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Cyclic GMP phosphodiesterase from bovine retina

Amino acid sequence of the α-subunit and nucleotide sequence of the corresponding cDNA

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The α-subunit primary structure of cyclic GMP phosphodiesterase has been determined by parallel analysis of the protein amino acid sequence and the corresponding cDNA nucleotide sequence. The enzyme α-subunit contains 858 amino acid residues, its N-terminal amino group being acetylated. The partial primary structure of the enzyme β-subunit has also been elucidated. A significant homology has been found between the α- and β-subunits of cGMP phosphodiesterase.

Cyclic GMP phosphodiesterase; Amino acid sequence; cDNA cloning; Nucleotide sequence; (Bovine retina)

1. INTRODUCTION

Cyclic GMP phosphodiesterase (PDE) as well as rhodopsin and transducin participates in transduction and amplification of the visual signal [1]. PDE from bovine retina consists of three subunits: α, β and γ, with molecular masses of 88, 84 and 10 kDa, respectively [2]. The enzyme catalytical subunits are α and β, the γ-subunit being an internal inhibitor of the protein enzymatic activity [3,4].

The protein amino acid sequence and the corresponding cDNA nucleotide sequence were analyzed in parallel to solve the enzyme γ-subunit primary structure [5]. The structure of large subunits was studied similarly.

The present paper describes determination of the amino acid sequence of PDE α-subunit (preliminary results published in [6]).

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00699

2. MATERIALS AND METHODS

The enzyme was isolated, and the γ-subunit was separated from α- and β-subunits as described in [5]. The mixture of α- and β-subunits was hydrolyzed with cyanogen bromide. The fragments obtained were fractionated by gel-filtration (Toyopearl HW-40 in 10% HCOOH). 10 homogeneous peptides were isolated from the low-molecular-mass fractions by HPLC on a reverse-phase column (Ultrasphere ODS, acetonitrile gradient in trifluoroacetic acid). The mixture of high-molecular-mass fragments was additionally digested with trypsin. Then the peptides were fractionated by gel-filtration and separated by HPLC

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on Ultrasphere ODS. Their amino acid sequence was established according to [5].

cDNA clone libraries in vector pUC 8 were produced by a poly(A)$^+$ fraction of RNA from bovine retina as described in [5]. To initiate synthesis of the first cDNA chain use was made of: (i) oligo(dT)$_{12-18}$; (ii) specific oligodeoxyribonucleotide probes; (iii) statistic mixture of primers (DNA hydrolysate with an average size of 10–20 nucleotides).

Oligodeoxyribonucleotide probes were synthesized by the phosphoramidite method on an Applied Biosystems synthesizer. The nucleotide sequence of DNA fragments was determined according to Maxam and Gilbert [7] and Sanger et al. [8]. The strategy of determining the nucleotide sequence was analogous to that used in [9].

3. RESULTS AND DISCUSSION

The attempts to elaborate a preparative method for separating α- and β-subunits failed due to similarity of their physicochemical properties. To study the primary structure a mixture of these subunits was hydrolyzed with cyanogen bromide and trypsin. Amino acid sequences of the peptides underlay in synthesizing a number of nucleotide probes. The former were used for screening the bovine retina cDNA clone library obtained by priming the first cDNA chain with oligo(dT). The screening with probe I (5′-CATGAGGGTCTCGT CCAT-3′) corresponding to cyanogen bromide peptide Asp-Glu-Thr-Leu-Hse allowed identification of three clones: pα55, pα154 and pα225 (fig.1).

The α- and β-subunits were analytically separated by SDS-electrophoresis in polyacrylamide gel according to Laemmli to determine the PDE subunit to which the nucleotide cDNA sequence of the isolated clones corresponded. The subunits were cleaved directly with cyanogen bromide in gel strips without pre-elution. Two peptides whose amino acid compositions and N-terminal residues corresponded exactly to sequences Asp-Glu-Thr-Leu-Met and Asn-Lys-Leu-Glu-Asn-Arg-Lys-Asp-Ile-Phe-Gln-Asp-Met, deduced from the nucleotide sequence of the fragments of cDNA clones pα55, pα154 and pα225, were isolated by HPLC from the α-subunit hydrolysate. These peptides were not found in the β-subunit hydrolysate. Thus the cDNA of the isolated clones encoded the PDE α-subunit.

To isolate clones containing cDNA of the α-subunit N-terminal part, a cDNA clone library was created using unique oligodeoxyribonucleotide probes I, II and III for priming cDNA synthesis, their structures corresponding to the 5′-terminal DNA region of clone pα154 (fig.1).

Analysis of 100000 recombinants revealed a great number of clones yielding a positive hybridization signal with probes II and III. Clones pα17 and pα40 were primarily chosen, but determination of their structure showed that neither of

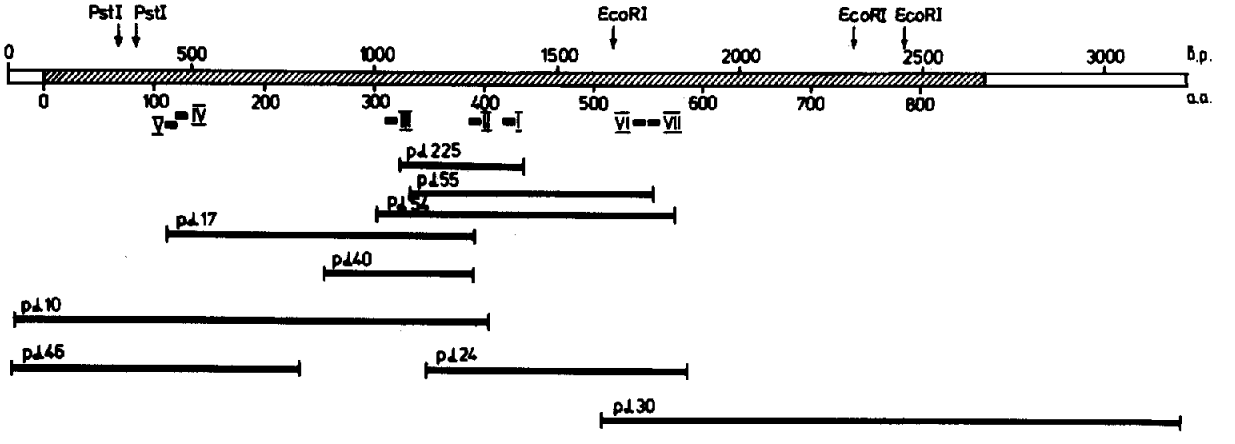

Fig.1. Location of cDNA fragments of the isolated clones in the restriction map of the PDE α-subunit cDNA. The cDNA part encoding the α-subunit is shaded. Black rectangles indicate nucleotide probes used in cDNA cloning.

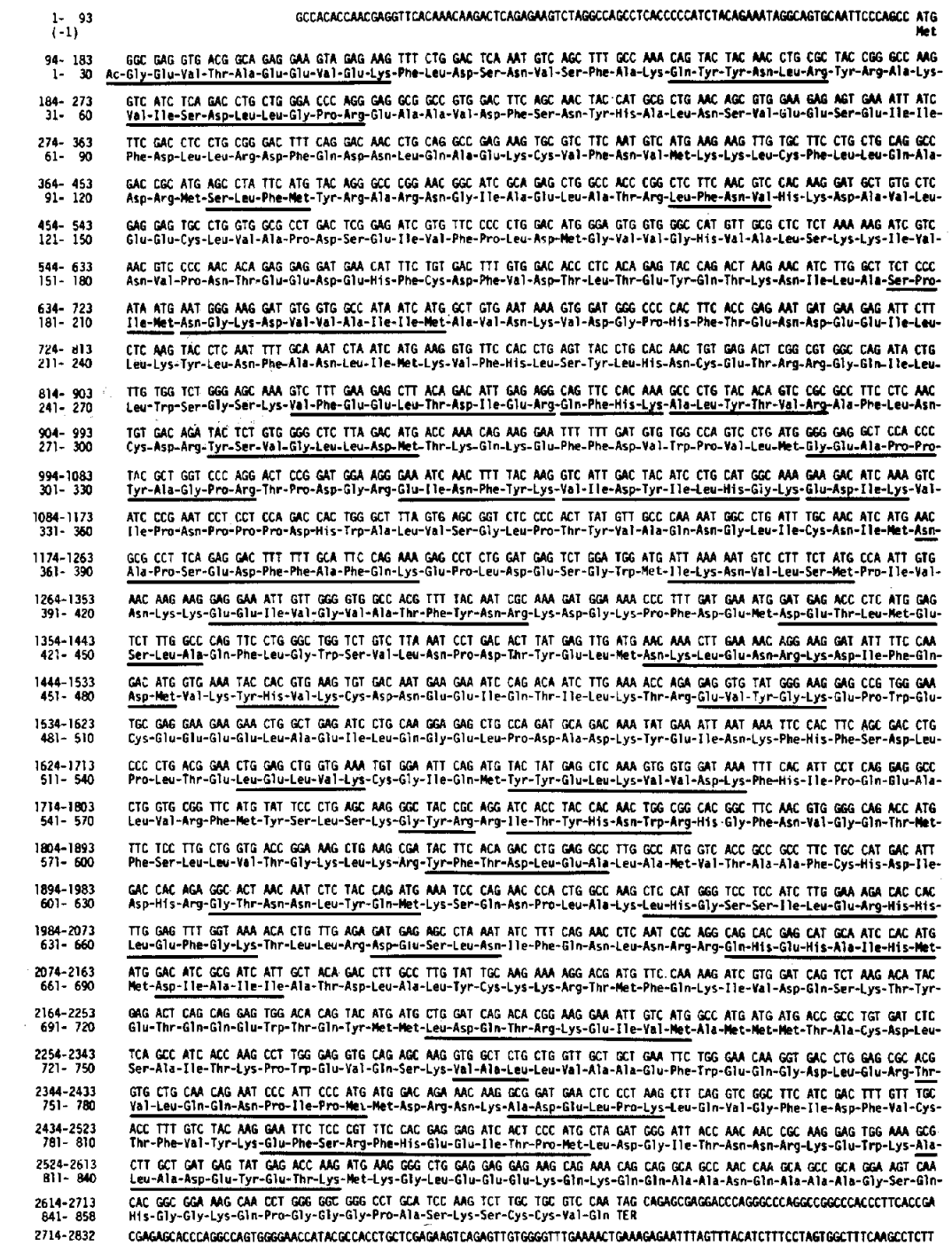

Fig.2. Nucleotide sequence of cDNA encoding the α-subunit of cyclic GMP phosphodiesterase from bovine retina, and the corresponding protein amino acid sequence. Amino acid sequences established by the α-subunit peptide analysis are underlined.

them contained a DNA region coding for the α-subunit N-terminal sequence. In search of such clones the cDNA 5'-region of clone pα17 structure was used for synthesizing two new oligodeoxyribonucleotide probes: IV and V (fig.1). These were utilized to screen a specific cDNA clone library. As a result, clones pα10, pα14 and pα46 were identified with the cDNA fragments sought.

To find the clones encoding the α-subunit C-terminal part use was made of probes VI and VII to screen the clone library created by statistic priming of the cDNA synthesis. Clones pα24 and pα30 were isolated (fig.1). Determination of the nucleotide sequence of the isolated clones allowed the reconstitution of the α-subunit cDNA sequence (3201 bp) (fig.2).

Triplet ATG (91–93) is the initiation codon of the PDE α-subunit, since on the one hand, it is the first ATG-codon located within the translation frame behind terminator TAG (70–72) and, on the other, it is a constituent of the PuCCATG sequence typical of initiation sites [10].

A peptide with a blocked N-terminal amino group was isolated from a tryptic hydrolysate.

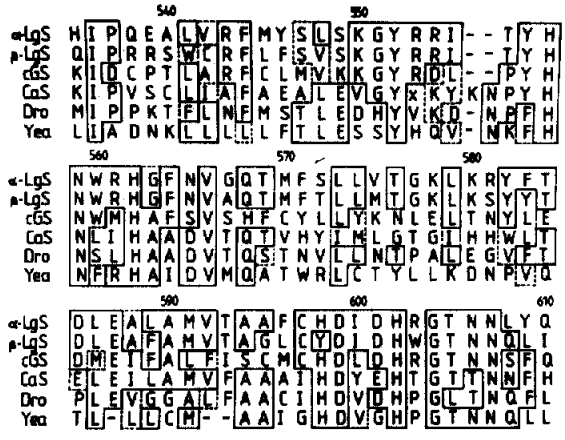

Fig.3. Homologous segments of the amino acid sequence of the cyclic nucleotide phosphodiesterases. α-LgS and β-LgS, α- and β-subunits of the light-dependent cyclic GMP phosphodiesterase from bovine retina; cGS, cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine heart; CaS, Ca^{2+}/calmodulin stimulated cyclic nucleotide phosphodiesterase from bovine brain; Dro, cAMP-specific phosphodiesterase from *Drosophila melanogaster*; Yea, cyclic nucleotide phosphodiesterase from yeast (*Saccharomyces cerevisiae*).

Amino acid and mass spectrometry analyses showed the peptide to correspond to the N-terminal peptide (1–10, fig.2) of the α-subunit, with the N-terminal amino group being acetylated. Thus, upon α-subunit post-translation modification the methionine N-terminal residue is cleaved (-1, fig.2) and the glycine residue next to it is acetylated. The PDE α-subunit amino acid sequence deduced from the cDNA nucleotide sequence contains 858 amino acid residues (fig.2) with a molecular mass of 99 261 Da. The structure is in perfect accord with the amino acid sequences of a large number of peptides from cyanogen bromide and tryptic hydrolysates.

Comparison of cDNA structures of the clones revealed some point differences, that sometimes led to distinctions in the amino acid sequences. For example, there were 4 clones isolated coding for methionine in position 380 and 2 for valine.

A series of clones encoding the β-subunit of the enzyme was also isolated from the clone libraries. Their structural analysis made it possible to establish over 70% of the protein amino acid sequence.

The amino acid sequences of these two subunits display close homology that, apparently, testifies to the existence of a common precursor.

Comparison of the α-subunit amino acid sequence from bovine retina with the known partial amino acid sequences of cyclic nucleotide phosphodiesterases from other sources [11] revealed close homology between the segments of the peptide chain of the α-subunit (535–671) and (719–778) adjoining the C-terminal and analogous segments of other phosphodiesterases located in different parts of polypeptide chains (fig.3). So the catalytical center in the α-subunit of cGMF phosphodiesterase is presumed to be located in the C-terminus of the molecule, while regulatory regions interacting with the γ-subunit of the enzyme and transducin are likely to be in the N-terminal part.

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