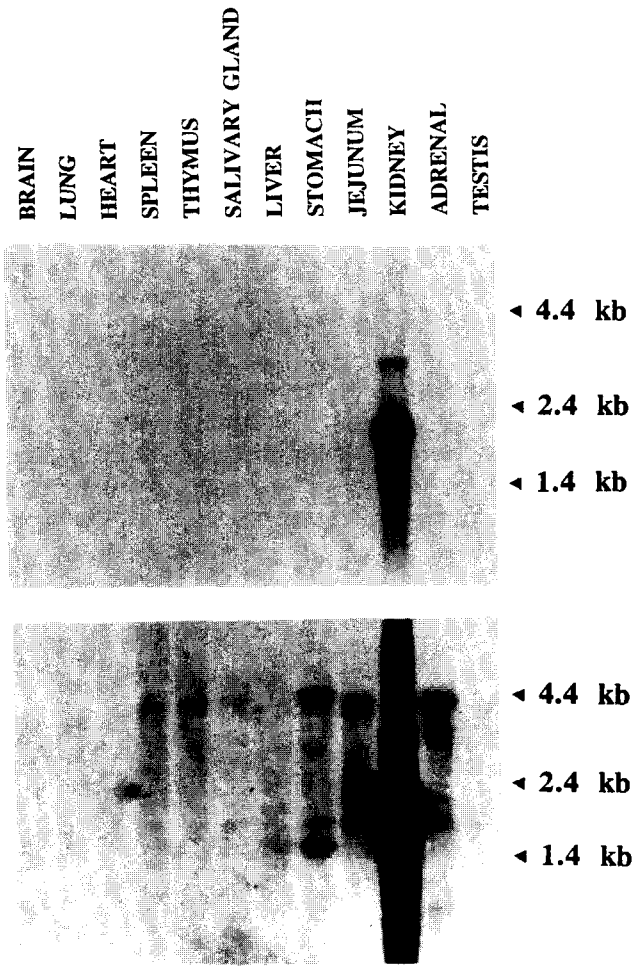

Fig. 3. Tissue distribution of RK-D mRNA. Northern blot of rabbit mRNA (5 μg/lane); the last two lanes contained total RNA (adrenal 25 μg and testis 15 μg). The blot was probed at high stringency with ^{32}P-labelled RK-D cDNA as described [3]. Positions of size standards are shown at right. Overnight exposure (top panel), 1 week exposure (bottom).

The tissue distribution of the message coding for RK-D is shown in Fig. 3. In an overnight exposure of the Northern blot a hybridization signal was found only in kidney. The predominant signal in kidney was approximately 2 kb, and there was a less intense signal at about 3 kb. Additional hybridization signals were seen in stomach, jejunum, and adrenal after a longer exposure of the Northern blot (1 week), suggesting that either a less abundant message or a closely related message is found in those organs. A previous study showed that RK-D message was more abundant in outer cortex than medulla, implying a proximal tubule distribution [3]. There are a number of sodium-coupled transporters located in the renal proximal tubule, which makes the number of potential substrates for RK-D quite large.

Preliminary studies to determine the function of RK-D suggest that RK-D does not transport substrates carried by the other known members of the SGLT family. Substrates were tested in both *Xenopus* oocytes and COS-7 cells expressing RK-D (results not shown), and included: α-methylglucose, glucose, mannose, *myo*-inositol, leucine, phenylalanine, serine, proline, succinate, uridine and uracil. There was no difference between control and RK-D expressing cells in the transport of these substrates.

In conclusion, this report describes the sequence and tissue distribution of a new member of the SGLT family of transporters, RK-D. Its sequence and secondary structure resembles that of the other family members, suggesting that RK-D is likely to be a sodium-coupled transporter. However, RK-D probably does not transport substrates carried by the other members of the SGLT family.

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