Cloning and Characterization of the cDNA Encoding the α-Subunit of cGMP-Phosphodiesterase in Canine Retinal Rod Photoreceptor Cells

Weiquan Wang, Gregory M. Acland, Gustavo D. Aguirre, Kunal Ray

The James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA

Rod photoreceptor cyclic GMP-phosphodiesterase (cGMP-PDE, EC 3.1.4.17) is a key enzyme in the phototransduction cascade of the vertebrate retina. The enzyme is composed of α- and beta-catalytic subunits, and two identical inhibitory gamma-subunits. Once the phototransduction cascade is initiated by the absorption of light by rhodopsin, activated cGMP-PDE rapidly hydrolyses cGMP, depletion of which shuts cGMP-gated cation channels in the plasma membrane. The consequent hyperpolarization of the photoreceptor outer segment represents a large signal amplification and generates the visual neural impulse. Aberrant function of cGMP-PDE is causally associated with retinal degenerative diseases in man and animals. Mutations in the genes for the α- and beta-subunits of cGMP-PDE (PDEA and PDE6B, respectively) cause retinitis pigmentosa (OMIM entry) in some human families [6,8,9]. Defects in PDE6B also cause retinal degeneration in the rd mouse [3,11] and the rd1 dog [5,13,14]. Rod-cone dysplasia 2 (rcd2), which affects the collie dog, also represents a defect of retinal cyclic GMP metabolism since retinal cGMP levels are significantly elevated and cGMP-PDE activity is deficient [16]; however, rcd2 is not caused by a defect in PDE6B [1]. Thus it is likely that the rcd2 locus codes for either another cGMP-PDE structural subunit [PDEA, or PDEG (the gene for the gamma-subunit of cGMP-PDE)], or for one of the other proteins in the phototransduction cascade that activates cGMP-PDE. Since the canine chromosomal locations of the rcd2 locus and the genes involved in phototransduction are not known, none of these candidate genes can be ruled out based on their map location relative to the rcd2 locus.

In order to identify the mutation responsible for the rcd2 disorder, we have begun to examine the different candidate genes that code for the phototransduction cascade proteins in the dog. The cDNAs for the α-subunit of cGMP-PDE (PDEα) have been cloned and characterized from man [12], mouse [2], and cow [10,12]. However, no information is available in the literature on the canine PDEα cDNA.

In this study we characterize the canine PDEα cDNA from normal dog, present evidence for usage of alternate polyadenylation sites to generate the two different transcripts described in multiple species [2,7,12], and compare the deduced amino acid sequences for conservation through evolution.

RESULTS

Initially two different segments of canine PDEα cDNA were obtained by reverse transcription (RT) and polymerase chain reaction (PCR) using total retinal RNA and consensus primer pairs (PDEA-1/PDEA-2 and PDEA-3/PDEA-4; Table 1) based on the coding regions of known PDEα cDNA sequences from other species. These RT-PCR amplified fragments were cloned (1-PDEA and 2-PDEA) in pCR II vector (Invitrogen; San Diego, CA) and sequenced to confirm authenticity of the retinal-specific PDE cDNA sequence. From the confirmed canine

Correspondence to: Kunal Ray, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853; email: kr22@cornell.edu
PDEα cDNA sequence, new canine-specific primers were designed to amplify both the 5’- and 3’-ends of canine PDEα cDNA from a canine retinal cDNA library by PCR (Figure 1).

To amplify the 5’-end of the cDNA, forward vector-specific (pBK-II) and PDEα-specific reverse (PDEα-6) primers were used. From the PCR products, the largest fragment showing evidence for PDEα specificity, based on PCR using internal primers, was cloned (5’-PDEα). To amplify the 3’-end of the cDNA, gene specific forward (PDEα-5) and vector specific reverse (T7) primers were used, and the amplified DNA fragment was cloned (3’-PDEα). The clone 3’-PDEα, however, lacked the poly (A) tail. We therefore designed a canine-specific primer (PDEα-9) from the 5’-end of the cDNA and used it in combination with the vector-specific reverse primer (pBK-V) for amplification of the entire open reading frame (ORF) and 3’-untranslated region (UTR) of PDEα cDNA from the cDNA library. The PCR resulted in amplification of two DNA fragments (3.0 and 3.2 kb), both of which hybridized to canine PDEα cDNA in Southern blots (data not shown). These two putative PDEα cDNA fragments (PDEα-L and PDEα-S) were cloned as described above.

Sequence of the PDEα cDNA was obtained from clones 1-PDEα, 2-PDEα, 5’-PDEα, 3’-PDEα, and PDEα-S, which contain overlapping fragments, from both directions. The identity of the larger clone (PDEα-L) as PDEα cDNA was confirmed by (a) partial sequencing (800 bp) of the 3’-end and two other upstream regions of the insert; (b) amplification of multiple overlapping fragments identical in size to those obtained from PDEα-S by PCR using the same set of primers; and (c) identical and predicted restriction enzyme digestion pattern of PCR amplified DNA fragments from both the clones (Figure 2). Sequences of all the primers used and their location in the canine PDEα cDNA or vector DNA are listed in Table 1.

Table 1. Sequence and location of primers used for PCR

<table>
<thead>
<tr>
<th>Primer sequence (5’ to 3’)</th>
<th>Source of Primer</th>
<th>Name of primer</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>gCTTCCCAACACTAATCAACC</td>
<td>pBkCMV</td>
<td>PDEα1</td>
<td>192-215</td>
</tr>
<tr>
<td>AGTGTGACAACTTGGGAGC</td>
<td>pBkCMV</td>
<td>PDEα2</td>
<td>815-836</td>
</tr>
<tr>
<td>GCGACTCACTATAGGGCGAATT</td>
<td>pBkCMV</td>
<td>PDEα3</td>
<td>1759-1786</td>
</tr>
<tr>
<td>ATGGTCTGCCCCACGTTGAAGCC</td>
<td>cPDEα</td>
<td>PDEα4</td>
<td>2393-2412</td>
</tr>
<tr>
<td>TCCACCCTATTCTGGTCCCA</td>
<td>cPDEα</td>
<td>PDEα5</td>
<td>1829-1851</td>
</tr>
<tr>
<td>TCCACCCTATTCTGGTCCCA</td>
<td>cPDEα</td>
<td>PDEα6</td>
<td>691-706</td>
</tr>
<tr>
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<td>cPDEα</td>
<td>PDEα7</td>
<td>380-404</td>
</tr>
<tr>
<td>AGACATTCTTGACACTCAAAG</td>
<td>cPDEα</td>
<td>PDEα8</td>
<td>137-160</td>
</tr>
<tr>
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<td>pBkCMV</td>
<td>PDEα9</td>
<td>2032-2054</td>
</tr>
<tr>
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<td>PDEα10</td>
<td>1303-1305</td>
</tr>
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<td>GCTCTGGCCGCGCCAT</td>
<td>pBkCMV</td>
<td>pBkCMV</td>
<td>1052-1067</td>
</tr>
</tbody>
</table>

Primers (PDEα-1 to PDEα-4) used for RT-PCR correspond to the human (h) PDEα cDNA sequence, selected from the consensus region in different species. All other PDEα primers correspond to canine (c) PDEα cDNA sequence. Location of all the PDEα primers are shown with respect to cPDEα cDNA sequence. Nucleotides (shown in lower case) in some primers have mismatches with the canine sequence because those primers were selected either from the human sequence, or from preliminary canine sequence. Phagemid vector (pBK-CMV) specific primers were selected either from the multiple cloning site or from the flanking region. The bold italicized region of primer pBK-V represents the linker used to make the canine cDNA library.

The two full length canine rod PDEα cDNAs are 2988 and 3233 nucleotides long, including the poly (A) tail (Figure 3). We have previously reported the presence of two distinct PDEα mRNAs, 3.3 and 3.0 kb, expressed in equivalent amounts in canine retina by northern blot [7]. Thus the sizes of the canine PDEα cDNAs described here (GenBank accession number U52868) are in complete agreement with our observation from northern analysis [7]. We have not formally determined the transcription start site; however, the clone containing the most upstream 5’-noncoding region contains 139 nucleotides in the 5’-UTR followed by the ATG codon for initiation of translation. The stop codon (TAA) corresponds to positions 2723 through 2725 of the cDNA sequence. The 3’-UTRs of the 3.0 kb and 3.3 kb transcripts, represented by clones PDEα-S and PDEα-L, are 263 and 508 nucleotides respectively. A non-consensus putative polyadenylation signal (AATAAA) is present in the corresponding locations of the smaller (PDEα-S) and larger (PDEα-L) clones. In PDEα-S this first polyadenylation signal (Figure 3, nucleotides 2948 through 2953) is 15 nucleotides upstream of the poly (A) tail.
In the larger clone (PDEA-L) there is a second polyadenylation signal (Figure 3, nucleotides 3187 through 3192) 23 nucleotides upstream of the poly (A) tail. The ORF of the canine sequence predicts a protein of 99.7 kDa containing 861 amino acids.

**Discussion**

Comparison of the ORF nucleotides of the canine PDEα cDNA with that of other species shows that they share similar nucleotide identity with the bovine (91.0%) and human (90.4%) sequences, and slightly lower identity with the mouse (86.5%) sequence. At the amino acid level, however, the similarity between the canine sequence and that of other species shows that it shares similar nucleotide identity with both species.

Figure 3. The nucleotide sequence of PDEα cDNA from canine retina (GenBank accession number U52868), and the deduced amino acid sequence. The position of the nucleotides and the deduced amino acids that are inserted into human and mouse sequences are shown in **bold**. The position of the nucleotides and the deduced amino acids that are inserted into human and mouse sequences are shown in **italics**.

The size of the transcript in mouse has not been reported. Since only a single ORF with high homology between different species has been identified for PDEα cDNAs, it is reasonable to assume that the inter- and intra-species difference in the sizes of the transcripts could be due to either different transcription start sites or use of alternative poly (A) addition sites. The sequence presented here clearly demonstrates that two differently sized canine PDEα transcripts are generated by use of different polyadenylation signals.
sites. The same mechanism could well account for the observed different transcript sizes within other species. The canine sequence does not contain the canonical polyadenylation signal (AATAAA); instead a similar sequence motif (ATTAAA) was identified in both appropriate locations (Figure 3). This alternative motif has been demonstrated to serve as a surrogate polyadenylation signal with ~80% efficiency [15]. A non-consensus polyadenylation signal (AATACA) has also been reported to be present in human PDEα cDNA [12].

It is noteworthy that while PDEαt cDNA clones from 3 other species contain an ORF capable of coding for a polypeptide of 859 amino acids, the canine PDEαt polypeptide is predicted to contain 861 amino acids. With respect to the human and mouse sequences, the two extra amino acids are located as the 849th (Ala) and 850th (Gly) residues (Figure 3). Similar to the comparison made between human and bovine PDEαt sequences [12], we noted that the differences in amino acid sequence among the four species (human, mouse, bovine, and canine) are clustered in the 225 N-terminal and 45 C-terminal residues (data not shown). The conserved region includes the domain present in several eukaryotic cyclic nucleotide phosphodiesterases [4]. Alignment of amino acid sequences of canine PDEαt and PDEβt shows a 72% overall identity, and the domains that are most dissimilar are at the N-terminus (first 50 residues) and C-terminus (last 30 residues).

The data presented here have been used to identify the possible mechanism for the presence of two transcripts for PDEαt in different species, and to compare nucleotide/amino acid identity among rod-specific PDEαt sequences of these species. Characterization of the wild type canine PDEαt cDNA sequence will allow us to detect PDEαt mutations in dogs affected with rcd2 or other inherited retinal degenerations. Because collie dogs affected with rcd2 have elevated retinal cGMP levels secondary to low PDE activity, we are currently investigating the possibility of a PDEαt mutation in this disease.

ACKNOWLEDGEMENTS


REFERENCES


The typographical corrections below were made to the article on the date noted. These changes have been incorporated in the article and the details are documented here. 29 March 1999: The text "retinitis pigmentosa (RP)" was changed to "retinitis pigmentosa (OMIM entry 268000)" in the first paragraph of the introduction. The abbreviations "Fig." and "no." were expanded to "Figure" and "number", respectively, throughout the article. The characters enclosing citations to references were changed from parentheses () to brackets []. Several instances of incorrect spacing between a word and punctuation have been corrected.