

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]).

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

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: α 55, α 154 and α 225 (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 α 55, α 154 and α 225, 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 α 154 (fig.1).

Analysis of 100 000 recombinants revealed a great number of clones yielding a positive hybridization signal with probes II and III. Clones α 17 and α 40 were primarily chosen, but determination of their structure showed that neither of

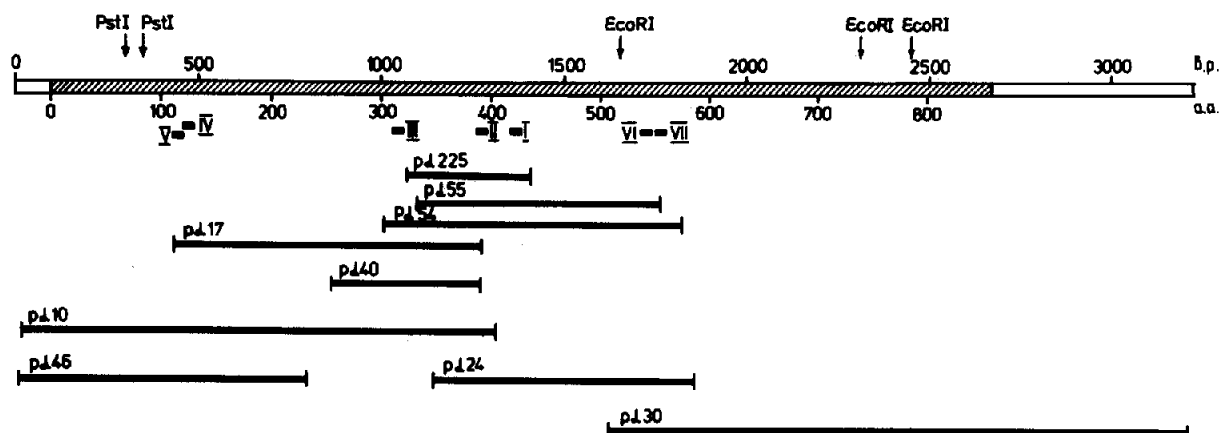


Fig.1. Location of cDNA fragments of the isolated clones in the restriction map of the PDE α -subunit cDNA. The cDNA part encoding the α -subunit is shaded. Black rectangles indicate nucleotide probes used in cDNA cloning.

1- 93 GCCACCAACGAGGTTCACAAACAGACTCAGAGAAGTCTAGGCCAGCTCACCCTCATCTACAGAAATAGGCATTCACAGCC ATG
 (-1) Met
 94- 183 GGC GAG GTG ACG GCA GAG GAA GTA GAG AAG TTT CTG GAC TCA AAT GTC AGC TTT GCC AAA CAG TAC TAC AAC CTG CGC TAC CGG GCC AAG
 1- 30 Ac-Gly-Glu-Val-Thr-Ala-Glu-Glu-Val-Glu-Lys-Phe-Leu-Asp-Ser-Asn-Val-Ser-Phe-Ala-Lys-Gln-Tyr-Tyr-Asn-Leu-Arg-Tyr-Arg-Ala-Lys-
 184- 273 GTC ATC TCA GAC CTG CTG GGA CCC AGG GAG GCG GCC GTG GAC TTC AGC AAC TAC CAT GCG CTG AAC AGC GTG GAA GAG AGT GAA ATT ATC
 31- 60 Val-Ile-Ser-Asp-Leu-Leu-Gly-Pro-Arg-Glu-Ala-Ala-Val-Asp-Phe-Ser-Asn-Tyr-His-Ala-Leu-Asn-Ser-Val-Glu-Glu-Ser-Glu-Ile-Ile-
 274- 363 TTC GAC CTC CTG CGG GAC TTT CAG GAC AAC CTG CAG GCC GAG AAG TGC GTC TTC AAT GTC ATG AAG TGG TGC TTC CTG CAG GCC
 61- 90 Phe-Asp-Leu-Leu-Arg-Asp-Phe-Gln-Asp-Asn-Leu-Gln-Ala-Glu-Lys-Cys-Val-Phe-Asn-Val-Met-Lys-Lys-Leu-Cys-Phe-Leu-Leu-Gln-Ala-
 364- 453 GAC CGC ATG AGC CTA TTC ATG TAC AGG GCG AAC GGC ATC GCA GAG CTG GCC ACC CGG CTC TTC AAC GTC CAC AAG GAT GCT GTG CTC
 91- 120 Asp-Arg-Met-Ser-Leu-Phe-Met-Tyr-Arg-Ala-Arg-Asn-Gly-Ile-Ala-Glu-Leu-Ala-Thr-Arg-Leu-Phe-Asn-Val-His-Lys-Asp-Ala-Val-Leu-
 454- 543 GAG GAG TGC CTG GTG GCG CCT GAC TCG GAG ATC GTG TTC CCC CTG GAC ATG GGA GTG GTG GGC CAT GTT GCG CTC TCT AAA AAG ATC GTC
 121- 150 Glu-Glu-Cys-Leu-Val-Ala-Pro-Asp-Ser-Glu-Ile-Val-Phe-Pro-Leu-Asp-Met-Gly-Val-Val-Gly-His-Val-Ala-Leu-Ser-Lys-Lys-Ile-Val-
 544- 633 AAC GTC CCC AAC ACA GAG GAG GAT GAA CAT TTC TGT GAC TTT GTG GAC ACC CTC ACA GAG TAC CAG ACT AAG AAC ATC TTG GCT TCT CCC
 151- 180 Asn-Val-Pro-Asn-Glu-Glu-Glu-Asp-Glu-His-Phe-Cys-Asp-Phe-Val-Asp-Thr-Leu-Thr-Glu-Tyr-Gln-Thr-Asn-Ile-Leu-Ala-Ser-Pro-
 634- 723 ATA ATG AAT GGG AAG GAT GTG GTG GCC ATA ATC ATG GCT GTG AAT AAA GTG GAT GGG CCC CAC TTC ACC GAG AAT GAT GAA GAG ATT CTT
 181- 210 Ile-Met-Asn-Gly-Lys-Asp-Val-Val-Ala-Ile-Ile-Met-Ala-Val-Asn-Lys-Val-Asp-Gly-Pro-His-Phe-Thr-Glu-Asn-Asp-Glu-Glu-Ile-Leu-
 724- 813 CTC AAG TAC CTC AAT TTT GCA AAT CTA ATC ATG AAG GTG TTC CAC CTG AGT TAC CTG CAC AAC TGT GAG ACT CGG CGT GGC CAG ATA CTG
 211- 240 Lys-Lys-Tyr-Lys-Asn-Phe-Ala-Asn-Leu-Ile-Met-Lys-Val-Phe-His-Leu-Ser-Tyr-Leu-His-Asn-Cys-Gln-Leu-Thr-Leu-Arg-Gly-Gln-Ile-Leu-
 814- 903 TTG TGG TCT GGG AGC AAA GTC TTT GAA GAG CTT ACA GAC ATT GAG AGG CAG TTC CAC AAA GCC CTG TAC ACA GTC CGC GCC TTC CTC AAC
 241- 270 Leu-Trp-Ser-Gly-Ser-Lys-Val-Phe-Glu-Glu-Leu-Thr-Asp-Ile-Glu-Arg-Gln-Phe-His-Lys-Ala-Leu-Tyr-Thr-Val-Arg-Ala-Phe-Leu-Asn-
 904- 993 TGT GAC AGA TAC TCT GTG GGG CTC TTA GAC ATG ACC AAA CAG AAG GAA TTT TTT GAT GTG TGG CCA GTC CTG ATG GGG GAG GCT CCA CCC
 271- 300 Cys-Asp-Arg-Tyr-Ser-Val-Gly-Leu-Leu-Asp-Met-Thr-Lys-Gln-Lys-Glu-Phe-Phe-Asp-Val-Trp-Pro-Val-Leu-His-Lys-Phe-His-Ile-Pro-Gln-Glu-Ala-
 994-1083 TAC GCT GGT CCC AGG ACT CGG GAT GGA AGG GAA ATC AAC TTT TAC AAG GTC ATT GAC TAC ATC CTG CAT GGC AAA GAA GAC ATC AAA GTC
 301- 330 Tyr-Ala-Gly-Pro-Arg-Thr-Pro-Asp-Gly-Arg-Glu-Ile-Asn-Phe-Tyr-Lys-Val-Ile-Asp-Tyr-Ile-Leu-His-Gly-Lys-Glu-Asp-Ile-Lys-Val-
 1084-1173 ATC CCG AAT CCT CCT CCA GAC CAC TGG GCT TTA GTG AGC GGT CTC CCC ACT TAT GTT GCC CAA AAT GGC CTG ATT TGC AAC ATC ATG AAC
 331- 360 Ile-Pro-Asn-Pro-Pro-Pro-Asp-His-Trp-Ala-Leu-Val-Ser-Gly-Leu-Pro-Thr-Tyr-Val-Ala-Gln-Asn-Gln-Leu-Ile-Cys-Ala-Leu-Ile-Met-Asn-
 1174-1263 GCG CCT TCA GAG GAC TTT TTT GCA TTC CAG AAA GAG CCT CTG GAT GAG TCT GGA TGG ATG ATT AAA AAT GTC CTT TCT ATG CCA ATT GTG
 361- 390 Ala-Pro-Ser-Glu-Asp-Phe-Phe-Ala-Phe-Gln-Lys-Glu-Pro-Leu-Asp-Glu-Ser-Gly-Trp-Met-Ile-Lys-Asn-Val-Leu-Ser-Met-Pro-Ile-Val-
 1264-1353 AAC AAG AAG GAG GAA ATT GTT GGG GTG GCC AGC TTT TAC AAT CGC AAA GAT GGA AAA CCC TTT GAT GAA ATG GAT GAG ACC CTC ATG GAG
 391- 420 Asp-Lys-Lys-Glu-Glu-Ile-Val-Gly-Val-Ala-Thr-Phe-Tyr-Asn-Arg-Lys-Asp-Gly-Lys-Pro-Phe-Asp-Glu-Lys-Phe-His-Ile-Pro-Gln-Glu-Ala-
 1354-1443 TCT TTG GCC CAG TTC CTG GGC TGG TCT GTC TTA AAT CCT GAC ACT TAT GAG TTG ATG AAC AAA CTT GAA AAC AGG AAG GAT ATT TTC CAA
 421- 450 Ser-Leu-Ala-Gln-Phe-Leu-Gly-Trp-Ser-Val-Leu-Asn-Pro-Asp-Thr-Tyr-Glu-Leu-Met-Asn-Lys-Leu-Glu-Asn-Arg-Lys-Asp-Ile-Phe-Gln-
 1444-1533 GAC ATG GTG AAA TAC CAC GTG AAG TGT GAC AAT GAA GAA ATC CAG ACA ATC TTG AAA ACC AGA GAG GTG TAT GGG AAG GAG CCG TGG GAA
 451- 480 Gly-Met-Val-Lys-Tyr-His-Val-Lys-Cys-Asp-Asn-Gln-Ile-Gln-Thr-Ile-Leu-Lys-Thr-Arg-Glu-Val-Tyr-Gln-Lys-Glu-Pro-Trp-Gln-
 1534-1623 TGC GAG GAA GAA GAA CTG GCT GAG ATC CTG CAA GGA GAG CTG CCA GAT GCA GAC AAA TAT GAA ATT AAT AAA TTC CAC TTC AGC GAC CTG
 481- 510 Cys-Glu-Glu-Glu-Glu-Leu-Ala-Glu-Ile-Leu-Gln-Gly-Glu-Leu-Pro-Asp-Ala-Asp-Lys-Tyr-Glu-Ile-Asn-Lys-Phe-His-Phe-Ser-Asp-Leu-
 1624-1713 CCC CTG ACG GAA CTG GAG CTG GTG AAA TGT GGA ATT CAG ATG TAC TAT GAG CTC AAA GTG GTG GAT AAA TTT CAC ATT CCT CAG GAG GGC
 511- 540 Pro-Leu-Thr-Glu-Leu-Glu-Glu-Leu-Val-Lys-Cys-Gly-Ile-Gln-Met-Tyr-Tyr-Glu-Leu-Lys-Val-Val-Asp-Lys-Phe-His-Ile-Pro-Gln-Glu-Ala-
 1714-1803 CTG GTG CGG TTC ATG TAT TCC CTG AGC AAG GCG TAC CGC AGG ATC ACC TAC CAC AAC TGG CGG CAC GGC TTC AAC GTG GGG CAG ACC ATG
 541- 570 Leu-Val-Arg-Phe-Met-Tyr-Ser-Leu-Ser-Lys-Gly-Tyr-Arg-Arg-Ile-Thr-Tyr-His-Asn-Trp-Arg-His-Gly-Phe-Asn-Val-Gly-Gln-Thr-Met-
 1804-1893 TTC TCG TTG CTG GTG ACC GGA AAG CTG AAG CGA TAC TCA CAC GAC CTG GAG GCG TTG GCG ATG GTC ACC GCG GCG TAT GCA CAT CAC ATG
 571- 600 Phe-Ser-Leu-Leu-Val-Thr-Gly-Lys-Leu-Lys-Arg-Tyr-Phe-Thr-Asp-Leu-Glu-Ala-Leu-Ala-Met-Val-Thr-Ala-Ala-Phe-Cys-His-Asp-Ile-
 1894-1983 GAC CAC AGA GGC ACT AAC AAT CTC TAC CAG ATG AAA TCC CAG AAC CCA CTG GCC AAG CTG CAT GGG TCC TCC ATC TTG GAA AGA CAC CAC
 601- 630 Asp-His-Arg-Phe-Thr-Asn-Asn-Tyr-Gln-Met-Lys-Ser-Gln-Asn-Pro-Leu-Ala-Lys-Leu-His-Gly-Ser-Ser-Ile-Leu-Glu-Arg-His-His-
 1984-2073 TTG GAG TTT GGT AAA ACA CTG TTG AGA GAT GAG AGC CTA AAT ATC TTT CAG AAC CTC AAT CGC AGG CAG CAG CAT GCA CAT CAC ATG
 631- 660 Leu-Glu-Phe-Gly-Lys-Thr-Leu-Leu-Arg-Asp-Glu-Ser-Leu-Asn-Ile-Phe-Gln-Asn-Leu-Asn-Arg-Arg-Gln-His-Glu-His-Ala-Ile-His-Met-
 2074-2163 ATG GAC ATC GCG ATC ATT GCT ACA GAC CTT GCC TTG TAT TGC AAG AAA AGG ACG ATG TTC CAA AAG ATC GTG GAT CAG TCT AAG ACA TAC
 661- 690 Met-Asp-Ile-Ala-Ile-Ile-Ala-Thr-Asp-Leu-Ala-Leu-Tyr-Cys-Lys-Lys-Arg-Thr-Met-Phe-Gln-Lys-Ile-Val-Asp-Gln-Ser-Lys-Thr-Tyr-
 2164-2253 GAG ACT CAG CAG GAG TGG ACA CAG TAC ATG ATG CTG GAT CAG ACA CGG AAG GAA ATT GTC ATG GCC ATG ATG ACC GCC TGT GAT CTC
 691- 720 Glu-Thr-Gln-Gln-Glu-Trp-Thr-Gln-Tyr-Met-Met-Leu-Asp-Gln-Thr-Arg-Lys-Glu-Ile-Val-Met-Ala-Met-Met-Met-Thr-Ala-Cys-Asp-Leu-
 2254-2343 TCA GCC ATC ACC AAG CCT TGG GAG GTG CAG AGC AAG GTG GCT CTG CTG GTT GCT GCT GAA TTC TGG GAA CAA GGT GAC CTG GAG CGC ACG
 721- 750 Ser-Ala-Ile-Thr-Lys-Pro-Trp-Glu-Val-Gln-Ser-Lys-Val-Ala-Leu-Leu-Val-Ala-Ala-Glu-Phe-Trp-Glu-Gln-Gly-Asp-Leu-Glu-Arg-Thr-
 2344-2433 GTG CTG CAA CAG AAT CCC ATT CCC ATG ATG GAC AGA AAC AAG GCG GAT GAA CTC CCT AAG CTT CAG GTC GGC TTC ATC GAC TTT GTT TGC
 751- 780 Val-Leu-Gln-Gln-Asn-Pro-Ile-Pro-Met-Met-Asp-Arg-Asn-Lys-Ala-Asp-Glu-Leu-Pro-Lys-Leu-Gln-Val-Gly-Phe-Ile-Asp-Phe-Val-Cys-
 2434-2523 ACC TTT GTC TAC AAG GAA TTC TCC GGT TTC CAC GAG GAG ATC ACT CCC ATG CTA GAT GGG ATT ACC AAC AAG CGC AAG GAG TGG AAA GCG
 781- 810 Thr-Phe-Val-Tyr-Lys-Glu-Phe-Ser-Arg-Phe-His-Glu-Glu-Ile-Thr-Pro-Met-Leu-Asp-Gly-Ile-Thr-Asn-Asn-Arg-Lys-Glu-Trp-Lys-Ala-
 2524-2613 CTT GCT GAT GAG TAT GAG ACC AAG ATG AAG GGG CTG GAG GAG GAG AAG CAG AAA CAG CAG GCA GCC AAC CAA GCA GCC GCA GGA AGT CAA
 811- 840 Leu-Ala-Asp-Glu-Tyr-Glu-Thr-Lys-Met-Lys-Gly-Leu-Glu-Glu-Glu-Lys-Gln-Gln-Ala-Ala-Asn-Gln-Ala-Ala-Ala-Gly-Ser-Gln-
 2614-2713 CAC GGC GGA AAG CAA CCT GGG GGC GGG CCT GCA TCC AAG TCT TGC GTC CAA TAG CAGAGCGAGGACCCAGGCCCGAGCCGCGCCACCTTCAACGA
 841- 858 His-Gly-Gly-Lys-Gln-Pro-Gly-Gly-Gly-Pro-Ala-Ser-Lys-Ser-Cys-Cys-Val-Gln TER
 2714-2832 CGAGAGCACCAGGCCAGTGGGAACCATACGCCACCTGCTCGAGAAGTCAGAGTTGTGGGTTTGAAGCTGAAGAGAATTATGTTACATCTTCTAGTGGCTTCAAGCCTCTT
 2833-2951 TTTCAAGCTGTAGAGACTTTTATTGAATGACCAACCAATTTTACCTTTCACAGACTCAACATTAGTGCCAGACTCAGCTGCTTACCTAGAATCTTTGTGTTCTAGACTTTTATT
 2952-3070 TATAGTACACAAATATGCAATCATTCACAAAGTAGAAGATTACCAATGAATTTGACTCTACCCATCATGCAGATTCTAATCAGGATTTTGTGCGAATTTGCTGCTGCTGCTT
 3071-3189 TAGAGTTTCTTTTCTTGTAGCTGAATATTTAAAAACAACTCCAGCATATCATTTGACTCTTCATTACGACCCCTAAGATGAATGGGTGCTCTCAACACATCATCATGA
 3190-3201 TCGATGCCACA

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 p α 17 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 p α 10, p α 14 and p α 46 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 p α 24 and p α 30 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.

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 99261 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 catalytic 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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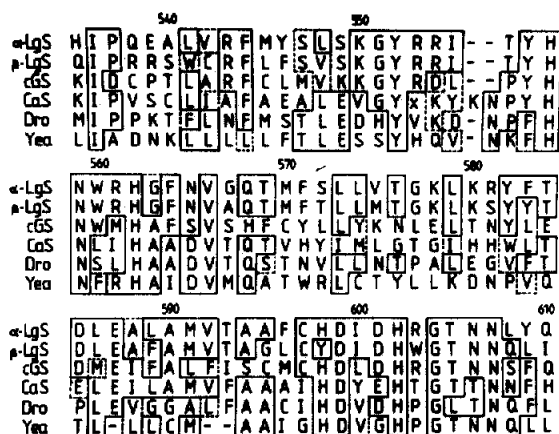


Fig.3. Homologous segments of the amino acid sequence of the cyclic nucleotide phosphodiesterases. α -LgS and β -LgS, α - and β -subunits of the light-dependent cyclic GMP phosphodiesterase from bovine retina; cGS, cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine heart; CaS, Ca^{2+}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

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