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Short Sequence-Paper

Cloning of a cGMP-gated cation channel from mouse kidney inner medullary collecting duct ☆

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Abstract

The cDNA sequence coding for the cGMP-gated cation channel expressed in the mouse kidney inner medullary collecting duct has been determined. The kidney cGMP-gated cation channel cDNA has an open reading frame of 2055 nucleotides and encodes a 685 amino acid protein. One cDNA clone is alternatively spliced thereby producing a deletion of 107 bp. Two differentially spliced 5′ untranslated regions were determined by 5′ RACE.

Keywords: Cation channel; Inner medullary collecting duct; Sodium ion transport; Reverse transcriptase–polymerase chain reaction; PCR; (Mouse kidney)

Sodium (Na^+) reabsorption by the renal inner medullary collecting duct (IMCD) is mediated by an amiloride-sensitive, 28 pS nonselective cation channel [1]. Atrial natriuretic peptide, via its' second messenger cGMP, inhibits Na^+ reabsorption across the IMCD by inhibiting the 28 pS cation channel [2,3]. Electrophysiological evidence suggests that the properties of the renal cGMP-gated cation channel are similar to cGMP-gated cation channels in photoreceptor, olfactory and other epithelia [2,4]. cGMP-gated cation channels do not discriminate among monovalent cations, have a single channel conductance of 28 pS (in the absence of divalent cations), and are regulated by cGMP [4]. However, two notable differences exist between rod photoreceptor and IMCD cGMP-gated cation channels. First, cGMP activates these channels in rod photoreceptors whereas cGMP inactivates these channels in the IMCD. Second, calcium decreases the single channel conductance of cGMP-gated cation channels in rod photoreceptor whereas calcium increases cGMP-gated cation channel activity in the IMCD [4].

cDNAs encoding cGMP-gated cation channels expressed in a variety of cell types have been cloned [4–6]. However, the molecular identity of the cGMP-gated cation channel in the IMCD is unknown. As a first step toward understanding the functional differences between rod photoreceptor and IMCD cGMP-gated cation channels, we have isolated a full-length cDNA encoding the cGMP-gated cation channel in the mouse IMCD.

Overlapping partial cDNA clones were obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) of poly(A^+) RNA isolated from a continuous cell line (mIMCD-K2) established from a mouse transgenic for simian virus 40 early-region genes, Tg (SV40E) Bri 7 [7]. First strand cDNA was synthesized using a Superscript II Pre-amplification cDNA Synthesis Kit (Gibco-BRL, Grand Island, NY) with DNase-treated poly(A^+) RNA (1 μg) as template and oligo(dT) or random hexamers as primers. Oligonucleotide primers corresponding to highly conserved regions of the mouse rod photoreceptor sequence [8] were used for PCR amplification of mIMCD-K2 cDNA. Optimal thermal cycling conditions were determined in preliminary experiments for each primer set. We obtained overlapping cDNA clones using the following oligonucleotide primer sets: IMCD1 (nucleotides 1–32) and IMCD2 (reverse complement of nucleotides 1024–1041) for Fragment A; IMCD3 (nucleotides 433–452) and IMCD4 (reverse complement of nucleotides 1338–1356) for fragment

☆ The nucleotide sequence data reported in this study have been submitted to the GenBank. The accession numbers are: U19717 for clone mIMCD-K2#1, U19715 for mIMCD-K2#2 and U19716 for mIMCD-K2#9.

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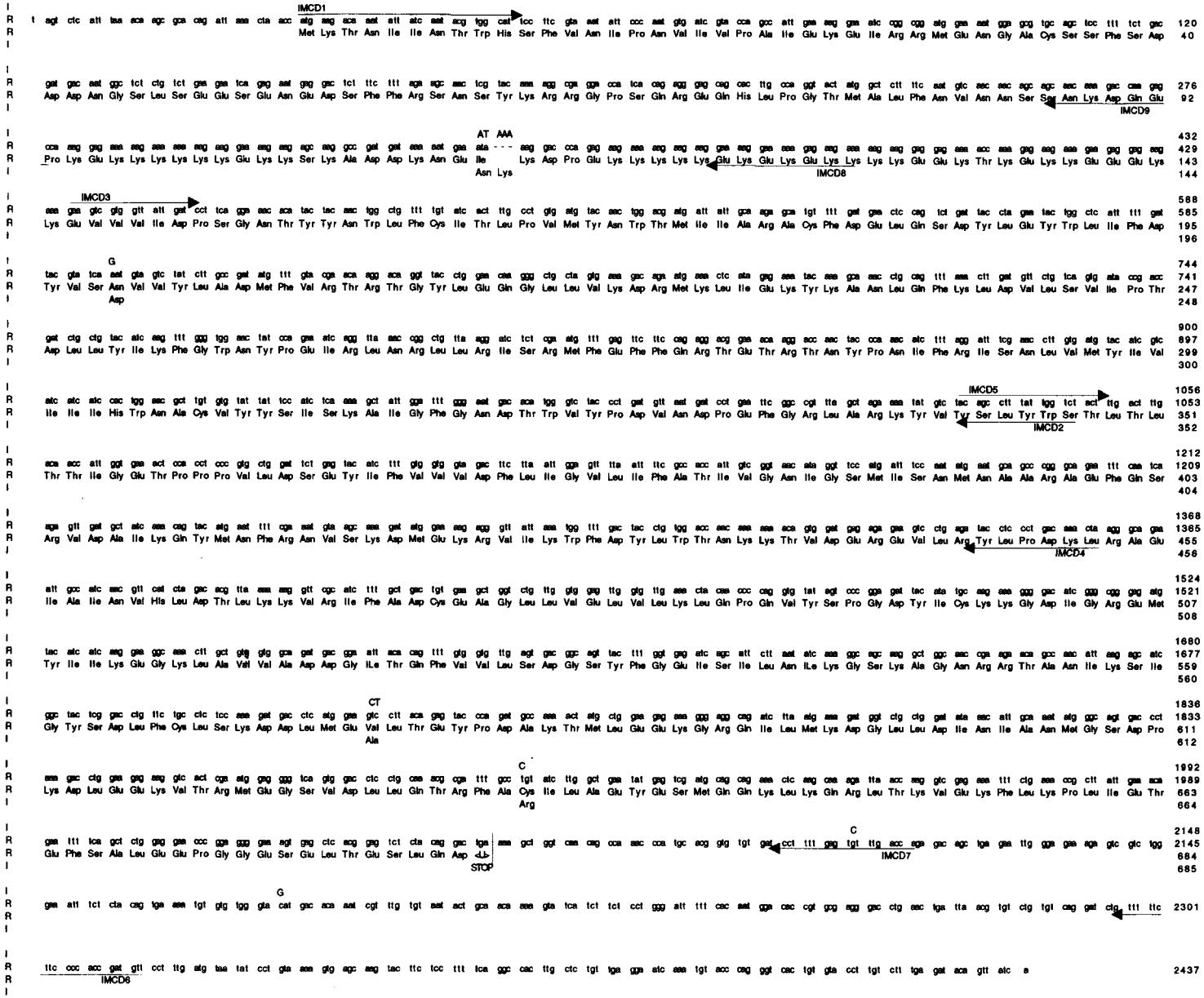

Fig. 1. Comparison of cDNA and deduced amino acid sequences of the mIMCD-K2 cGMP-gated cation channel (I) with mouse rod photoreceptor (R). Differences between mIMCD-K2 and mouse rod photoreceptor nucleotide and amino acid sequences are indicated in the first and fourth rows. Identical residues are represented by an empty space. Gaps in the sequence are represented by a dashed line. PCR primers are indicated by underlined or overlined sequences. Position 1 has been assigned to the first nucleotide of the first start codon (ATG) downstream of a stop codon. A vertical line follows the stop codon that terminates the open reading frame.

B; and IMCD5 (nucleotides 1025–1045) and IMCD6 (reverse complement of nucleotides 2294–2316) for fragment C (Fig. 1). Control PCR reactions included no reverse transcriptase and no cDNA template. As an additional control, β-actin was amplified by RT-PCR using intron-exon junction spanning primers (nucleotides 475–945 and reverse complement of nucleotides 1236–1266) [9].

PCR amplification of β-actin resulted in a single 351 bp product, whereas amplification of genomic DNA was predicted to yield a PCR product for β-actin of 792 bp. As expected, no PCR reaction products were visualized in the absence of reverse transcriptase or cDNA template.

In separate reactions for each primer set, a PCR product of the expected size was obtained. Excised gel fragments were purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI) and subcloned into the pCR II vector using the TA Cloning System (InVitrogen, San Diego, CA). Three or more independent clones of each fragment were sequenced in both directions by the dideoxynucleotide chain termination method using [α-^{35}S]dATP and T7 DNA polymerase (Sequenase, version 2.0, United States Biochemical, Cleveland, OH). A full-length cGMP-gated cation channel cDNA was assembled by in vitro recombinant PCR, restriction enzyme digestion and ligation.

The 5′ untranslated region of the cGMP channel cDNA was obtained by PCR amplification of 5′ cDNA ends (5′ RACE) synthesized with random hexamers or an antisense gene-specific primer. Following ligation of an AmpliFINDER anchor (Clontech, Palo Alto, CA), multiple products were amplified under suboptimal conditions using the manufacturer's anchor primer (Clontech) and a gene-specific primer (IMCD 8; Fig. 1). The PCR products were gel purified and used as template in a second PCR reaction using the anchor primer and a nested gene-specific primer (IMCD 9; Fig. 1). Optimization of the reaction conditions resulted in the amplification of two distinct 5′ RACE products. These products were subcloned into the pCR II vector and sequenced.

The cGMP-gated cation channel cDNA has an open reading frame of 2055 nucleotides (Fig. 1). The cDNA open reading frame differs by 9 nucleotides from the cDNA encoding the cGMP-gated cation channel in mouse rod photoreceptor [8]. Nucleotide differences within the coding region were noted at positions 338 and 339 (TA to AT), 595 (A to G), 1724 (T to C), 1725 (C to T) and 1900 (T to C). These substitutions result in 4 amino acid changes (Ile to Asn, Asn to Asp, Val to Ala, and Cys to Arg, respectively). We also observed a 3 nucleotide insertion (340, 341, and 342) in the renal cDNA. As indicated in Fig. 1, these nucleotides result in the insertion of a lysine.

The photoreceptor cDNA sequence was obtained from C57BL/6 mice [8]. To determine if the sequence differences between photoreceptor and kidney are due to strain differences, cDNA was synthesized from poly(A^+)RNA isolated from C57BL/6 mouse kidney and amplified by PCR. The cDNA sequence was identical in mIMCD-K2 clones and C57BL/6 kidney clones. Thus, we conclude that differences in nucleotide sequence between photoreceptor and kidney are tissue specific.

Sequence analysis also revealed one cDNA clone with a 107 bp deletion. This region corresponds to exon 9 of the human rod photoreceptor DNA sequence [10], suggesting that the cGMP cation channel poly (A^+) RNA may be alternatively spliced in the renal IMCD.

As shown in Fig. 2, the 5′ RACE products are only 29% homologous between −67 and −17 nucleotides but share sequence identity from −16 to +90 nucleotides. Both 5′ RACE products are preceded by an upstream stop codon (TGA,TAG) and have a partial Kozak consensus sequence (ACC) preceding the initiation start codon (ATG). When compared with the predicted intron-exon junction sequences of the human rod photoreceptor (10), the 5′ untranslated region of the mIMCD-K2 cGMP-gated cation channel cDNA is differentially spliced at a site corresponding to the second intron.

The open reading frame of the cDNA clone isolated from mIMCD-K2 cells is ~99% identical to the cDNA

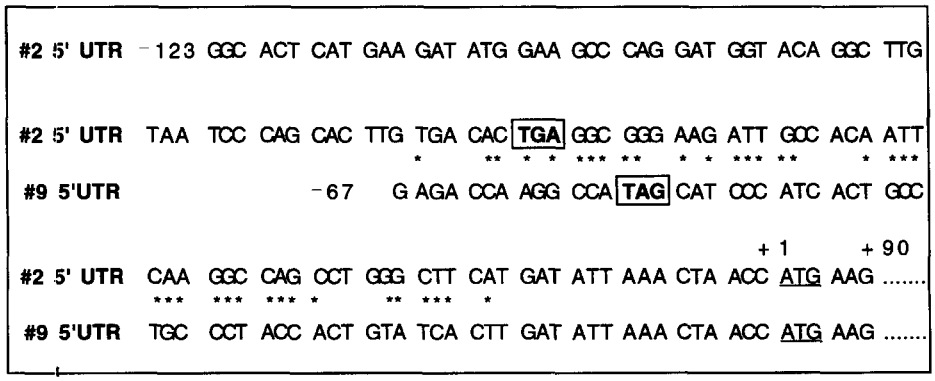

Fig. 2. Illustration of the 5′ untranslated regions of two different cGMP-gated cation channel cDNA sequences using a single cDNA template. Differences in nucleotide sequence between the two clones are indicated by an asterisk. The first nucleotide of the initiator methionine codon (ATG), 58 nucleotides downstream of an in-frame stop codon (TGA) in clone 2 and 52 nucleotides downstream (TAG) in clone 9, has been assigned position +1.

encoding the cGMP-gated cation channel in mouse rod photoreceptor [8]. The differences in sequence are likely due to tissue specific differences rather than mouse strain differences. These minor differences in sequence may be responsible for the different properties of the cGMP-gated cation channel in rod and kidney. A single amino acid change in the retinal K^+ channel results in a change in gating properties, rendering the channel insensitive to divalent cations [11]. Functional differences between photoreceptor and IMCD cGMP-gated cation channel may also result from post-translational events. For example, the non-conserved change in residue 636 in the cDNA isolated from IMCD results in an additional proteolytic consensus sequence that may result in a truncated protein with altered function. It is also possible that the mouse IMCD channel, like the channel in photoreceptor, is a heteromultimeric complex and that the channel in the IMCD is composed of different β subunits from photoreceptor thereby conferring upon the IMCD channel different kinetic, pharmacological and biophysical properties. Experiments are in progress to evaluate these possibilities.

Interestingly, two different 5′ untranslated regions in the mouse IMCD cGMP-gated cation channel cDNAs were observed. Differential splicing of the 5′ untranslated regions of the cGMP-gated cation channel gene may results in different regulatory elements mediating hormone-dependent transcriptional activation.

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