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Calmodulin-independent bovine brain adenylate cyclase

Amino acid sequence and nucleotide sequence of the corresponding cDNA

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An individual catalytic component of calmodulin-independent adenylate cyclase has been isolated from bovine brain cortex. Affinity chromatography on an immunosorbent was used. The amino acid sequence of adenylate cyclase as well as the corresponding nucleotide sequence of the cDNA has been determined. cDNA of adenylate cyclase encodes a protein consisting of 834 amino acid residues and the signal peptide (19 amino acid residues). A series of adenylate cyclase isoforms has been found. A homology between adenylate cyclases from bovine brain, *E. coli* and *Bordetella pertussis* has been revealed.

Adenylate cyclase; Monoclonal antibody; Amino acid sequence; cDNA cloning; Nucleotide sequence

1. INTRODUCTION

The adenylate cyclase system of the majority of eukaryotic cells is a membrane enzymatic complex functioning as a transmitter of hormonal signals into the cell. The system includes three components: receptor, GTP-binding proteins and the actual adenylate cyclase or catalytic component [1]. The latter has been poorly characterized thus far due to its instability and the small amounts occurring in tissues.

This work deals with the isolation of calmodulin-independent adenylate cyclase from bovine brain and deciphering of its primary structure by parallel analysis of the amino acid sequence of the protein and nucleotide sequence of the corresponding cDNA (preliminary results in [2]).

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2. MATERIALS AND METHODS

Membranes of brain cortex were isolated according to [3] and membrane proteins were solubilized with 1% Lubrol PX or 0.6% CHAPS. The adenylate cyclase preparation used for immunization was obtained by chromatography on DEAE Sephacel [4]. Monoclonal antibodies were produced as in [5] by using mouse lines BALB/c for immunization [4]. Adenylate cyclase activity was determined according to [6].

Monoclonal antibodies were immobilized on CNBr-Sepharose. Affinity chromatography on immunosorbent was performed as described in [4] and on calmodulin-Sepharose as in [7]. For protein structural studies, adenylate cyclase was additionally purified by SDS-PAGE with subsequent electroelution of the corresponding band [8].

Adenylate cyclase (4 nmol) was digested with lysylendopeptidase from *Achromobacter liticus*. Peptides were fractionated by gel filtration (Toyopearl HW40, 10% acetic acid in 20% acetonitrile) and the following reverse-phase HPLC (Ultrasphere ODS, acetonitrile gradient in 0.1% trifluoroacetic acid). Cloning and hybridization were performed as in [9]. The nucleotide sequence of DNA was determined using the Maxam-Gilbert technique according to a solid-phase modification [10].

3. RESULTS AND DISCUSSION

The adenylate cyclase catalytic component was

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isolated by chromatography of the Lubrol solubilizate of bovine cortex membranes on the immunosorbent [4]. When preparing the immunosorbent, use was made of monoclonal antibody Mab-1 as a ligand. This antibody was obtained upon immunization of mice with the adenylate cyclase preparation partially purified by DEAE-Sephacel chromatography [4]. The antibody inhibits the enzymatic activity of adenylate cyclase and is able to sorb enzyme after immobilization on protein A-Sepharose. The preparation eluted from the immunosorbent contains one major polypeptide (130 ± 10 kDa) of molecular mass corresponding to that of adenylate cyclase [11,12] and two minor components (180 ± 10 and 40 ± 2 kDa). Unfortunately, the eluted adenylate cyclase preparation lost its activity during the course of chromatography on the immunosorbent due to the severe conditions necessary for elution. To prove that the 130 kDa protein represents a catalytic component of the adenylate cyclase system, an antiserum was prepared against this protein electroeluted from polyacrylamide. The resulting polyclonal antibodies effectively inhibit adenylate cyclase activity and after immobilization on protein A-Sepharose are capable of binding the enzyme from solution [4].

It was shown that the monoclonal antibodies Mab-1 bind solely to the form of adenylate cyclase that does not bind to calmodulin-Sepharose. We therefore isolated the calmodulin-independent enzyme form.

Immunoblotting shows that of three proteins (180, 130 and 40 kDa) the two of greater molecular masses cross-react with both the monoclonal antibody and polyclonal antibodies to the 130 kDa protein.

For structural investigations we employed the homogeneous proteins (130 and 180 kDa) obtained from PAG by electroelution. The N-terminal sequences of both polypeptides appeared to be identical: Leu-Gln-Val-Asp-Ile-Val-Pro-Ser-Gln-Gly-Glu-Ile-Ser-Val-...

To cleave the adenylate cyclase polypeptide chain of 130 kDa we applied lysylendopeptidase from *A. liticus*. The hydrolysate was fractionated by gel filtration on Toyopearl HW40 followed by HPLC on Ultrasphere ODS. A total of 18 individual peptides were isolated and the sequence of 228 amino acid residues was elucidated [4]. According to the amino acid sequence of the peptide (Lys)-Trp-Tyr-Asp-Ala-Lys, a mixture of oligodeoxyribonucleotide probes I 5′-AA$^{G}_{A}$TGGTA$^{T}_{C}$GA$^{T}_{C}$ GC was synthesized. These probes were employed for screening a bovine brain cDNA clone library produced with a statistical mixture of oligonucleotides as primer (dispersed primer). Clone pAc 111 was found to give a positive hybridization signal. To search for other clones, use was made of the method described in [2]. Clones pAc I, 5, 2.1, 13, 18 and 32 were selected using probes I and II from the clone library constructed via the use of oligodeoxyribonucleotide probes III and IV as primers, their structures corresponding to the regions of clone pAc 111 cDNA. Simultaneously, by employing probes III–V in the clone libraries produced using dispersed and oligo(dT) primers, clones pAc 3, 4, 43, 21.I, 22, 2.3 and 87 were determined. Another specific clone library was generated to disclose cDNA clones of the protein N-terminal region, probes VI and VII being applied as primers revealing clones pAc 12, 19.2 and 19.4.

Fig.1 depicts the location of the isolated adenylate cyclase cDNA fragments. The deciphered nucleotide sequence of these fragments resulted in reconstitution of the entire cDNA struc-

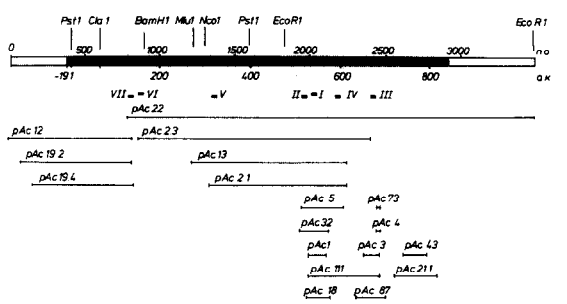

Fig.1. Location of cDNA fragments of isolated clones on a partial restriction map of adenylate cyclase cDNA. Filled rectangles indicate nucleotide probes used in cDNA cloning. The cDNA part encoding the adenylate cyclase is filled.

Fig.2. Nucleotide sequence of cDNA encoding calmodulin-independent adenylate cyclase from bovine brain and the corresponding amino acid sequence of the enzyme polypeptide chain. The amino acid sequence derived from peptide analysis is underlined. Nine potential sites of *N*-linked glycosylation are marked.

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   1-  68                                                                 TGATGTGAGGCACTATTTGTGGCCGGCGTGGTGGAAGGACACAGTGAGGTTCTCACCCCCGCCCCCCG
  69- 187   CCCCCAGCTCCCATCCCCAGTTCCATCAAAGCGAACCCGGGCCAGCGCAAGGATCTCCGAGTTGCTAGTGTGCTGAGGCTGGGACTGTCACTCATTCTCCGATCAGCGCGTGAACGACT
 188- 306   CTCGGCAGCGGCTGGCAGGAAACAATTCTACAAAAATAATCGTACTCAGCCTGGCAATTGTCTGCCCCTAGGTCTGTTGCTCTGCCGCCGTCCACACTCGCTGCAAGGGGGGGGCAGAG
 307- 406   AATTTACCGCGGCAAGAACATCCCTCCCGGCCAGCAGATTACA ATG CTG CAA ACT AAG AAT CTC ATC TGG ACT TTG TTT TTC CTG GGA ACT GCA GTT TCT
  -1- -19                                               Met-Leu-Gln-Thr-Lys-Asn-Leu-Ile-Trp-Thr-Leu-Phe-Phe-Leu-Gly-Thr-Ala-Val-Ser
 407- 496   CTG CAA GTG GAT ATT GTT CCC AGC CAA GGG GAA ATC AGC GTG GGA GAG TCC AAA TTC TTC CTG TGC CAA GTG GCC GGA GAT GCC AAA GAT
   1-  30   Leu-Gln-Val-Asp-Ile-Val-Pro-Ser-Gln-Gly-Glu-Ile-Ser-Val-Gly-Glu-Ser-Lys-Phe-Phe-Leu-Cys-Gln-Val-Ala-Gly-Asp-Ala-Lys-Asp-
 497- 586   AAA GAC ATC TCC TGG TTC TCG CCC AAC GGA GAG AAG CTC ACC CCA AAC CAG CAG CGG ATC TCG GTG GTA TGG AAC GAT GAC TCC TCC TCC
  31-  60   Lys-Asp-Ile-Ser-Trp-Phe-Ser-Pro-Asn-Gly-Glu-Lys-Leu-Thr-Pro-Asn-Gln-Gln-Arg-Ile-Ser-Val-Val-Trp-Asn-Asp-Asp-Ser-Ser-Ser-
 587- 676   ACC CTC ACC ATC TAC AAC GCC AAC ATC GAT GAT GCC GGC ATC TAC AAG TGT GTG GTC ACT GCG GAG GAT GGC ACC GAG TCA GAG GCC ACG
  61-  90   Thr-Leu-Thr-Ile-Tyr-Asn-Ala-Asn-Ile-Asp-Asp-Ala-Gly-Ile-Tyr-Lys-Cys-Val-Val-Thr-Ala-Glu-Asp-Gly-Thr-Glu-Ser-Glu-Ala-Thr-
 677- 766   GTC AAC GTG AAG ATC TTC CAG AAG CTC ATG TTC AAG AAC GCG CCA ACC CCA CAG GAG TTC AGG GAG GGA GAA GAT GCC GTG ATC GTG TGT
  91- 120   Val-Asn-Val-Lys-Ile-Phe-Gln-Lys-Leu-Met-Phe-Lys-Asn-Ala-Pro-Thr-Pro-Gln-Glu-Phe-Arg-Glu-Gly-Glu-Asp-Ala-Val-Ile-Val-Cys-
 767- 856   GAT GTG GTC AGC TCC CTC CCC CCA ACC ATC ATC TGG AAG CAC AAA GGC CGA GAT GTC ATC CTG AAA AAA GAT GTC CGA TTC ATA GTC CTG
 121- 150   Asp-Val-Val-Ser-Ser-Leu-Pro-Pro-Thr-Ile-Ile-Trp-Lys-His-Lys-Gly-Arg-Asp-Val-Ile-Leu-Lys-Lys-Asp-Val-Arg-Phe-Ile-Val-Leu-
 857- 946   ACC AAC AAC TAC CTC CAG ATC CGG GGC ATC AAG AAA ACA GAT GAG GGC ACT TAC CGC TGT GAG GGC AGG ATC CTT GCC CGC GGG GAG ATC
 151- 180   Thr-Asn-Asn-Tyr-Leu-Gln-Ile-Arg-Gly-Ile-Lys-Lys-Thr-Asp-Glu-Gly-Thr-Tyr-Arg-Cys-Glu-Gly-Arg-Ile-Leu-Ala-Arg-Gly-Glu-Ile-
 947-1036   AAC TTC AAG GAC ATT CAG GTC ATC GTG AAT GTG CCA CCC ACC GTC CAG GCC AGA CAG AGC ATC GTG AAT GCC ACC GCC AAC CTC GGC CAG
 181- 210   Asn-Phe-Lys-Asp-Ile-Gln-Val-Ile-Val-Asn-Val-Pro-Pro-Thr-Val-Gln-Ala-Arg-Gln-Ser-Ile-Val-Asn-Ala-Thr-Ala-Asn-Leu-Gly-Gln-
1037-1126   TCC GTC ACC CTC GTG TGC AAC GCC GAA GGC TTC CCA GAG CCC ACC GTG AGC TGG ACG AAG GAC GGG GAG CAG ATA GAG AAT GAG GAG GAC
 211- 240   Ser-Val-Thr-Leu-Val-Cys-Asn-Ala-Glu-Gly-Phe-Pro-Glu-Pro-Thr-Val-Ser-Trp-Thr-Lys-Asp-Gly-Glu-Gln-Ile-Glu-Asn-Glu-Glu-Asp-
1127-1216   GAG AAG TAC CTG TTC AGC GAC GAC AGC TCC GAG CTG ACC ATC AGG AAG GTG GAC AAG AAC GAC GAG GCT GAG TAC GTC TGC ATT GCC GAG
 241- 270   Glu-Lys-Tyr-Leu-Phe-Ser-Asp-Asp-Ser-Ser-Glu-Leu-Thr-Ile-Arg-Lys-Val-Asp-Lys-Asn-Asp-Glu-Ala-Glu-Tyr-Val-Cys-Ile-Ala-Glu-
1217-1306   AAC AAG GCG GGC GAG CAG GAC GCG TCC ATC CAC CTC AAG GTC TTC GCA AAA CCC AAA ATC ACC TAC GTA GAG AAC CAG ACT GCC ATG GAA
 271- 300   Asn-Lys-Ala-Gly-Glu-Gln-Asp-Ala-Ser-Ile-His-Leu-Lys-Val-Phe-Ala-Lys-Pro-Lys-Ile-Thr-Tyr-Val-Glu-Asn-Gln-Thr-Ala-Met-Glu-
1307-1396   CTG GAG GAA CAG GTC ACT CTT ACC TGT GAA GCC TCG GGA GAC CCC ATT CCC TCA ATC ACC TGG AGA ACA TCC ACC CGA AAC ATC AGC AGT
 301- 330   Leu-Glu-Glu-Gln-Val-Thr-Leu-Thr-Cys-Glu-Ala-Ser-Gly-Asp-Pro-Ile-Pro-Ser-Ile-Thr-Trp-Arg-Thr-Ser-Thr-Arg-Asn-Ile-Ser-Ser-
1397-1486   GAA GAA AAG GCT TCG TGG ACT CGA CCA GAG AAG CAA GAG ACC CTG GAC GGG CAC ATG GTG GTG CGC AGC CAC GCC CGC GTG TCG TCC CTG
 331- 360   Glu-Glu-Lys-Ala-Ser-Trp-Thr-Arg-Pro-Glu-Lys-Gln-Glu-Thr-Leu-Asp-Gly-His-Met-Val-Val-Arg-Ser-His-Ala-Arg-Val-Ser-Ser-Leu-
1487-1576   ACG CTG AAG AGC ATC CAG TAC ACC GAC GCC GGG GAG TAT GTC TGC ACT GCC AGC AAC ACC ATC GGC CAG GAC TCC CAG TCC ATG TAC CTC
 361- 390   Thr-Leu-Lys-Ser-Ile-Gln-Tyr-Thr-Asp-Ala-Gly-Glu-Tyr-Val-Cys-Thr-Ala-Ser-Asn-Thr-Ile-Gly-Gln-Asp-Ser-Gln-Ser-Met-Tyr-Leu-
1577-1666   GAA GTG CAA TAT GCC CCC AAG CTG CAG GGC CCT GTG GCT GTG TAC ACT TGG GAG GGG AAC CAG GTG AAC ATC ACC TGC GAG GTG TTT GCC
 391- 420   Glu-Val-Gln-Tyr-Ala-Pro-Lys-Leu-Gln-Gly-Pro-Val-Ala-Val-Tyr-Thr-Trp-Glu-Gly-Asn-Gln-Val-Asn-Ile-Thr-Cys-Glu-Val-Phe-Ala-
1667-1756   TAC CCC AGT GCC ACT ATC TCG TGG TTC CGA GAC GGT CAG CTG CTG CCC AGC TCC AAC TAC AGC AAC ATC AAG ATC TAC AAC ACC CCG TCC
 421- 450   Tyr-Pro-Ser-Ala-Thr-Ile-Ser-Trp-Phe-Arg-Asp-Gly-Gln-Leu-Leu-Pro-Ser-Ser-Asn-Tyr-Ser-Asn-Ile-Lys-Ile-Tyr-Asn-Thr-Pro-Ser
1757-1846   GCC AGC TAC CTG GAG GTG ACC CCA GAC TCT GAA AAT GAT TTT GGA AAC TAC AAC TGT ACC GCA GTG AAC CGC ATC GGA CAG GAG TCT TTG
 451- 480   Ala-Ser-Tyr-Leu-Glu-Val-Thr-Pro-Asp-Ser-Glu-Asn-Asp-Phe-Gly-Asn-Tyr-Asn-Cys-Thr-Ala-Val-Asn-Arg-Ile-Gly-Gln-Glu-Ser-Leu-
1847-1936   GAA TTC GTC CTT GTC CAA GCA GAT ACT CCA TCC TCA CCA TCC ATC GAC CAG GTG GAG CCA TAC TCT AGC ACA GCA CAG GTG CAA TTT GAT
 481- 510   Glu-Phe-Val-Leu-Val-Gln-Ala-Asp-Thr-Pro-Ser-Ser-Pro-Ser-Ile-Asp-Gln-Val-Glu-Pro-Tyr-Ser-Ser-Thr-Ala-Gln-Val-Gln-Phe-Asp
1937-2026   GAG CCA GAG GCC ACA GGC GGA GTG CCC ATC CTC AAA TAC AAA GCT GAG TGG AGA GCA ATG GGC GAG GAG GTG TGG CAC TCC AAG TGG TAT
 511- 540   Glu-Pro-Glu-Ala-Thr-Gly-Gly-Val-Pro-Ile-Leu-Lys-Tyr-Lys-Ala-Glu-Trp-Arg-Ala-Met-Gly-Glu-Glu-Val-Trp-His-Ser-Lys-Trp-Tyr-
2027-2116   GAT GCC AAG GAA GCC AGC ATG GAG GGC ATT GTC ACC ATC GTG GGC CTC AAG CCC GAG ACG ACG TAC GCA GTC CGG CTG GCG GCC CTC AAC
 541- 570   Asp-Ala-Lys-Glu-Ala-Ser-Met-Glu-Gly-Ile-Val-Thr-Ile-Val-Gly-Leu-Lys-Pro-Glu-Thr-Thr-Tyr-Ala-Val-Arg-Leu-Ala-Ala-Leu-Asn-
2117-2206   GGC AAG GGC CTG GGC GAG ATC AGC GCC GCC TCT GAG TTC AAG ACG CAG CCA GTC CGG GAA CCC AGT GCA CCT AAG CTC GAA GGG CAG ATG
 571- 600   Gly-Lys-Gly-Leu-Gly-Glu-Ile-Ser-Ala-Ala-Ser-Glu-Phe-Lys-Thr-Gln-Pro-Val-Arg-Glu-Pro-Ser-Ala-Pro-Lys-Leu-Glu-Gly-Gln-Met-
2207-2296   GGA GAG GAT GGA AAC TCT ATT AAG GTG AAG CTG ATC AAG CAA GAC GAC GGC GGC TCC CCC ATC CGA CAC TAC CTG GTC AAA TAC CGA GCG
 601- 630   Gly-Glu-Asp-Gly-Asn-Ser-Ile-Lys-Val-Lys-Leu-Ile-Lys-Gln-Asp-Asp-Gly-Gly-Ser-Pro-Ile-Arg-His-Tyr-Leu-Val-Lys-Tyr-Arg-Ala-
2297-2386   CTC TCC TCC GAG TGG AAA CCA GAG ATC AGG CTC CCG TCT GGC AGT GAC CAC GTC ATG CTC AAG TCC CTG GAC TGG AAT GCC GAG TAC GAG
 631- 660   Leu-Ser-Ser-Glu-Trp-Lys-Pro-Glu-Ile-Arg-Leu-Pro-Ser-Gly-Ser-Asp-His-Val-Met-Leu-Lys-Ser-Leu-Asp-Trp-Asn-Ala-Glu-Tyr-Glu-
2387-2476   GTC TAT GTG GTG GCC GAG AAC CAG CAG GGC AAG TCC AAA GCG GCG CAC TTT GTG TTC AGG ACC TCG GCC CAG CCC ACA GCC ATC CCA GCC
 661- 690   Val-Tyr-Val-Val-Ala-Glu-Asn-Gln-Gln-Gly-Lys-Ser-Lys-Ala-Ala-His-Phe-Val-Phe-Arg-Thr-Ser-Ala-Gln-Pro-Thr-Ala-Ile-Pro-Ala-
2477-2566   AAC GGC AGC CCC ACC TCG GGC CTG AGC ACT GGC GCC ATC GTG GGC ATC CTC GTC GTG ACC TTC GTC CTG CTC CTG GTG GCC GTG GAC GTC
 691- 720   Asn-Gly-Ser-Pro-Thr-Ser-Gly-Leu-Ser-Thr-Gly-Ala-Ile-Val-Gly-Ile-Leu-Val-Val-Thr-Phe-Val-Leu-Leu-Leu-Val-Ala-Val-Asp-Val-
2567-2656   ACC TGC TAC TTC CTG AAC AAG TGT GGC CTG CTC ATG TGC ATT GCC GTC AAC TTG TGT GGC AAA GCC GGG CCC GGA GCC AAG GGC AAG GAC
 721- 750   Thr-Cys-Tyr-Phe-Leu-Asn-Lys-Cys-Gly-Leu-Leu-Met-Cys-Ile-Ala-Val-Asn-Leu-Cys-Gly-Lys-Ala-Gly-Pro-Gly-Ala-Lys-Gly-Lys-Asp-
2557-2746   ATG GAG GAG GGC AAA GCG GCC TTC TCG AAA GAT GAG TCC AAG GAG CCT ATC GTG GAG GTG CGA ACG GAG GAG GAG CGG ACC CCG AAT CAC
 751- 780   Met-Glu-Glu-Gly-Lys-Ala-Ala-Phe-Ser-Lys-Asp-Glu-Ser-Lys-Glu-Pro-Ile-Val-Glu-Val-Arg-Thr-Glu-Glu-Glu-Arg-Thr-Pro-Asn-His-
2747-2836   GAC GGA GGG AAA CAC ACG GAG CCG AAC GAG ACC ACG CCG CTG ACG GAG CCC GAG AAG GGT CCC GTA GAA GCA AAG CCG GAG ACA GAA ACG
 781- 810   Asp-Gly-Gly-Lys-His-Thr-Glu-Pro-Asn-Glu-Thr-Thr-Pro-Leu-Thr-Glu-Pro-Glu-Lys-Gly-Pro-Val-Glu-Ala-Lys-Pro-Glu-Thr-Glu-Thr-
2837-2930   AAG CCT GCG CCA GCC GAA GTC CAG ACG GTC CCC AAC GAT GCC ACA CAA ATA AAG GTG AAT GAG AGC AAA GCG TGA TGGGTGACGAGACACCAGC
 811- 834   Lys-Pro-Ala-Pro-Ala-Glu-Val-Gln-Thr-Val-Pro-Asn-Asp-Ala-Thr-Gln-Ile-Lys-Val-Asn-Glu-Ser-Lys-Ala TER
2931-3049   AAAGATCAAAATATAAAGTGACACAACAGCTTCTCCAGAGCATCTCCAATATCTCACACACACACACACACACACACACAATACACACACACACAATCTCATTCCTCTAGTATCTTT
3050-3168   TGCCTTTAAAGAAAAAAAACACTAAGCAGATCAACATAAAATCTCCTTTTTGTAGATTTATAGAAAGGGTTCCTTTGTTACGCACTCACTTGTAAGAAAATAAGACAAAAACGTAAAAC
3169-3287   CCACAGCCAAACTAGGACACTCGTTCCCTGAAACCATTTAAAAATCGAACAAAAGGACCCCAAATTAAGAATCTAGGAAGCTCAGAAGAAAAAAAAAGAATCTAGGTTCAGGATTTTGG
3288-3406   TGTGCCGAACCGGCACAGACCGGGTTCAGAGAAATAACTTCTAGACGCTAAGACTAATCGAATGAACAAAGTCCAAGTTTATTTTTCTACTTTCAGTCGAGTTTGGACTCTGTGAAACC
3407-3525   TCATAAATAAGTTATAATTTCTGTTCACTTTGTATTTGTTCAGTATGCAAAGTGTGTCGCCCTTCCTAGCTGAATTCAATTCCCACATAGACTCTTGTTTTGTAGGAAGAATGCCAAAC
3526-3574   GCAGCTTCTGGGGGTAGATCTCAATTTGCAGTATTCGGATTTCTTTTCT
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ture (fig.2). The adenylate cyclase amino acid sequence (1–14) derived from the cDNA structure coincided with the N-terminal amino acid sequence of the protein.

Triplet ATG (350–352) is an initiation codon: (i) this is the first ATG codon within the translation frame behind terminator TAA (224–226); (ii) the structure of the signal peptide (Met-Leu-Gln-Thr-Lys-Asn-Leu-Ile-Trp-Thr-Leu-Phe-Phe-Leu-Gly-Thr-Ala-Val-Ser) derived from cDNA corresponds to the generalized structure for sequences of this type [13].

The amino acid sequence determined contains 834 amino acid residues and has a molecular mass of 91 735 kDa. The validity of this structure is indicated by the correspondence to the amino acid sequences of the isolated peptides. Comparison of the cDNA structures of the isolated clones revealed some differences. Hence, together with clones pAc 13, 2.1 and 5, whose nucleotide sequences correspond to the peptide amino acid sequences, we isolated clones pAc 2.3 and 22 with the deletion of 30 nucleotides (1405–1434), in which the triplet CGG (2171–2173), coding for arginine, is sub-

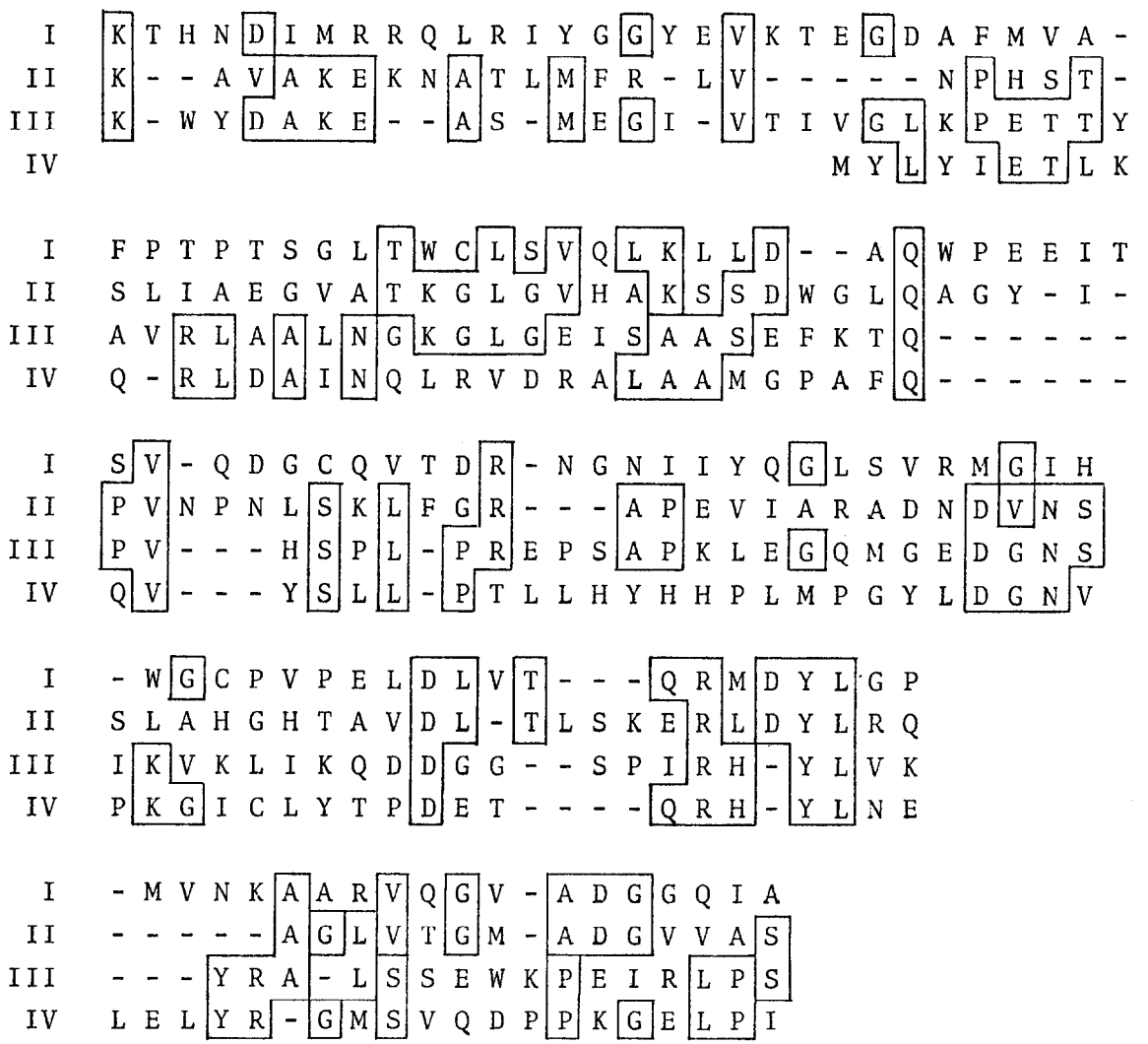

Fig.3. Homologous regions of adenylate cyclase sequence from I, *Saccharomyces cerevisiae* (1693–1814); II, *Bordetella pertussis* (28–139); III, bovine brain (538–643, the given structure corresponds to that of pAc 111); IV, *Escherichia coli* (1–95). Identical amino acid residues are boxed.

stituted by the sequence CAAGGG encoding the dipeptide Gln-Gly. We also isolated clone pAc 111 with the insertion of 15 nucleotides (between nucleotides 2170 and 2171) encoding the peptide His-Ser-Pro-Leu-Pro. All this demonstrates the mRNA to be of several types encoding diverse isoforms of adenylate cyclase.

A considerable degree of structural homology of the bovine brain adenylate cyclase polypeptide chain region of 106 amino acid residues (538–643) with the N-terminal regions of adenylate cyclases from *Escherichia coli* [14] (29 identical amino acids) and *Bordetella pertussis* [15] (33 identical amino acids) has been determined. At the same time, homology of the bovine and *Saccharomyces cerevisiae* [16] adenylate cyclases as well as enzymes from *E. coli* and *B. pertussis* is not as evident (16 and 12 amino acids, respectively) (fig.3).

Since the N-terminal regions of adenylate cyclase from *E. coli* [14] and *B. pertussis* [15] are known to be responsible for the enzyme catalytic activity, the homologous regions of calmodulin-independent adenylate cyclase from bovine brain might also enter the catalytic site. This is proved by the presence of the sequence Ala-X-X-X-X-Gly-Lys-Ser (amino acid residues 665–672) following the homologous region in the adenylate cyclase structure from bovine brain, being typical of the nucleotide-binding site [17].

Amino acid sequence analysis of adenylate cyclase according to Kyte and Doolittle [18] revealed one distinct hydrophobic fragment consisting of amino acid residues 702–738, apparently, being transmembrane. The protein also contains several regions of less distinct hydrophobicity (185–228, 562–583). Bovine brain adenylate cyclase is known to be a glycoprotein; nine potential glycosylating regions were found in the amino acid sequence (fig.2). These regions are mostly located in the mid- and C-terminal protein domain, some being probably outside the membrane. The location of the adenylate cyclase polypeptide chain relative to the plasma membrane is the aim of further studies.

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