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The human rod photoreceptor cGMP phosphodiesterase β-subunit

Structural studies of its cDNA and gene

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cDNA clones encoding the β-subunit of the photoreceptor cGMP phosphodiesterase (PDE) were isolated from a human retina library and their sequence was determined. The encoded polypeptide consists of 854 amino acid residues with a calculated molecular mass of 98,416 Da. Alignment of the deduced amino acid sequence with the earlier analysed α-, β- and α′-subunits of bovine and mouse PDEs demonstrates a high homology. Two overlapping recombinant λ phage clones containing 26 kb of the human PDE β-subunit gene were isolated from the genomic library. A total nucleotide sequence of exons 4-22 of the PDE β-subunit gene was established which completely corresponded to the cDNA structure. According to sequence analysis no potential possibility for alternative splicing of the β-subunit gene was observed between exons 20 and 21 which led to the formation of the β′-subunit as described for mouse PDE. Polymerase chain reaction (PCR) experiments also confirm the absence of the PDE β′-subunit in human retina.

Cyclic GMP phosphodiesterase; cDNA cloning; Polymerase chain reaction; Alternative splicing; Human retina

1. INTRODUCTION

Visual signaling in rod photoreceptor cells is triggered upon absorption of a photon by rhodopsin. The phototransduction cascade leads to rapid hydrolysis of cGMP by activated cGMP phosphodiesterase (PDE) which in turn results in closure of cGMP-gated cation channels in the plasma membrane, leading to hyperpolarization of the cell [1]. Rod PDE is thought to be a peripherally membrane-bound heterotrimeric enzyme $\alpha\beta\gamma_2$ [2,3].

Evidence has been received recently that the degenerative process in retinal degeneration (rd) of mouse is caused by a defect in the β-subunit of the rod cGMP PDE [4]. Studies of the human photoreceptor cell proteins at a molecular level might facilitate disclosure of the molecular and genetic defects in the human visual system.

Earlier we have determined the primary structures of the PDE α-, β- and γ-subunits from bovine retina [5–7] as well as the structure and organization of the PDE γ-subunit gene from human retina [8].

This paper deals with the cloning and elucidation of the cDNA primary structure of the human PDE β-subunit* and cloning of the corresponding gene.

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Abbreviation: CAAX, Cys-aliphatic-aliphatic-X.

*The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number no. X66142.

2. MATERIALS AND METHODS

The human retinal cDNA library prepared (Clontech) by oligo$(dT)_{12-18}$ priming in bacteriophage λgt11 was a gift of Dr. M. Applebury (Department of Ophthalmology, University of Chicago). The human genomic library in λEMBL3 was a gift of Dr. A. Grishin (Shemyakin Institute of Bioorganic Chemistry, Moscow). DNA manipulations were carried out by standard methods [9]. Oligonucleotide probes I (1,120–1,149 nucleotides; 5′-ATGAATGCCCCTGCCGACGAAATGTTCAAT), II (1,531–1,557 nucleotides; 5′-CTCATAGATGTCGAACTTGGCGGGCCC) and III (2,542–2,568 nucleotides; 5′-GTTGCAGATTTCTGTGCCCACTTTCTT) corresponding to the bovine PDE β-subunit sequence were synthesized [6]. Nucleotide sequences were determined by the Maxam and Gilbert method in solid phase modification [10].

The PCR was performed with a Gene AMP DNA amplification reagent kit from Perkin-Elmer, USA. The cDNA from the human retinal library was used as a template for in vitro amplification with primers I and III, and the thermal profile was used as 94°C for 30 s, 55°C for 2 min, and 72°C for 2 min for a total of 30 cycles. The amplified products were separated by agarose gel electrophoresis and cloned into the pSP65 vector by the *Hin*cII restriction site.

3. RESULTS AND DISCUSSION

Oligodesoxyribonucleotide probe I and the bovine

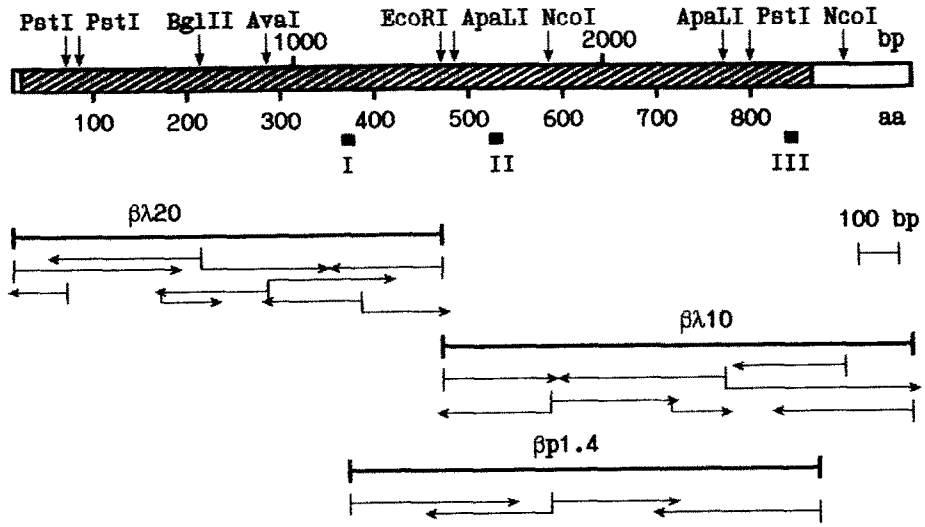

Fig. 1. Location of insertions of isolated clones in the restriction map of the phosphodiesterase β-subunit cDNA and sequencing strategy. The coding region is hatched. Black squares indicate nucleotide probes used in the work.

PDE β-subunit cDNA fragment *Nco*I–*Hin*dIII (1,815–2,580 nucleotides) [6] were used as hybridized probes for screening the cDNA clone library from human retina. Two hybridization-positive clones (βλ20 and βλ10) were isolated from 1.2×10^6 plaques and their inserts were used for sequence analysis (Fig. 1). A comparison of the polypeptide of fragment structures deduced from the nucleotide sequences of cDNA inserts with the amino acid sequences of the human and mouse PDE β-subunits revealed that the βλ20 and βλ10 inserts encoded the protein N- and C-terminal parts were abutting. To confirm the central region structure of the cDNA, clones βp1.4 and βp2.5 were obtained by PCR using primers I and III. Their nucleotide sequences were identical and coincided with those of the corresponding regions in clones βλ20 and βλ10 (Fig. 1).

Fig. 2 shows the 2,833 nucleotide sequence of the human cDNA of the PDE β-subunit. Its comparison with the PDE β-subunit cDNA structures from bovine and mouse shows that triplet ATG (6–8) is an initiation codon. Codon 854, specifying leucine, is followed by the termination codon (2,568–2,570). Thus, the PDE β-subunit amino acid sequence deduced from the cDNA nucleotide sequence consists of 854 residues with a calculated molecular mass of 98,416 Da. The amino acid sequence of the human PDE β-subunit has 91.1% and 92.4% identity with the PDE β-subunits of bovine [6] and mouse [11], respectively; 71.9%, 72% and 71.1% identity with the PDE α-subunits of human [12], bovine [5] and mouse [11], respectively, and 63.2% identity with the cone PDE α′-subunit from bovine retina [13].

The catalytic region for cyclic nucleotide hydrolysis has been established based on the strong conservation of amino acid sequence in all members of the phosphodiesterase family [5,6,13–15]. It corresponds to the 250-residue domain and lies in the C-terminal half of the protein chain (556–778 amino acid residues). Elements that may contribute to forming a guanine nucleotide-binding site are observed in each of the photoreceptor subunits. These include a glycine-rich region that may form a loop for cyclic nucleotide binding (residues 562–577), an Mg^{2+}-binding element DX_2G (residues 601–604), and NKXD element that has been suggested to specify binding for the guanine ring (residues 763–766) [6,13,16,17]. In the β-subunit, the NKXD element has been replaced by NKXA.

The rod PDE β-subunit from human retina, like the rod α-, β-subunits and the cone PDE α′-subunit from bovine retina, has an internal homologous repeat of residues in its N-terminal half. These regions (residues 90–253 and 297–465) seem to be the sites of noncatalytic cGMP binding [18].

The domains that are most dissimilar are at the N-terminus (first 85 residues) and at the C-terminus (last 30 residues). The last 4 residues at the C-terminus conform to the CAAX consensus motif for multistep post-translational processing involving lipidation, proteolysis and carboxymethylation [19].

When studying the β-subunit PDE cDNA structure from mouse retina W. Baehr revealed cDNA clones, their inserts encoded both the β-subunit and a new β′-subunit [20]. The mRNA of the mouse PDE β′-subunit was suggested to be the result of an alternative splicing (Fig. 3), it encoded the protein differing from the mouse PDE β-subunit, and is 55 amino acid shorter in its C-terminal part.

We have also attempted to search cDNA clones encoding for a similar β′-subunit of human cGMP phosphodiesterase. To study a probable alternative splicing of the human PDE β-subunit cDNA (in position 2,357) we used the primers I and III for four PCR-independent experiments. The 1,500 bp long products

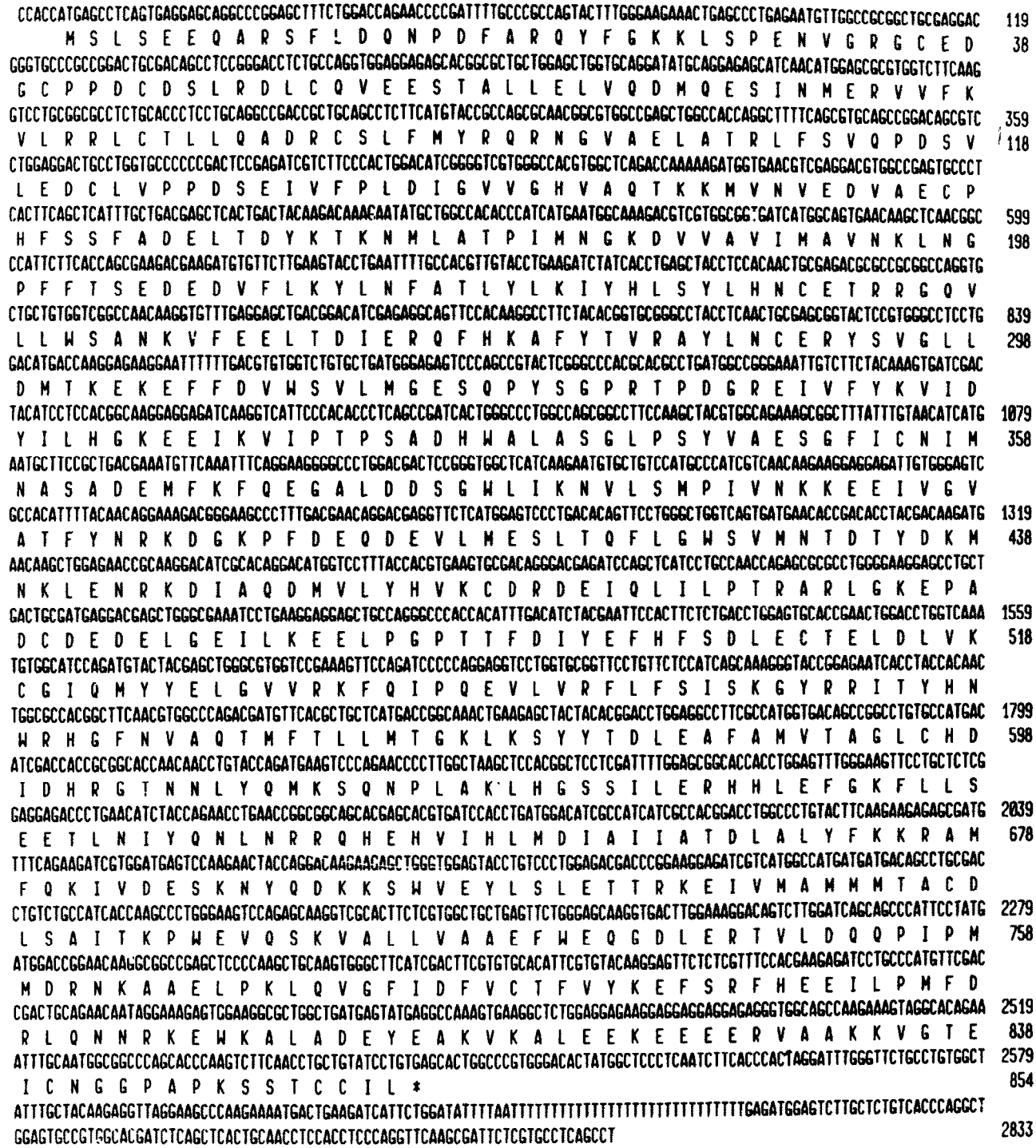

Fig. 2. Nucleotide sequence of the cDNA and deduced amino acid sequence of the rod phosphodiesterase β-subunit from human retina.

of the amplification reaction were assumed to contain cDNA fragments encoding the β- and β'-subunits. About 100 hybridized clones were selected by probe II, their plasmid DNAs being isolated. No clone with a restriction map corresponding to that of the human PDE β'-subunit was found. Furthermore the partial nucleotide sequences of four cDNA fragments from the independent amplification reaction confirmed that the PCR products corresponded only to the human PDE β-subunit.

The nucleotide sequence of the corresponding region of the human PDE β-subunit gene was determined for further studies on the location of probable alternative splicing. Two phages were earlier isolated from the genomic clone library. Their overlapping inserts contained a 26 kb long fragment of the human PDE β-

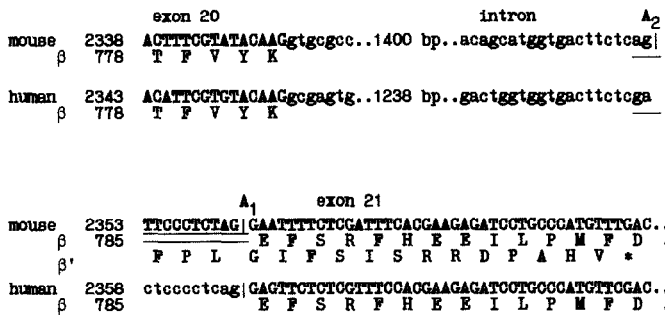

Fig. 3. Comparison of nucleotide sequences of exon–intron margins between genome exons 20 and 21 of the human and mouse PDE β-subunit as well as of the partial amino acid sequences of the human PDE β-subunit and the mouse PDE β- and β'-subunits. A_1 and A_2, sites of alternative splicing for the PDE β and β'-subunits, respectively. Underlined: one line, substitution of AG with GA; two lines, insertion of 10 nucleotides found only in the cDNA of the mouse PDE β'-subunit.

subunit gene (unpublished data). Hybridization analysis and use of synthetic oligodesoxyribonucleotide probes as well as determination of the nucleotide sequence and its comparison with the cDNA structure evidenced that these DNA fragments contained 19 exons of the gene and that their primary structures totally coincided with the cDNA structure.

The gene fragments we cloned did not contain the first three exons if to compare with the exon–intron organization of the mouse PDE β-subunit gene [4]. The 1,700 bp long subfragment *Xho*I–*Xba*I containing exons 20 (84 bp) and 21 (150 bp) was subcloned and its total nucleotide sequence was determined. The size of the intron located between exons 20 and 21 is 1,238 bp long. A comparison of the structures of exon-intron boundary regions in the genes of human and mouse PDE β-subunits (Fig. 3) testified the absence of an additional sequence $(Y)_nXAG$ (Y = pyrimidine, X = any nucleotide) typical of the intron 3'-termini which was present in the mouse gene (A_2, Fig. 3). Dinucleotide AG in the human gene is replaced by GA and consequently alternative splicing here seems to be impossible. No sequence of 10 additional nucleotides, present only in the mouse PDE β'-subunit cDNA (underlined in Fig. 3), was observed between exons 20 and 21.

The conclusion is drawn from the presented data that the β'-subunit of cGMP phosphodiesterase is absent in human retina.

We also localized exon 7. In mouse, the defect causing the rd-mutation that resulted in the degeneration of mouse retina was observed in the analog exon. The primary structure of gene intron regions adjoining this exon was elucidated and this may shed light on the existence of genetic prerequisites for such mutations in the gene of the human PDE β-subunit. Cloning of the human gene and knowledge of its genomic organization will allow assessment of the role of this gene in the cause of human retinopathies.

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