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Primary structure of bovine cerebellum GTP-binding protein G_{39} and its effect on the adenylate cyclase system

Yu.A. Ovchinnikov, V.Z. Slepak, A.N. Pronin, A.B. Shlensky, N.B. Levina, V.L. Voeikov and V.M. Lipkin

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Science, ul. Miklukho-Maklaya, 16/10, 117871 GSP Moscow V-437, USSR

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The primary structure of bovine cerebellum GTP-binding protein α-subunit, protein G_{39}, was determined by parallel analysis of the protein amino acid sequence and the corresponding cDNA nucleotide sequence. The protein consists of 354 amino acid residues and has a molecular mass of 40064 Da. High homology between G_{39} and other G-proteins, especially rat brain G_o, was shown. An assumption is made that certain brain adenylate cyclase system properties are determined by the presence of G_{39}.

GTP-binding protein; Adenylate cyclase; Amino acid sequence; cDNA cloning; Nucleotide sequence; (Bovine cerebellum)

1. INTRODUCTION

Signal transduction from cell surface receptors to membrane inner surface effector proteins is mediated through GTP-binding regulatory G-proteins. Membrane adenylate cyclase activity regulation involves stimulating (G_s) and inhibiting (G_i) proteins [1]. In 1984, a new G-protein, G_o, of unknown function was isolated from bovine brain [2]. G-proteins are $\alpha\beta\gamma$-heterotrimers. Their specificity is determined by α-subunits responsible for binding and hydrolysis of GTP and affecting target enzyme activity.

α-subunit amino acid sequences were defined in a number of G-proteins, in particular, G_s and G_i from bovine [3-5] and rat [6] brain. A partial structure was determined for rat brain G_o-protein. High structural homology of G-proteins was shown [6].

Recently, we isolated a low K_m GTPase, 39 kDa protein G_{39}, from bovine cerebellum cortex. This protein is attributed to nervous tissue and is capable of interacting with the G-protein $\beta\gamma$-subunit [7].

This paper presents the amino acid sequence of G_{39} established by parallel analysis of the protein amino acid sequence and respective cDNA nucleotide sequence. Some functional properties of the protein are discussed. (Preliminary results are published in [8].)

Correspondence address: Yu.A. Ovchinnikov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, ul. Miklukho-Maklaya, 16/10, 117871 GSP Moscow V-437, USSR

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00709

2. MATERIALS AND METHODS

G_{39} and G-protein $\beta\gamma$-subunits from bovine cerebellum cortex were isolated as described in [7].

G_{39} was cleaved by trypsin, *St. aureus* protease and cyanogen bromide. Peptides were isolated by reverse-phase HPLC on C_{18}-columns; some fractions were additionally purified on a TSK-2000 col-

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umn. N-terminal sequences of the peptides were determined using automatic Edman degradation on a 470A sequencer (Applied Biosystems) or manually with amino acid dansyl derivatives identification. The 37 kDa peptide, obtained from G_{39} limited proteolysis [7], was subjected to electrophoresis in a polyacrylamide gel with subsequent electroblotting on a glass fibre filter modified by aminopropyl groups according to [9], then its N-terminal sequence was determined.

Bovine cerebellum mRNA isolation, cDNA synthesis and cloning in plasmid pUC8, and clone library screening were performed as described in [10]. For priming of the first cDNA chain use was made of either one specific oligodesoxyribonucleotide probe or a random primer (DNA hydrolyzate, mean size 10–20 nucleotides).

Oligodesoxyribonucleotide probes were synthesized by the phosphoramidite technique on a 380B DNA synthesizer (Applied Biosystems). DNA was sequenced by the Maxam and Gilbert method [11].

The effect of G_{39} on the adenylate cyclase system was studied by its reconstitution with rat reticulocyte plasma membranes. 20 μl of reticulocyte membranes (40 μg of membrane protein) were mixed at 4°C with 5 μl (1 μg) of G_{39} and 5 μl (1 μg) of $\beta\gamma$-subunit (or 5 μl of buffer). 20 μl of buffer containing 3×10^{-4} M GTP or 3×10^{-4} M GTP plus 10^{-5} M isoproterenol was added to the mixture and incubated for 30 min at 30°C. Then 20 μl medium containing [α-^{32}P]ATP and ATP-regenerating system was added to incubation mixture and adenylate cyclase activity was measured as described in [7].

3. RESULTS AND DISCUSSION

Revealed sequences of the G_{39} peptide were homologous to the primary structure of G_s-, G_i- and, in particular, G_o. From the amino acid sequences of the G_{39} peptide, that differed most significantly in structure from G_s and G_i, a series of unique nucleotide probes was synthesized. When deducing probe nucleotide sequences, the cDNA nucleotide sequence of other G-proteins was taken into consideration.

(Glu)-Asp-Ala-Ala-Ala-Tyr-Ile (298–304, fig. 1)
5′-GATGTAGGCAGCTGCGTCTTC-3′ probe I

Ala-Lys-Tyr-Tyr-Leu-Asp-Ser (153–159, fig.1)
5′-CTGTCCAGGTAGTATTTGGC-3′ probe II

Probe I was used as a primer to synthesize a single-stranded cDNA when constructing the bovine cerebellum cDNA library. Screening of 40 000 colonies from the library by probe II revealed 5 positive clones. Clone Gα21.20 contains a cDNA insert of 799 base pairs long corresponding to a cDNA fragment encoding the central part of the G_{39} polypeptide chain (nucleotides 28–826, fig. 1). The rest of the clones contained shorter cDNA fragments.

To identify clones encoding C- and N-terminal regions of the G_{39} molecule the library, constructed by random priming, was screened. Clone Gα21.20 cDNA insert, labeled by nick-translation, and probe I were used for screening. Analysis of 100 000 transformants yielded 23 positive clones. Restriction analysis was applied to select clones presumably encoding G_{39} C- and N-terminal regions. Clone GαR4.2 contains the insert encoding the G_{39} C-terminal and mRNA 3′-untranslated region (nucleotides 715–1129). Clone GαR16.2 encodes the mRNA 5′-untranslated region and a significant portion of the G_{39} amino acid sequence (nucleotides −164–916, fig. 1).

Fig. 1 shows the complete G_{39} amino acid sequence deduced from the cDNA nucleotide sequence. The G_{39} polypeptide chain contains 354 amino acid residues, the calculated molecular mass of the protein being 40 064 Da. The structure was proved by its correspondence to amino acid sequences in a great number of peptides, it is especially important in case of G-proteins, the proteins with high structural homology.

The amino acid sequence of bovine cerebellum G_{39} protein displays extremely high homology to rat brain G_o protein. Only five differences were found: in G_{39} Ile, Ala, Pro, Thr and Ala residues occupied positions 93, 104, 122, 292 and 306, respectively, and the respective G_o sequence involved Val, Ser, Ala, Pro and Thr residues.

When this manuscript was in preparation an article [12] describing cDNA cloning of G_o from bovine retina appeared. The structure of this cDNA is identical to our G_{39} cDNA structure with only two differences: there is no residue G in position −65 of G_o 5′-untranslated region; the struc-

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-164                                                                  GGGGGG
-158   TTCTGGTTTCTCGACATTTTTGTTTTCAGCCAAGGGGAGGCTATCGTGATTTTTTCCCCTTTGAGCCCAGGTTCTGCTC
 -79   TTTGGGGGGGTGGGGGGCGCGCCGAGCCGGGGAGCCGTGCCAGCAGAGTCGTGCGGGCTGTGGCAGGGAAGGGGCCACC
    I   Met Gly Cys Thr Leu Ser Ala Glu Glu Arg Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu
    I   ATG GGA TGT ACT CTG AGC GCA GAG GAG CGA GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG

   21   Lys Asn Leu Lys Glu Asp Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly
   61   AAA AAC CTC AAA GAG GAT GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG

   41   Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu Asp Gly
  121   GCT GGA GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAC GAA GAC GGC

   61   Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val Tyr Ser Asn Thr Ile Gln Ser
  181   TTC TCT GGC GAA GAC GTG AAG CAG TAC AAG CCC GTG GTC TAC AGC AAC ACC ATC CAG TCC

   81   Leu Ala Ala Ile Val Arg Ala Met Asp Thr Leu Gly Ile Glu Tyr Gly Asp Lys Glu Arg
  241   CTG GCA GCC ATC GTC CGG GCC ATG GAC ACT CTG GGC ATC GAG TAC GGT GAC AAG GAG CGA

  101   Lys Ala Asp Ala Lys Met Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe
  301   AAG GCC GAC GCC AAG ATG GTG TGT GAC GTG GTG AGT CGG ATG GAA GAC ACG GAG CCC TTC

  121   Ser Pro Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile Gln Glu Cys
  361   TCT CCA GAG CTG CTC TCC GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC CAG GAG TGC

  141   Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Lys Tyr Tyr Leu Asp Ser Leu
  421   TTC AAC AGG TCC CGG GAA TAT CAG CTC AAC GAC TCT GCC AAA TAC TAC CTG GAC AGC CTG

  161   Asp Arg Ile Gly Ala Ala Asp Tyr Gln Pro Thr Glu Gln Asp Ile Leu Arg Thr Arg Val
  481   GAC CGG ATC GGG GCC GCC GAC TAC CAG CCC ACC GAG CAG GAC ATC CTC CGA ACC AGG GTC

  181   Lys Thr Thr Gly Ile Val Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe
  541   AAA ACC ACC GGC ATC GTA GAA ACC CAC TTC ACA TTC AAG AAC CTC CAC TTC AGG CTG TTC

  201   Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Asp Val Thr
  601   GAC GTC GGG GGC CAG CGG TCT GAG CGC AAG AAG TGG ATT CAC TGC TTC GAG GAC GTC ACG

  221   Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln Val Leu His Glu Asp Glu Thr
  661   GCC ATC ATC TTC TGT GTC GCG CTC AGC GGC TAT GAC CAG GTG CTC CAC GAA GAC GAG ACC

  241   Thr Asn Arg Met His Glu Ser Leu Met Leu Phe Asp Ser Ile Cys Asn Asn Lys Phe Phe
  721   ACG AAC CGC ATG CAC GAG TCT CTC ATG CTC TTC GAC TTC ATC TGT AAC AAC AAG TTC TTC

  261   Ile Asp Thr Ser Ile Ile Leu Phe Leu Asn Lys Lys Asp Leu Phe Gly Glu Lys Ile Lys
  781   ATC GAT ACC TCC ATC ATT CTC TTC CTC AAC AAG AAA GAT CTC TTT GGG GAG AAG ATC AAG

  281   Lys Ser Pro Leu Thr Ile Cys Phe Pro Glu Tyr Thr Gly Ser Asn Thr Tyr Glu Asp Ala
  841   AAG TCA CCT CTG ACC ATC TGC TTT CCC GAG TAC ACA GGC TCC AAC ACC TAT GAA GAC GCC

  301   Ala Ala Tyr Ile Gln Ala Gln Phe Glu Ser Lys Asn Arg Ser Pro Asn Lys Glu Ile Tyr
  901   GCC GCC TAC ATC CAA GCA CAA TTT GAA AGC AAA AAC CGC TCA CCC AAC AAA GAA ATT TAT

  321   Cys His Met Thr Cys Ala Thr Asp Thr Asn Asn Ile Gln Val Val Phe Asp Ala Val Thr
  961   TGT CAC ATG ACT TGT GCC ACA GAC ACG AAT AAT ATC CAG GTG GTA TTC GAC GCT GTC ACC

  341   Asp Ile Ile Ile Ala Asn Asn Leu Arg Gly Cys Gly Leu Tyr stop
 1021   GAC ATC ATC ATT GCC AAC AAC CTC CGG GGC TGC GGC TTG TAC TGA CCTCTTGTCCTGTATAGCA

 1085   ACCTATTTGACTGCTTCACGGACTCTTTGCTGTTGACGATCTCCT
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Fig. 1. cDNA nucleotide sequence encoding G_{39} and the corresponding protein amino acid sequence. Amino acid sequences established by the G_{39} peptide analysis are underlined.

tural part of G_o cDNA involves a T residue in position 788 instead of a C residue in G_{39}, this means that Thr is replaced with Ile. This makes possible the assumption that the bovine cerebellum G_{39} protein is an analogue of the G_o protein, both of them possibly performing a similar function.

The true G_o function has not yet been revealed though it was shown to affect various cellular systems [13–16]. It appeared to be of interest to find out whether G_{39} affects the adenylate cyclase system.

An appropriate model for this purpose is rat reticulocyte membranes containing adenylate cyclase which is highly sensitive to GTP and β_2-adrenoreceptor agonist stimulation [17], G_{39} of the membranes is not revealed by antibodies [7]. The addition of G_{39} to reticulocyte membranes produced an increase of adenylate cyclase activity measured in the presence of GTP and a decrease of the isoproterenol stimulating effect. As a result enzyme stimulation by isoproterenol drops from 11–12-times over to only 4.5 (fig. 2). When the $\beta\gamma$-subunit, in a quantity equimolar to G_{39}, was added to the incubation mixture its effects were neutralized (not shown). However, the effect of G_{39} on the adenylate cyclase system may be attributed not only to its interaction with the $\beta\gamma$-subunit. G_{39} and G_i affect in different ways signal transmission from β_2-adrenoreceptor to adenylate cyclase: introducing G_i into the liposome-reconstituted adenylate cyclase system increases adenylate cyclase stimulation by the hormone [18].

Interestingly, in brain membranes where the G_{39} content is high, the basal activity of adenylate cyclase is higher, but its stimulation by hormones is much lower compared to somatic tissues devoid of G_{39} [19,20]. We assume that one of the G_{39} functions may be to act as a buffer, i.e. cAMP level stabilization in neurons in cases of sharp increase of blood catecholamine concentration, for example, under stress conditions.

A number of authors hold an opinion based on indirect indications that the G-protein $\beta\gamma$-subunit interacts with the N-terminal region of α-subunits [22,23]. However, when comparing G_{39} structure with structures of G_s, G_i, $T_{\alpha 1}$ and $T_{\alpha 2}$, we noted a comparatively high variability in the N-terminal fragments of these proteins. Moreover, when treating G_{39} with trypsin in the presence of GppNHp we obtained an N-terminal-deficient

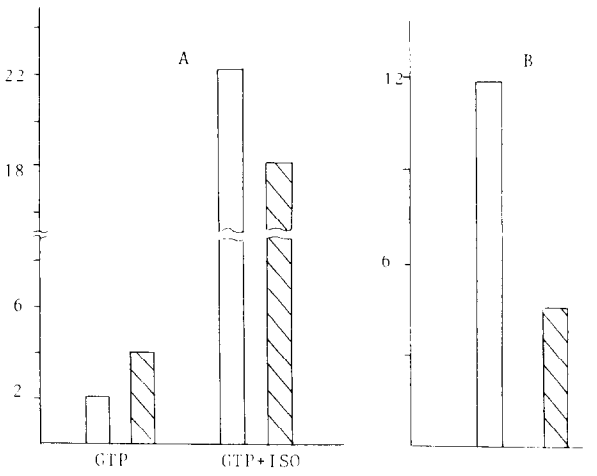

Fig. 2. Effect of G_{39} on rat reticulocyte membrane adenylate cyclase stimulation (hatched columns). Open columns, control. (A) Y-axis adenylate cyclase activity, relative units. The basal activity of adenylate cyclase (1 relative unit) equals 2.2 pmol cAMP per mg membrane protein per min. (B) Y-axis relative stimulation by isoproterenol (ISO) and GTP compared to the activity measured in the presence of GTP.

fragment of 37 kDa [7]. Its N-terminal sequence was determined using an automatic sequencer (amino acids 22–42, fig. 1). The 37 kDa fragment appeared to retain GTPase activity, 40% being stimulated by the $\beta\gamma$-subunit [24]. These data make the conclusion of the $\beta\gamma$-subunit interaction with the N-terminal region of α-subunits, questionable.

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