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γ-Subunit of mouse retinal cyclic-GMP phosphodiesterase: cDNA and corresponding amino acid sequence

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The cDNA nucleotide and corresponding amino acid sequences of the γ-subunit of cyclic-GMP phosphodiesterase (cGMP-PDE$_\gamma$) from mouse retina have been determined. The cDNA translated region was found to be 91.5% homologous to the cDNA coding region for the enzyme from bovine retina [(1986) FEBS Lett. 204, 288–292]. On Northern blots of normal mouse retinal RNAs this cDNA hybridized the cGMP-PDE$_\gamma$ mRNA which is 900 bp long. The mouse γ-subunit contains 87 amino acid residues which share 97.7% homology with the bovine polypeptide [(1986) FEBS Lett. 204, 288–292]. Only two amino acids have been changed, Ala 8 to Gly and Met 17 to Ile.

Cyclic-GMP phosphodiesterase: cDNA cloning; Nucleotide sequence; Amino acid sequence; (Mouse retina)

1. INTRODUCTION

Cyclic-GMP phosphodiesterase (cGMP-PDE, EC 3.1.4.17) is a key enzyme in the phototransduction process of rod photoreceptors [2] and in their normal functioning and health. A deficiency in cGMP-PDE activity has been shown to cause accumulation of cGMP in visual cells of the *rd* mouse [3] which is affected with an inherited retinal disease characterized by blindness. These elevated cGMP levels are associated with degeneration of the *rd* photoreceptors.

cGMP-PDE from bovine retina has been well characterized. It consists of three subunits, the α- (88 kDa) and β- (84 kDa) subunits have catalytic activity, and the γ- (11 kDa) subunit has inhibitory properties [4,5]. The latter is formed by 87 amino acids of which the sequence has been deduced from the cDNA sequence recently established by Ovchinnikov et al. [1]. In contrast to the bovine enzyme, little is known about cGMP-PDE from mouse retina. Since we are interested in determining the molecular abnormality underlying the defect in cGMP-PDE activity in the *rd* mouse, and this could result from changes in the composition/structure of the α/β-subunits or from an excess of γ-subunit, we started by cloning the cGMP-PDE subunits from a normal mouse cDNA library. Comparison of their nucleotide and corresponding amino acid sequences with those of cGMP-PDE subunits of *rd* retinas will hopefully allow us to pinpoint any modifications at the DNA level and to determine whether post-translational changes are responsible for the *rd* mutation. Here, we report the characterization of the cGMP-PDE$_\gamma$ from normal mouse retina.

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y00746

2. MATERIALS AND METHODS

Normal C57Bl/6N (+/+) mice, 3–4 weeks old, were obtained from Simonson (Gilroy, CA). Retinas were dissected quickly after killing and frozen on dry ice.

Total RNA was isolated from frozen retinas using the method

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of Chirgwin et al. [6] with some modifications. Retinas were homogenized in 4 M guanidinium thiocyanate and centrifuged for 5 min at 10000 rpm in an Eppendorf microfuge at room temperature. The supernatant was layered on an equal volume of 5.7 M CsCl and ultracentrifuged in a Beckman table-top TL-100 centrifuge using a TLS-55 rotor at 30000 rpm for 6 h at 20°C. The resulting RNA pellet was dissolved in 10 mM Tris-EDTA buffer, pH 7.5, and precipitated with ethanol.

A mouse retinal cDNA library was made from total retinal RNA using bacteriophage cloning vector λgt10. The method used was adapted from that of Gubler and Hoffman [7] and followed the steps described by Davis et al. [8]. About 50000 recombinant clones were screened with a (γ-^{32}P)-5′-end-labeled 30-mer probe, which corresponds to nucleotides 91–120 of the cDNA sequence of cGMP-PDE$_\gamma$ [1]. This oligonucleotide probe was synthesized by the phosphoramidite method on an Applied Biosystems 380A DNA synthesizer [9] and was purified by polyacrylamide gel electrophoresis [10] and HPLC [11]. A positive clone was plaque-purified and its DNA was isolated and digested with *Eco*RI. The inserted DNA was purified by electrophoresis and subcloned into the *Eco*RI site of M13mp18. This cDNA was sequenced by the standard dideoxy method using [α-^{35}S]thio-dATP and Sequenase as described in the protocol booklet Sequenase (US Biochemicals).

Mouse retinal RNA obtained from 3–4-week-old animals was denatured in formamide, electrophoresed on a 1.5% agarose-formaldehyde gel [12] and transferred to a nylon membrane [13]. The Northern blot was hybridized with (α-^{32}P)-labeled cGMP-PDE$_\gamma$ cDNA (0.42 kb) isolated from the mouse retinal library in a solution containing 6 × SSC, 4 × Denhardt's solution, salmon sperm DNA (300 μg/ml), 40 mM Tris-HCl (pH 7.5) and 0.2% SDS at 60°C for 16–20 h. After hybridization the blot was washed twice for 20 min each in 2 × SSC + 0.1% SDS at 57°C, twice for 20 min each in 0.3 × SSC + 0.1% SDS at 55°C and once with 0.1 × SSC + 0.1% SDS at room temperature. Finally, the Northern blot was exposed at −80°C to Kodak XAR-5 film with an intensifying screen (DuPont Cronex Lighting Plus). Determination of the size of hybridized RNAs was made by comparison with the migration of eukaryotic ribosomal RNAs (28,18 S) in the same gel and also to an RNA ladder (BRL) consisting of 1.77, 1.52, 1.28, 0.78, 0.53, 0.40, 0.28 and 0.16 kb RNAs.

3. RESULTS AND DISCUSSION

Analysis of 50000 recombinants from a normal mouse retinal cDNA library after hybridization with the (γ-^{32}P)-labeled 30-mer probe synthesized for the purpose of isolating the γ-subunit of cGMP-PDE revealed a positive clone which was found to have a 0.48 kb cGMP-PDE$_\gamma$ insert. The complete nucleotide sequence of the isolated cDNA fragment containing 482 bp is shown in fig.1. This sequence was confirmed by analyzing each of the strands of the cDNA. We sequenced at least three M13 clones with each of the two possible DNA orientations. Our cDNA fragment has the complete coding moiety of the gene for cGMP-PDE$_\gamma$ (261 bp) and 121 bp of the 5′- and 100 bp of the 3′-untranslated regions, respectively. Although the bovine cGMP-PDE$_\gamma$ cDNA reported by Ovchinnikov et al. [1] has the same number of base

```
    -121
     CCAGATCTCAGGAAGCCACAGCGCCGGTTATCTGTCCAGTGCTTGCCTGCATGAGGAC
                                                            -1
ACCAGCCCAGCCTGACAGAGTCCAGAAGCTAAGGGTCACTGCAGTGTCTCTGCCAGCCTCACC

ATG AAC CTG GAG CCA CCC AAG GGT GAG ATT CGG TCA GCC ACC CGG GTG      1-48
Met Asn Leu Glu Pro Pro Lys Gly Glu Ile Arg Ser Ala Thr Arg Val      1-16

ATA GGA GGA CCA GTC ACC CCC AGG AAA GGA CCA CCT AAG TTT AAG CAG     49-96
Ile Gly Gly Pro Val Thr Pro Arg Lys Gly Pro Pro Lys Phe Lys Gln     17-32

CGG CAA ACC AGG CAG TTC AAG AGC AAG CCC CCC AAG AAA GGC GTG CAA     97-144
Arg Gln Thr Arg Gln Phe Lys ser Lys Pro Pro Lys Lys Gly Val Gln     33-48

GGG TTT GGG GAT GAC ATC CCT GGA ATG GAA GGC CTG GGG ACA GAT ATC    145-192
Gly Phe Gly Asp Asp Ile Pro Gly Met Glu Gly Leu Gly Thr Asp Ile     49-64

ACC GTC ATC TGC CCT TGG GAG GCC TTC AAT CAC CTA GAG CTG CAC GAG    193-240
Thr Val Ile Cys Pro Trp Glu Ala Phe Asn His Leu Glu Leu His Glu     65-80

CTG GCC CAG TAT GGC ATC ATT TAG TCAGATCCCTGCTATGTGAGCCCTGGGAAGA    241-295
Leu Ala Gln Tyr Gly Ile Ile TER                                    81-87

AACCTGCTGAAGACTCCCTCCCCCCTCTGCCAACCCGTGGAATTGTAATATGGTTAAGCTGTT    296-358

CTT                                                                359-361
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Fig.1. Nucleotide sequence of the γ-subunit of cGMP-PDE from mouse retina and the corresponding amino acid sequence of the protein.

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Bovine:   1  ................GCGTGAGGGAGTCCAGAAGCTGAAGGTCACTGC   33
                              ** ***  ************ * *********
Mouse:   51  ATGAGGACACCAGCCCAGCCTGACAGAGTCCAGAAGCTAAGGGTCACTGC  100

         34  GGGATCTCTGCCAACCTGGCCATGAACCTGGAGCCACCCAAGGCCGAGAT   83
              * ********* ***  ************************  *****
        101  AGTGTCTCTGCCAGCCTCACCATGAACCTGGAGCCACCCAAGGGTGAGAT  150

         84  CCGGTCGGCCACCAGGGTGATGGGGGGACCCGTCACTCCCAGGAAAGGGC  133
              ***** ****** ******* ** ***** ***** ********** *
        151  TCGGTCAGCCACCCGGGTGATAGGAGGACCAGTCACCCCCAGGAAAGGAC  200

        134  CCCCGAAATTTAAGCAGCGGCAAACCAGGCAGTTCAAGAGCAAGCCCCCC  183
             * ** ** ******************************************
        201  CACCTAAGTTTAAGCAGCGGCAAACCAGGCAGTTCAAGAGCAAGCCCCCC  250

        184  AAGAAAGGTGTCCAAGGGTTTGGTGATGACATCCCTGGAATGGAAGGCCT  233
             ******** ** ********** ************************
        251  AAGAAAGGCGTGCAAGGGTTTGGGGATGACATCCCTGGAATGGAAGGCCT  300

        234  GGGAACAGACATCACCGTCATCTGCCCGTGGGAGGCCTTCAACCACCTGG  283
             *** ***** ***************** ************** ***** *
        301  GGGGACAGATATCACCGTCATCTGCCCTTGGGAGGCCTTCAATCACCTAG  350

        284  AGCTGCACGAGCTGGCCCAGTACGGCATCATCTAGCCCTGGACCCCCGCC  333
             ********************** ******** *** *   ** *** **
        351  AGCTGCACGAGCTGGCCCAGTATGGCATCATTTAGTC..AGATCCCTGCT  398

        334  CTCAGCCCCTCTACTCCGCTGCCCACCCTGACCCCCTGCTCAAGATTCCT  383
              * *  **                      *** **** * *   * **
        399  ATGTGAGCCCTGGGAAGAAACCTGCTGAAGACTCCCTCCCCCCTCTGCCA  448

        384  GTGAGGAGAGCTGTGCCCCGGGAGGTCCAGAGTGTCTGGATTGTGTCTGG  433
                * **  ***     **     *     *
        449  ACCCGTGGAATTGTAATATGGTTAAGCTGTTCTT.............. 482
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Fig.2. Nucleotide sequences of cGMP-PDE$_\gamma$ cDNAs from mouse and bovine retinas. The symbol (*) shows homology. The initiation codons ATG and the termination codons TAG are underlined.

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Bovine: Met-Asn-Leu-Glu-Pro-Pro-Lys-Ala-Glu-Ile-Arg-Ser-Ala-Thr-Arg  1-15
         *   *   *   *   *   *   *       *   *   *   *   *   *   *
Mouse:  Met-Asn-Leu-Glu-Pro-Pro-Lys-Gly-Glu-Ile-Arg-Ser-Ala-Thr-Arg  1-15

        Val-Met-Gly-Gly-Pro-Val-Thr-Pro-Arg-Lys-Gly-Pro-Pro-Lys-Phe  16-30
         *       *   *   *   *   *   *   *   *   *   *   *   *   *
        Val-Ile-Gly-Gly-Pro-Val-Thr-Pro-Arg-Lys-Gly-Pro-Pro-Lys-Phe  16-30

        Lys-Gln-Arg-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys  31-45
         *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
        Lys-Gln-Arg-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys  31-45

        Gly-Val-Gln-Gly-Phe-Gly-Asp-Asp-Ilu-Pro-Gly-Met-Glu-Gly-Leu  46-60
         *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
        Gly-Val-Gln-Gly-Phe-Gly-Asp-Asp-Ilu-Pro-Gly-Met-Glu-Gly-Leu  46-60

        Gly-Thr-Asp-Ilu-Thr-Val-Ilu-Cys-Pro-Trp-Glu-Ala-Phe-Asn-His  61-75
         *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
        Gly-Thr-Asp-Ilu-Thr-Val-Ilu-Cys-Pro-Trp-Glu-Ala-Phe-Asn-His  61-75

        Leu-Glu-Leu-His-Glu-Leu-Ala-Gln-Tyr-Gly-Ile-Ile TER          76-87
         *   *   *   *   *   *   *   *   *   *   *   *
        Leu-Glu-Leu-His-Glu-Leu-Ala-Gln-Tyr-Gly-Ile-Ile TER          76-87
```

Fig.3. Amino acid sequences of cGMP-PDE$_\gamma$ from mouse and bovine retinas. Asterisks indicate homology.

pairs in its coding region, the 5′-untranslated region is shorter (54 bp) and the 3′-end is longer (518 bp). The termination codon TAG of the mouse cDNA is in positions 262–264 as in the bovine cDNA [1]. We have not found the polyadenylation signal in the 3′-untranslated sequence because our clone does not have the entire 3′-untranslated region.

The deduced amino acid sequence of cGMP-PDE$_\gamma$ is also shown in fig.1. The polypeptide chain of the mouse retinal enzyme has the same number of amino acids as the bovine γ-subunit [1]. The 87 amino acids correspond to a molecular mass of 9.7 kDa and have the following composition: Asp, 3; Asn, 2; Thr, 5; Ser, 2; Glu, 6; Gln, 5; Pro, 10; Gly, 11; Ala, 3; Cys, 1; Val, 4; Met, 2; Ile, 7; Leu, 5; Tyr, 1; Phe, 4; His, 2; Lys, 8; Arg, 5; Trp, 1. The mouse cGMP-PDE γ-subunit is a basic protein. Ten of the basic amino acid residues are present in the polypeptide chain region corresponding to positions 24–45 which has been previously shown to be important for cGMP-PDE$_\gamma$ inhibitory activity [14]. Comparison of the nucleotide sequences of mouse and bovine [1] cGMP-PDE$_\gamma$ subunit is outlined in fig.2. There is 91.75% homology in the nucleotides of the coding regions. When a similar comparison is made between the deduced amino acid sequences of mouse and bovine [1] cGMP-PDE$_\gamma$, as shown in fig.3, the amino acid homology is 97.7%. Only two amino acids at positions 8 and 17 (fig.3) were found to be different in the mouse and bovine enzymes. In the mouse protein these amino acids are Gly 8 and Ile 17, while in the bovine protein they are Ala 8 and Met 17. This shows that cGMP-PDE$_\gamma$ is a highly conserved protein.

We used our 0.48 kb cGMP-PDE$_\gamma$ cDNA as a probe to obtain the mRNA that codes for the enzyme of mouse retina. After (γ-^{32}P) labeling of the probe we hybridized it to the Northern blot of mouse retinal total RNA. A very intense band of about 0.90 kb was detected (fig.4). However, two other bands of about 3.5 and 5.0 kb with very low intensity were also observed. These bands remained even after washing the blot at a stringency of 0.2 × SSC + 0.1% SDS at 60°C (fig.4) and may be either stable nuclear precursors of the γ-subunit of cGMP-PDE mRNA or other mRNAs coding for retinal proteins which have some homology to cGMP-PDE$_\gamma$.

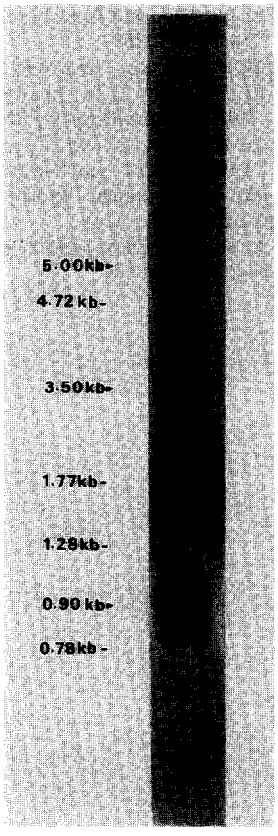

Fig.4. mRNA hybridization to the γ-subunit of cGMP-PDE cDNA. Mouse (3–4-week-old) total retina RNA (15 μg) was denatured, electrophoresed, transferred to a nylon membrane, hybridized to a (α-^{32}P)-labeled cGMP-PDE$_\gamma$ cDNA probe (0.48 kb) and washed as described in section 2.

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