Human cone-specific cGMP phosphodiesterase α′ subunit: complete cDNA sequence and gene arrangement**

Elena A. Feshchenko[a], Svetlana G. Andreeva[a], Veronika A. Suslova[a], Evgeniya V. Smirnova[a], Vasily E. Zagranichny[b], Valery M. Lipkin[a,b,*]

[a]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya St. 16/10, 117871 GSP Moscow V-437, Russia*

[b]*Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia*

Received 11 January 1996

Abstract Four independent phage clones containing the fragments of cone-specific cGMP phosphodiesterase (PDE) α′ subunit (PDEα′) cDNA were isolated from the human cDNA library. The screening of the genomic library resulted in isolation of four independent phage clones with the fragments of human cone PDEα′ gene including 5′-flanking region and exons ranged from 1 to 14 (overall 32 kilobases). Structural studies of the clones made it possible to establish the complete human cone PDEα′ cDNA structure (3455 base pairs). The encoding polypeptide consists of 858 amino acid residues with a calculated molecular mass of 99169 Da. The deduced amino acid sequence displays high homology to the earlier analyzed catalytic α, β and α′ subunits of bovine, human, chicken and mouse photoreceptor PDEs.

Key words: Cyclic GMP phosphodiesterase (PDE); cDNA cloning; Human retina; Nucleotide sequence

1. Introduction

The enzymatic cascade of phototransduction reactions involved in photoreception of vertebrate photoreceptor cells starts with the absorption of a photon by photosensitive protein, rhodopsin, and ends in lowering cGMP concentration and the closing cGMP-dependent cation channels in the plasma membrane leading to cell hyperpolarization. Light-activated rhodopsin catalyses the formation of the transducin α subunit complex with GTP [1]. This complex, in turn, activates cGMP phosphodiesterase (PDE) [2], a key component in the phototransduction cascade in photoreceptor cells.

PDE is a peripheral heterotetrameric membrane protein consisting of two types of subunits, large catalytic and small inhibitory. PDE in vertebrate rods responsible for low-light black-and-white vision is represented by a heterotetramer $\alpha\beta\gamma_2$ consisting of two different catalytic α [3] and β [4] subunits and two identical inhibitory γ subunits [5]. In mammalian color-sensitive cones PDE contains two identical catalytic α′ subunits [6]. Complete cDNA primary structures of bovine photoreceptor PDE subunits were elucidated [3–6] by partial protein sequencing and cDNA cloning. The structures and organization of human rod PDE β [7] and γ [8] subunit genes were also determined.

Some degenerative processes in mice, dog and human retinas are caused by defects in the genes encoding rod PDE β subunit [9–11]. Studies of the human genes coding for photoreceptor cell proteins at a molecular level might facilitate disclosure of the molecular and genetic defects in the human visual system. Here, we report the nucleotide sequence and organization of the gene encoding the human cone PDEα′.

2. Materials and methods

The human retinal cDNA library in bacteriophage λgt10 was a kind gift of Dr. J. Nathans (Johns Hopkins University). The human genomic library in λFIX was a kind gift of Dr. D. Smirnov (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry). Bovine PDEα′ cDNA was kindly presented by Prof. M. Applebury (Harvard Medical School). The full-length bovine PDEα′ cDNA [6] and its fragments, *Nco*I–*Eco*RI (1–850), *Eco*RI–*Eco*RI (851–1509) and *Eco*RI–*Eco*RI (1510–2947), corresponding to the protein N-terminal, middle and C-terminal regions, respectively, were radiolabelled using the Nick-translation kit (Amersham), then were used for human genomic and cDNA libraries screening. DNA manipulations were carried out by conventional methods [12]. Nucleotide sequences were determined by the standard dideoxynucleotide chain termination method using the Sequenase kit (USB).

3. Results and discussion

Human retinal cDNA library (2×10^6 plaques) was screened with different bovine PDEα′ cDNA fragments. Four independent clones (α′g1, α′g3.1, α′g37 and α′g45) containing fragments of cDNA encoding cone PDEα′ were isolated (Fig. 1). The comparison of the established cDNA nucleotide sequences and of the corresponding sequences of bovine and chicken cone PDEα′ cDNAs [6,13] revealed that the cDNA structure lacks 5′-untranslating region and nucleotides coding for 147 N-terminal amino acids.

Human genomic library (about 1.6×10^6 independent recombinant clones) was screened with the same bovine PDEα′ cDNA probes. Four positive clones (α′f11, α′f21, α′f11.3 and α′f4) were isolated (Fig. 1). The inserts of the clones were characterized by standard restriction endonuclease mapping procedures using *Hin*dIII, *Eco*RI, *Bam*HI and *Sal*I. According to digest analysis the inserts overlapped. The DNA fragments positive in hybridization were subcloned into the plasmid vector pSP65 for further detailed restriction mapping and sequencing.

Fig. 2 outlines the complete primary structure of human cone PDEα′ cDNA deduced from data on the cDNA and genomic clone nucleotide sequencing. Its comparison with bo-

*Corresponding author. Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia. Fax: (7) (095) 310-7007. E-mail: lipkin@lhr.siobc.ras.ru

**The nucleotide sequence presented here has been submitted to the EMBL/GeneBank database under the accession number X94354.

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

SSDI S0014-5793(96)00104-4

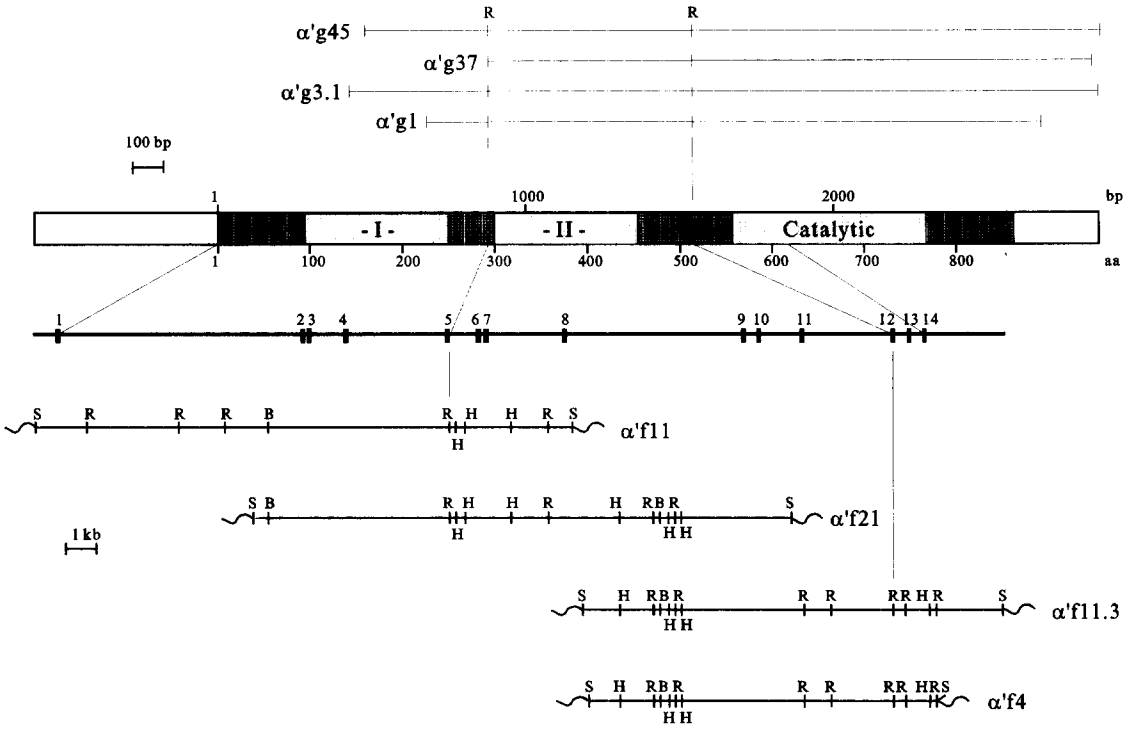

Fig. 1. Location of insertions of isolated cDNA clones in the restriction map of human cone PDEα′ cDNA and physical map and genomic organization of exons 1–14 of human cone PDEα′ gene. α′g45, α′g37, α′g3.1 and α′g1 (above) are cDNA clones. α′f11, α′f21, α′f11.3 and α′f4 (below) are genomic clones. cDNA base numbering: A in initiating ATG refers as the first nucleotide. The cDNA part encoding PDEα′ polypeptide is shaded, putative functional domains (two for non-catalytic cGMP binding, I and II; and one catalytic site for cyclic nucleotides hydrolysis) are indicated. Open boxes represent cDNA 5′ and 3′ untranslated regions. Solid rectangles designate exons from 1st to 14th (out of scale). Abbreviations: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; bp, base pairs; kb, thousand of base pairs; aa, amino acid residues.

Fig. 2. Nucleotide sequence of the cDNA and deduced amino acid sequence of human cone PDEα′. cDNA base numbering as in Fig. 1. The potential TATA-like boxes, CCAAT motifs and polyadenylation signal are underlined. Nucleotides and amino acid residues differing from or absent in earlier published structure [17] (see text) are boxed. Arrow indicates the position of cytosine insertion in [17].

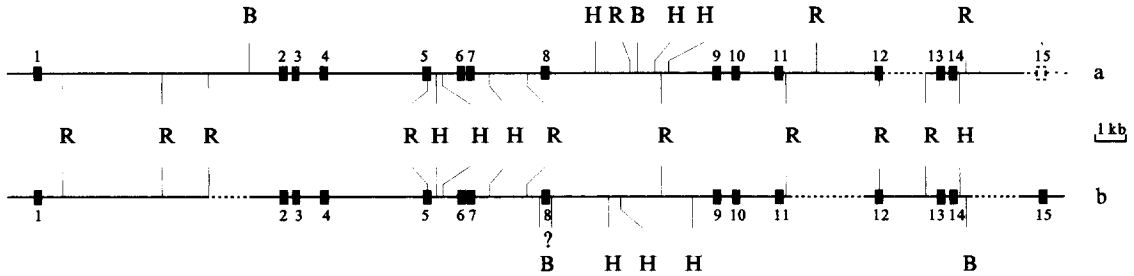

Fig. 3. Alignment of human cone PDEα′ gene physical maps described here (line a) and previously published [17] (line b). All designations are as in Fig. 1. Dashed lines represent gaps introduced to match exons and common restriction sites, which are indicated between the lines. Restriction sites unique for each of the structures are indicated above line a and below line b, respectively.

vine and chicken cone PDEα′ cDNA structures shows that triplet ATG (1–3) is an initiation codon. Two TATA-like sequences (TAAT) are present 232 and 267 bp upstream the translation start. Two potential CCAAT motifs are found 421 and 431 bp upstream this point. Codon 858, specifying leucine, is followed by the termination codon (2575–2577). Polyadenylation signal is found at positions 2742–2747. Thus, human cone PDEα′ amino acid sequence deduced from the cDNA structure consists of 858 residues with calculated molecular mass of 99169 Da. Human cone PDEα′ has 89.1% and 82.7% homology to bovine [6] and chicken [13] cone PDEα′, respectively; 63.8% homology to human rod PDE α subunit [14]; and 64.5% homology to human rod PDE β subunit [7]. Human cone PDEα′, like mammalian visual PDEs, has an internal homologous repeat of the amino acid sequence in its N-terminal part. These regions (residues 94–255 and 300–468, Fig. 1) appear to be the sites of non-catalytic cGMP binding. The catalytic region for cyclic nucleotide hydrolysis lies in the C-terminus of the protein chain (560–783 amino acid residues; Fig. 1) [4]. The last 4 residues at the C-terminus conform to the CAAX consensus motif for multistep post-translational processing covering lipidation, proteolysis and carboxymethylation [15].

Sequence analysis of genomic clones and comparison of the structure obtained with the corresponding sequences of bovine cone PDEα′ cDNA [6] and human rod PDE β-subunit gene [7] indicated the presence of fourteen N-terminal exons and 5′-flanking region. Table 1 presents data available on the exon/intron organization of this 5′ part of human PDEα′ gene. Exon–intron border regions for 13 introns coincide with a splice site canonical sequence and follow the GT/AG rule for exon–intron junctions [16]. Intron 9 5′-splice donor site contains GC instead of GT, but this is not exceptional.

Data on the PDEα′ gene structure have been published by the end of our work [17]. Comparing these two sequences seven point mismatches in exons were identified, four of them resulted in amino acid changes at positions 116 (Asp(GAT) to Val(GTA)), 270 (Thr(ACA) to Ser(TCA)) and 565 (Arg(CGG) to Gln(CAG)), the rest were silent nucleotide replacements (A_{252} for G, G_{1098} for A and A_{2485} for C). Several discrepancies in cDNA 5′ and 3′ untranslated regions were also found (adenosines absent at cDNA positions −551, −603 and −605 in published structure [17]; replacements of C_{-312} for G, A_{-306} for T, G_{-195} for C and T_{2861} for A). Cytosine was not identified between T_{2818} and A_{2819} (summarized in Fig. 2).

In addition, we established the differences in PDEα′ gene physical maps and organization (summarized in Fig. 3). Intron 1 is about 1300 bp longer than previously described, it also contains the site for *Bam*HI absent in [17]. Intron 8 is dramatically dissimilar to the structure published in the restriction site positions and order, although its length agrees with the described one. The absence of the site for *Bam*HI near exon 8 is confirmed by either direct DNA sequencing (about 500 bp upstream and 350 bp downstream exon 8) or restriction mapping of two independent clones (Fig. 1). The same is true for the presence of *Hin*dIII site located 250 bp downstream *Eco*RI, the only common site for both intron 8 structures. Intron 11 is of about 3000 bp in length (250 bp in [17]). Intron 12 of 233 bp in length (2200 bp in [17]) was

Table 1
Exon/intron organization of human cone PDEα′ gene

Exon number	Positions in cDNA[a]	Intron number	Intron size, bp[b]
1	1–480	1	8000
2	481–633	2	106
3	634–723	3	1000
4	724–864	4	3500
5	865–939	5	950
6	940–1004	6	100
7	1005–1071	7	2400
8	1072–1119	8	5500
9	1120–1269	9	500
10	1270–1413	10	1354
11	1414–1482	11	3100
12	1483–1629	12	233
13	1630–1737	13	362
14	1738–1847	14	min. 2500

[a]cDNA base numbering as in Fig. 1.
[b]Intron 2, 6, 10, 12 and 13 sizes were determined by direct sequencing, sizes of other introns were established by restriction mapping.

entirely sequenced. Intron 14 should be no shorter than 2500 bp (if 3′ part of clone α′f11.3 insert is not a result of scrambling). The fragment *Eco*RI–*Sal*I (of about 2200 bp) obtained from this clone lacks exon 15 although intron 14 appeared to be of 1100 bp (this was established in PCR experiment to bridge the gap between the inserts of phage clones available). Revealed differences in human cone PDEα′ gene restriction map as well as above coding sequence mismatches could occur due to genetic polymorphism.

Acknowledgements: The authors thank Dr. T. V. Rakitina for her kind assistance and Dr. N.S. Bystrov for oligonucleotides synthesis. The research described in this publication was made possible in part by Grants MU5000 and MU5300 from the International Science Foundation. This research was also supported by the Russian Fundamental Research Fund (project code 93-04-7310). The research of V. Zagranichny was supported in part by an International Research Scholar's award from the Howard Hughes Medical Institute.

References

[1] Stryer, L. (1986) Annu. Rev. Neurosci. 9, 87–119.
[2] Fung, B.K.-K., Hurley, J.B. and Stryer, L. (1981) Proc. Natl. Acad. Sci. USA 78, 152–156.
[3] Ovchinnikov, Yu.A., Gubanov, V.V., Khramtsov, N.V., Ishchenko, K.A., Zagranichny, V.E., Muradov, K.G., Shuvaeva, T.M. and Lipkin, V.M. (1987) FEBS Lett. 223, 169–173.
[4] Lipkin, V.M., Khramtsov, N.V., Vasilevskaya, I.A., Atabekova, N.V., Muradov, K.G., Gubanov, V.V., Li, T., Johnston, J.P., Volpp, K.J. and Applebury, M.L. (1990) J. Biol. Chem. 265, 12955–12959.
[5] Ovchinnikov, Yu.A., Lipkin, V.M., Kumarev, V.P., Gubanov, V.V., Khramtsov, N.V., Akhmedov, N.B., Zagranichny, V.E. and Muradov, K.G. (1986) FEBS Lett. 204, 288–292.
[6] Gillespie, P.G. and Beavo, J.A. (1988) J. Biol. Chem. 263, 8133–8141.
[7] Khramtsov, N.V., Feshchenko, E.A., Suslova, V.A., Shmukler, B.E., Terpugov, B.E., Rakitina, T.V., Atabekova, N.V. and Lipkin, V.M. (1993) FEBS Lett. 327, 275–278.
[8] Piriev, N.I., Khramtsov, N.V. and Lipkin, V.M. (1994) Gene 151, 297–301.
[9] McLaughlin, M.E., Sandberg, M.A., Berson, E.L. and Dryja, T.P. (1993) Nature Genet. 4, 130–134.
[10] Suber, M.L., Pittler, S.J., Qin, N., Wright, G.C., Holcombe, V., Lee, R.H., Craft C.M., Lolley, R.N., Baehr, W. and Hurwitz, R.L. (1993) Proc. Natl. Acad. Sci. USA 90, 3968–3972.
[11] Bowes, C., Li, T., Danciger, M., Baxter, L.C., Applebury, M.L. and Farber, D.B. (1990) Nature 347, 677–680.
[12] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular cloning. A laboratory manual. N.-Y.: Cold Spring Harbor.
[13] Semple-Rowland, S.L. and Green, D.A. (1994) Exp. Eye Res. 59, 365–372.
[14] Pittler, S.J., Baehr, W., Wasmuth, J.J., McConnell, D.G., Champagne, M.S., VanTuinen, P., Ledbetter, D. and Davis, R.L. (1990) Genomics 6, 272–283.
[15] Maltese, W.A. (1990) FASEB J. 4, 3319–3328.
[16] Sharp, P.A. (1981) Cell 23, 643–646.
[17] Piriev, N.I., Viczian, A.S., Ye, J., Kerner, B., Korenberg, J.R. and Farber, D.B. (1995) Genomics 28, 429–435.