Primary structure of the α-subunit of bovine adenylate cyclase-inhibiting G-protein deduced from the cDNA sequence

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The primary structure of the α-subunit of the adenylate cyclase-inhibiting G-protein (G_i) has been deduced from the nucleotide sequence of cloned DNA complementary to the bovine cerebral mRNA encoding the polypeptide. A much higher degree of amino acid sequence homology is observed between the α-subunits of G_i and transducin (68%) than between those of G_i and the adenylate cyclase-stimulating G-protein (G_s) (43%) or between those of transucin and G_s (42%).

Adenylate cyclase *G-protein* *cDNA* *Cloning* *Nucleotide sequence* *Transducin* *ADP-ribosylation site*

1. INTRODUCTION

A family of membrane-associated G-proteins are essential for transducing signals generated at cell surface receptors into changes in cellular function and metabolism [1]. These proteins are composed of 3 subunits termed α, β and γ. The α-subunit is responsible for binding guanine nucleotides and is unique to each G-protein. G_i mediates hormonal inhibition of adenylate cyclase [1]. Here, the primary structure of the α-subunit of G_i has been deduced by cloning and sequencing cDNA encoding it. The amino acid sequence homology observed among the α-subunits of G_i, G_s [2] and transducin [3–6] is discussed in terms of the function of G-proteins.

2. MATERIALS AND METHODS

G_i was purified from bovine cerebrum as in [7], except that it was separated from G_o [7,8] by chromatography on a DEAE-Toyopearl column (1.4 × 13 cm, Toyo Soda). G_i was eluted ahead of G_o from the column with a linear gradient of NaCl (0–0.25 M) in 20 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA, 1 mM dithiothreitol and 0.6% (w/v) Lubrol PX. The purified G_i was treated with guanosine 5′-(3-*O*-thio)triphosphate to dissociate it into the α-subunit and a complex of the β- and γ-subunits, concentrated with a hydroxyapatite column and then subjected to gel permeation HPLC on a TSK G2000SW column (0.75 × 60 cm, Toyo Soda) as in [9]. The G_i α-subunit thus obtained was further purified by rechromatography on the same column.

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Abbreviations: G-protein, guanine nucleotide-binding protein; G_i, adenylate cyclase-inhibiting G-protein; G_s, adenylate cyclase-stimulating G-protein; G_o, a G-protein purified from brain; HPLC, high-performance liquid chromatography; IAP, islet-activating protein

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The procedure for cloning pGα28 has been described [2]. DNA sequencing was carried out by the method of Maxam and Gilbert [10].

3. RESULTS AND DISCUSSION

Clone pGα28 was isolated from a cDNA library derived from bovine cerebral cortex poly(A)$^+$ RNA [2]. This clone hybridized with the two oligodeoxyribonucleotide probes synthesized on the basis of the pentapeptide sequences contained in the α-subunits of both bovine transducin and G_o. The cDNA insert of clone pGα28 encodes an amino acid sequence that is homologous with the sequences of bovine transducin [3] and G_s [2] and the known partial sequence of bovine G_o [11], but not identical. In the hope of identifying the protein encoded by this cDNA clone, we carried out partial amino acid sequence analysis of the α-subunit of G_i purified from bovine cerebrum. Tryptic peptides from the G_i α-subunit were isolated by reverse-phase HPLC and subjected to sequence analysis with a gas-phase sequencer (fig.1). Eight peptide sequences were thus determined.

Fig.2 shows the 3099-nucleotide sequence [excluding the poly(dA) tract] of the cDNA insert of clone pGα28. All 8 peptide sequences determined were found to be encoded by the cDNA sequence

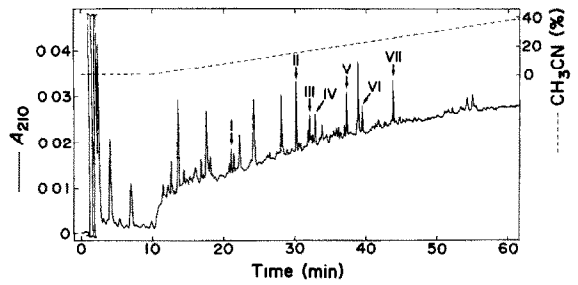

Fig.1. Partial amino acid sequence analysis of the α-subunit of bovine G_i. Approx. 8.2 μg purified bovine G_i α-subunit was digested by trypsin and then subjected to reverse-phase HPLC. Seven fractions (I–VII) corresponding to absorbance peaks were collected and analysed for amino acid sequence with a gas-phase sequencer [12]. Fraction II proved to be a mixture of two peptides (IIa,IIb). The sequences determined were as follows (in one-letter code): I, MHESMK; IIa, IDFGDSAR; IIb, DLHFK; III, MFDVGGQR; IV, DLFEEK; V, IAQPNYIPTQQDVLR; VI, LLLLGA; VII, EYQL. For experimental details, see [3].

in the same reading frame (amino acid residues 36–41, 93–100, 145–148, 162–176, 193–197, 198–205, 243–248 and 272–277). This reading frame was used to deduce the primary structure of the G_i α-subunit (fig.2). The assignment of the translational initiation site to the methionine codon composed of nucleotides 1–3 is based on the alignment of the deduced amino acid sequence with the sequences of the α-subunits of transducin and G_s (fig.3). This assignment is supported by the fact that the nucleotide sequence surrounding this ATG triplet agrees with the favoured sequence that flanks functional initiation codons in eukaryotic mRNAs [17,18]. The possibility that the initiating methionine is located upstream of the 5′-end of the cDNA insert of clone pGα28 cannot be excluded. A translational termination codon (TGA) occurs in frame after the 354th codon specifying phenylalanine. Thus, the α-subunit of bovine G_i consists of 354 amino acid rsidues (including the initiating methionine) and has a calculated M_r of 40359, which agrees with the reported value [7,8]. Blot hybridization analysis of bovine cerebral cortex poly(A)$^+$ RNA with a G_i α-subunit cDNA probe exhibited a hybridizable RNA species with an estimated size of approx. 3900 nucleotides (fig.4).

The α-subunit of bovine G_i shows 68 and 43% amino acid sequence homology with the α-subunits of bovine transducin and G_s, respectively, whereas that between the α-subunits of bovine transducin and G_s is 42% (fig.3); gaps have been counted as one substitution regardless of their length. Some of the regions that are highly conserved among the 3 G-protein α-subunits exhibit sequence homology with elongation factor Tu and *ras* p21 proteins and correspond to functional regions of G-proteins [21–24]. The segment comprising positions 42–60 in the aligned sequences (fig.3; the numbering hereafter refers to the aligned sequences as shown in this figure) is homologous with the region of elongation factor Tu and *ras* proteins that is proposed as being involved in interaction with the phosphate groups of the GDP ligand through the side chain of the lysine corresponding to that at position 53 [22–24]. The segment comprising positions 171–175 is homologous with the region of elongation factor Tu and *ras* proteins including the aspartic acid (corresponding to that at position 173) that may form a salt bridge with an Mg^{2+}

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5'--TGGCCGGCGTCAGGAGGAATTCGAACGCCTGCATCCAGAAAGAAAGAATTCACCTGTGTTTCGAGGCAGCGCGCCGGACTTCGAGGGAGCGGCAGCCAGCTTTCGCTCCTGGCACA   -1
  1                                10                                20                                30
Met Gly Cys Thr Leu Ser Ala Glu Asp Lys Ala Ala Val Glu Arg Ser Lys Met Ile Asp Arg Asn Leu Arg Glu Asp Gly Glu Lys Ala
ATG GGC TGT ACG CTG AGC GCC GAG GAC AAG GCG GCG GTG GAG CGG AGT AAG ATG ATC GAC CGG AAC CTC CGC GAG GAT GGC GAG AAG GCG    90
                                40                                50                                60
Ala Arg Glu Val Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu Ala Gly
GCG CGC GAG GTC AAG CTG CTG CTG CTC GGT GCT GGT GAA TCT GGG AAA AGT ACA ATT GTG AAG CAA ATG AAA ATT ATC CAT GAA GCT GGT   180
                                70                                80                                90
Tyr Ser Glu Glu Glu Cys Lys Gln Tyr Lys Ala Val Val Tyr Ser Asn Thr Ile Gln Ser Ile Ile Ala Ile Ile Arg Ala Met Gly Arg
TAT TCA GAA GAG GAA TGT AAG CAG TAC AAA GCT GTG GTC TAC AGT AAC ACC ATC CAG TCA ATT ATC GCT ATC ATT AGG GCC ATG GGG AGA   270
                               100                               110                               120
Leu Lys Ile Asp Phe Gly Asp Ser Ala Arg Ala Asp Asp Ala Arg Gln Leu Phe Val Leu Ala Gly Ala Ala Glu Glu Gly Phe Met Thr
TTG AAG ATT GAC TTC GGT GAC TCA GCC CGG GCG GAT GAT GCC CGC CAA CTC TTT GTG CTT GCT GGC GCT GCA GAG GAA GGT TTT ATG ACT   360
                               130                               140                               150
Ala Glu Leu Ala Gly Val Ile Lys Arg Leu Trp Lys Asp Ser Gly Val Gln Ala Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp
GCA GAA CTT GCT GGA GTT ATA AAG AGA CTT TGG AAA GAC AGT GGT GTA CAA GCC TGC TTC AAC AGA TCC CGA GAG TAC CAG CTT AAT GAT   450
                               160                               170                               180
Ser Ala Ala Tyr Tyr Leu Asn Asp Leu Asp Arg Ile Ala Gln Pro Asn Tyr Ile Pro Thr Gln Gln Asp Val Leu Arg Thr Arg Val Lys
TCT GCA GCA TAC TAT TTG AAT GAT TTG GAC AGA ATT GCA CAA CCA AAT TAT ATT CCA ACT CAA CAA GAT GTT CTC AGA ACT CGA GTG AAA   540
                               190                               200                               210
Thr Thr Gly Ile Val Glu Thr His Phe Thr Phe Lys Asp Leu His Phe Lys Met Phe Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys
ACC ACA GGA ATT GTT GAG ACC CAT TTT ACT TTC AAA GAT CTT CAT TTT AAA ATG TTT GAT GTG GGA GGA CAG AGA TCT GAG CGG AAG AAA   630
                               220                               230                               240
Trp Ile His Cys Phe Glu Gly Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Asp Tyr Asp Leu Val Leu Ala Glu Asp Glu Glu Met
TGG ATT CAT TGC TTC GAA GGA GTG ACC GCC ATC ATC TTC TGT GTG GCG CTG AGT GAC TAT GAC CTG GTT CTA GCT GAA GAT GAA GAA ATG   720
                               250                               260                               270
Asn Arg Met His Glu Ser Met Lys Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile Ile Leu Phe Leu Asn Lys
AAC CGA ATG CAT GAA AGC ATG AAG TTA TTC GAC AGC ATA TGT AAC AAC AAA TGG TTT ACA GAT ACA TCT ATT ATA CTT TTT CTG AAC AAG   810
                               280                               290                               300
Lys Asp Leu Phe Glu Glu Lys Ile Lys Lys Ser Pro Leu Thr Ile Cys Tyr Pro Glu Tyr Ala Gly Ser Asn Thr Tyr Glu Glu Ala Ala
AAG GAT CTC TTT GAA GAA AAA ATC AAG AAG AGC CCT CTC ACT ATA TGC TAT CCA GAA TAT GCA GGC TCA AAC ACA TAT GAA GAG GCA GCT   900
                               310                               320                               330
Ala Tyr Ile Gln Cys Gln Phe Glu Asp Leu Asn Lys Arg Lys Asp Thr Lys Glu Ile Tyr Thr His Phe Thr Cys Ala Thr Asp Thr Lys
GCG TAC ATT CAG TGT CAG TTT GAA GAC CTC AAT AAG AGA AAG GAC ACA AAG GAA ATA TAC ACC CAC TTC ACG TGC GCC ACG GAC ACC AAG   990
                               340                               350
Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys Gly Leu Phe
AAC GTG CAG TTC GTC TTT GAT GCC GTA ACA GAC GTC ATC ATA AAA AAT AAC CTA AAA GAC TGT GGT CTC TTC TGA GTGTTGGCGGCAAATGGTAA  1085
AATGCATTTTCAAACCAAATGAGTACTTACATGTGGATCTCTCTAGACTAGAGTCTTGCAGCAACACAGAATGTAGTATATGGCGAGTGCATCTGGGACCTGACCAAAGCTGTTCTATTT  1205
GTTTTTTTTTTAACTGAAAGTAATGGAAGGACCTTTCGTAAGTGTGAGAGGTGGTCCTGCAGTGTGAAACTAAGGGCAGTGTTAAAGCTGGGCTCTAGTGTACGGATGACTTCTACATAC  1325
ATGTAAATATGCAAATGTATGTATACATGTATTTATGACTTTAGTTTTCCACATTACTTTTAGACATTCAGTAAGCGGCAACTTATAATTTTAGCGTGGTGGCTTTGGAAATAACAGAAA  1445
TATTAAGTACTTTGTACTGAATGACAGACTATTGTCATGTTTGCCAGTTCTAAACAGCTTTATTTATGTTTCTGTCCTGTAAATTTTTAAGTACAATGATTAATATTGGGACACATTGCA  1565
GCTCTTGTCTTGATTATATGTAGTATACTTGTAATCATAAATGTTATTTGTACAAACATTGCACAGACTATTTTAATAACATGATTTGTTCTTTAAATTTATGTGTTTTATTGAAATGTT  1685
CTTGAAGAAGATGACTATACCTGCCTTTGGATCAGTTAAACACTGTATGCATTTCAGTTTTTCTTTTAAGGGTGCATGCTAATCTGATTCTACATTAGATTTGGTTTAAAAACTGTAAAA  1805
TGCAGGTTTCTGAGGATATACATATAGACTTATAAACACTTAATTTTTATTCAGTTGGTTTGTTTTCACTTTGAATTTTAATATTTGGATGGTATTCATGCATTGCCTTAAAGGTGATGC  1925
CAAGTTAATTTTTATACACTTCAAATAACTACATTTTTATTTATAAGTAAGTTTAGGTGGGTGCAAGAGCATTTTTGTGGGTAAAAAATAAGTTAGCATAATGAATTTTGAGACATTCAT  2045
TTGTGTATTTCCTTTGGGAAATCCCTTGTACGTACCATACATGACAGCTCTTTGTTCGGAAGGTAACAGGAAAGACCTCGAAGATTCTGCTACCGATAAAATGCAGCCTTTAAATTCACA  2165
TATGTAAGTAACTAGTTTCAAATTTAATTATCACACTATATTAAAATTACTTTTTTCCCTGAGATTATTCAAATTTCCTCCACTTGCTTGAGTTTGTATTATTTCTTTAAACTGTGCTAT  2285
TATCTCTGAGAATGAAATGGGCAATTACACTTAGGAAAATGAGTAATCGTATTTAATTAAGTTAGCAATTGTATGTATTCTCAAGTAAGTATTACATTTTTGCTAGATATTAAAATTTTG  2405
ATAGTCACTTTTGTTTTAATTATTCACAGTATCTCTCTATGACCTTGTTTTCAGCAAAGTGAACAGCATTCCATACCTTACTTCTCTTTTTTTACTCATCTTAAAAACATTATGTAGTGT  2525
TTCAATAAACTTTGTGGGTAAGTAGTTTCTAAATTTAGTTGTGTGTTATCATTTTGTTGAGGTCTATTTGTTGCAGTGTGTGTGTGTTTGTGTGTGTGTATGTAACCAGAAACTACATTT  2645
ACATCTGTTTCATTTGGGGGATTTTTCCTTTTTGTAATGTAAAGAAGTTCAAAGTTATCAGAATTCTTTAAATAAATGTGTTAGTTTAGATTCTTTATGTGCCTTTCATGAAAGAAATGT  2765
TTTCATTAATTTTATGGATAGAAGACCTGTTGTATTCATCTTATGAAGCTATGTATGAAATTCAACTGTCCTGTGAATCGATTTGAATCATGAGAAATAACAGCTTAAAAAGCCACAAAG  2885
AAGCACATATTGGTGACCACCATTGATGAATTCCTGAACTTTACTCTGTGTAATTGTGTTACTAATAAAATCTAATAAATTCGGATTTTTAAAATTTT--3'
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Fig.2. Nucleotide sequence of the cDNA encoding the α-subunit of bovine G_i. Nucleotide residues are numbered in the 5'- to 3'-direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5'-side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each line is given. The deduced amino acid sequence of the G_i α-subunit is shown above the nucleotide sequence, and amino acid residues are numbered beginning with the initiating methionine. The 5'-terminal sequence presented does not extend to the 5'-end of the mRNA. The 3'-terminal sequence shown is followed by a poly(dA) tract connected with the vector DNA sequence [13]. The 3'-noncoding region contains 4 and 2 copies of the polyadenylation signals AATAAA [14] (nucleotides 2529–2534, 2716–2721, 2949–2954 and 2959–2964) and ATTAAA [15] (nucleotides 2205–2210 and 2394–2399), respectively.

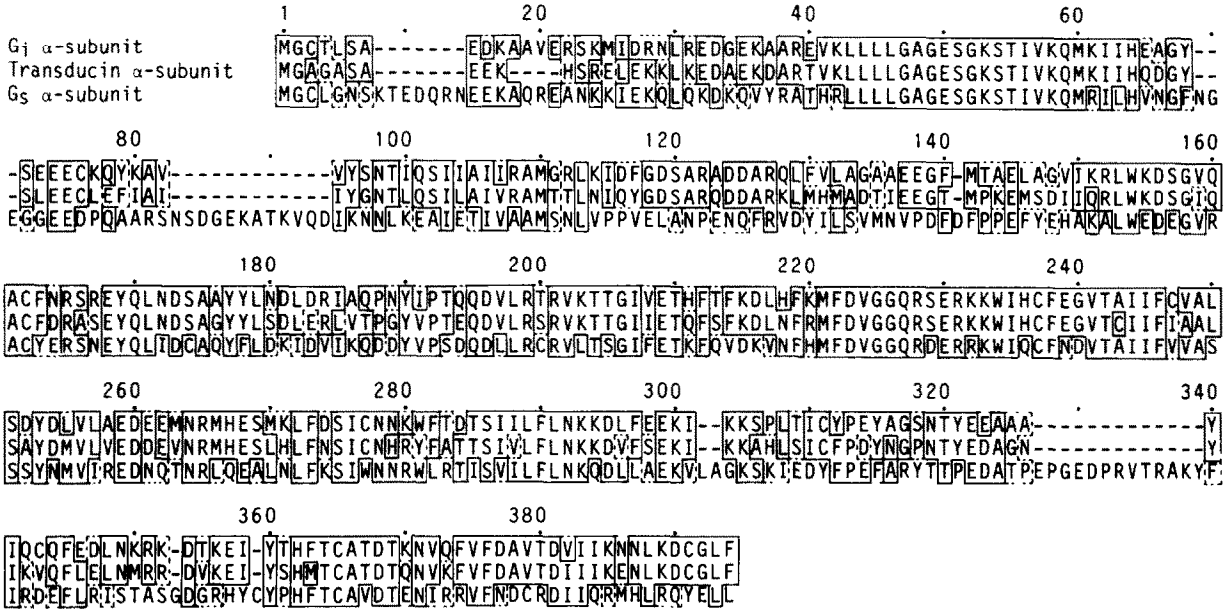

Fig.3. Alignment of the amino acid sequences of the α-subunits of bovine G_i (top), transducin (middle) and G_s (bottom). The one-letter amino acid notation is used. The sequence data for the α-subunits of transducin and G_s have been taken from [3] and [2], respectively. Sets of identical residues are enclosed by solid lines and of conservative residues by dashed lines. Conservative amino acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W [16]. Gaps (–) have been inserted to achieve maximum homology. The positions in the aligned sequences including gaps are numbered beginning with that of the initiating methionine.

located close to the β-phosphate group of the GDP ligand [22–24]. Furthermore, the two regions of *ras* proteins mentioned above are thought to be involved in GTPase activity [25–27]. The segment comprising positions 287–300 is homologous with the region of elongation factor Tu and *ras* proteins that is implicated in interaction with the guanine ring through the side chains of the asparagine and the aspartic acid corresponding to those at positions 292 and 295 [22–24].

The hydropathy profile [28] and the predicted secondary structures [29] of the G_i α-subunit are generally similar to those of the α-subunits of transducin [3] and G_s [2]. The region comprising positions 241–251 of all 3 G-protein α-subunits represents a highly hydrophobic segment with predicted secondary structure. This region corresponds to one of the β-strands proposed as being located in the vicinity of the guanine nucleotide-binding site of elongation factor Tu and *ras* proteins [23]. It is also possible that this region is involved in hydrophobic interaction with other subunits of the G-proteins, with receptor or effector proteins or with the plasma membrane.

The carboxy-terminal nonapeptide sequence of the transducin α-subunit (positions 386–394, fig.3) has been identified as the site that is ADP-ribosylated by IAP [11,30]. The ADP-ribose is linked to the cysteine at position 391 [30]. The G_i α-subunit, which is also ADP-ribosylated by IAP [31], contains a cysteine at the corresponding position, and the carboxy-terminal region of the G_i α-subunit is highly homologous with that of the transducin α-subunit. This is consistent with the finding that antibodies against the carboxy-terminal peptide of M_r 5000 of the transducin α-subunit cross-react with the G_i α-subunit [32].

The observation that the α-subunit of G_i shows higher sequence homology with that of transducin compared to G_s may suggest a functional similarity between G_i and transducin. In fact, it has been reported that G_i, like transducin, exhibits

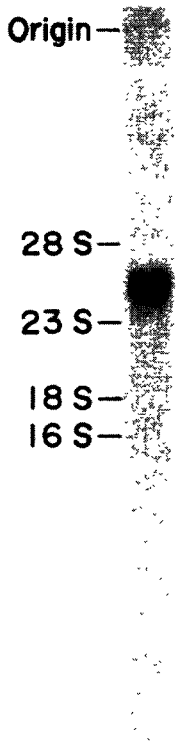

Fig.4. Autoradiogram of blot hybridization analysis of bovine cerebral cortex poly(A)$^+$ RNA with a G_i α-subunit cDNA probe. Poly(A)$^+$ RNA was isolated as in [2], and analysed by the procedure in [19]; the amount used was 15 μg. The hybridization probe was the *Bst*NI(−7)-*Bst*NI(695) fragment excised from clone pGα28 and labelled by nick-translation [20] with [α-^{32}P]dCTP; the restriction sites are identified by numbers indicating the 5′-terminal nucleotide generated by cleavage. The size markers were bovine and *Escherichia coli* rRNA.

rhodopsin-stimulated GTPase activity [33,34] and that transducin, like G_i, inhibits G_s-stimulated adenylate cyclase activity [35].

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