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Biochemistry

Sequence analysis of cDNA and genomic DNA for a putative pertussis toxin-insensitive guanine nucleotide-binding regulatory protein α subunit

(signal transduction/ADP-ribosylation)

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ABSTRACT We have isolated cDNA clones from rat C6 glioma cells coding for several guanine nucleotide-binding regulatory protein (G protein) α subunits (G_α). The cDNA clones were then used to isolate human chromosomal genes. Among human genomic clones isolated by cross-hybridization with the rat cDNA for the α subunit of the inhibitory G protein G_{i2}, termed $G_{i2\alpha}$, a clone designated λHGi62 was found to contain a sequence that is highly homologous but distinct from any of the known G_α sequences, and we have tentatively designated this sequence $G_{x\alpha}$. We have searched a rat brain cDNA library with the $G_{x\alpha}$ sequence and isolated a cDNA clone containing a rat sequence similar to human $G_{x\alpha}$. The cDNA contained a single open reading frame of 1065 nucleotides coding for a protein of 355 amino acids with a calculated molecular weight of 40,879. The amino acid sequence of rat $G_{x\alpha}$ shows 66% and 40% similarity with rat $G_{i2\alpha}$ and rat $G_{s\alpha}$ (the α subunit of the stimulatory G protein), respectively. By RNA blot hybridization analysis, mRNA of ≈3.2 kilobases was detected mainly in brain. Interestingly, the deduced amino acid sequence of $G_{x\alpha}$ predicts that the $G_{x\alpha}$ protein may be refractory to modification by pertussis toxin since the cysteine residue in the fourth position from the C terminus of pertussis toxin-sensitive G_α is replaced by isoleucine.

Guanine nucleotide-binding regulatory proteins (G proteins) are involved in a variety of receptor-mediated signal-transduction systems (for a review, see ref. 1). Several G proteins have been purified and characterized: G_s and G_i are involved in stimulation and inhibition, respectively, of adenylate cyclase activity, whereas transducin (G_t) activates cGMP phosphodiesterase in response to photosignal transduction. Another G protein (G_o), which is abundant in brain, may function in neuronal responses, although its precise function has not yet been clarified.

G proteins are heterotrimers, composed of α, β, and γ subunits. The α subunit (G_α), which binds GTP, is specific to each G protein, whereas common β and γ subunits (G_β and G_γ, respectively) may be found in some G proteins. The α subunits of some G proteins are substrates for ADP-ribosylation catalyzed by cholera toxin (2, 3) and pertussis toxin (4). The α subunit of G_s ($G_{s\alpha}$) is ADP-ribosylated by cholera toxin, whereas the α subunits of G_i and G_o ($G_{i\alpha}$ and $G_{o\alpha}$, respectively) are modified by pertussis toxin. The α subunit of G_t ($G_{t\alpha}$) is ADP-ribosylated by both cholera and pertussis toxins. The sensitivity toward these toxins has often been used as a criterion for involvement of G proteins in certain cellular functions and also used for visualization of G_α polypeptides after $NaDodSO_4$/polyacrylamide gel electrophoresis by labeling with a radioactive NAD. Thus, it has been shown that the pertussis toxin-sensitive G proteins are

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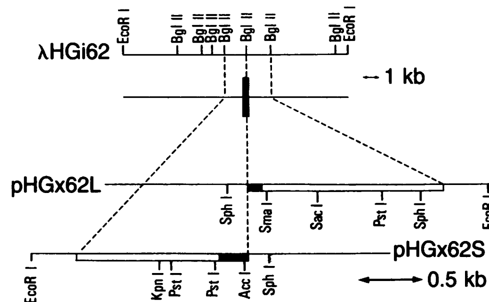

FIG. 1. Restriction maps of λHGi62, pHGx62L, and pHGx62S inserts. The coding region of a putative exon of $G_{x\alpha}$ is indicated by shaded bars. *Bgl* II fragments (1.6 and 1.4 kb) of λHGi62 were subcloned to the *Bam*HI site of pBR327. Open bars indicate inserts in the pHG plasmids.

responsible for not only inhibition of adenylate cyclase but also for activation of phospholipase C (5) and phospholipase A_2 (6) as well as for gating of K^+ (7–9) and Ca^{2+} (10, 11) channels. The molecular heterogeneity of substrates for pertussis toxin was identified by gel electrophoresis of labeled ADP-ribosylated proteins (12, 13). It has also been suggested that a species of pertussis toxin-insensitive G protein is involved in the activation of phospholipase C (1, 14).

Much effort has been focused on the cloning and sequence determination of cDNAs coding for the various G_α polypeptides. Thus, cDNAs for $G_{s\alpha}$ from bovine adrenal gland (15), bovine brain (16), rat C6 glioma cells (17), and human liver (18) have been reported. $G_{o\alpha}$ cDNA was cloned from rat C6 glioma cells (17) and bovine retina (19); two $G_{t\alpha}$ cDNAs were cloned from bovine retina, one coding for the α subunit of G_t from rod cells ($G_{t1\alpha}$) (20–22) and the other for the α subunit of G_t from cone cells ($G_{t2\alpha}$) (23). As for $G_{i\alpha}$ cDNAs, at least three subtypes have been reported. In this paper, we adopted the nomenclature used by Gilman (1) and Jones and Reed (24). Thus, $G_{i1\alpha}$ is the predominant $G_{i\alpha}$ species in mammalian brain, for which the cDNA sequence from bovine brain was reported by Nukada *et al.* (25) and the cDNA sequence from the human brain was reported by Bray *et al.* (26). $G_{i2\alpha}$ was cloned from rat C6 glioma cells by Itoh *et al.* (17) and then cloned from mouse macrophages (27), from human monocytes (28), and from human T lymphocytes (29). The third $G_{i\alpha}$ subtype $G_{i3\alpha}$ was cloned from human granulocytes (30), human liver (31), human T lymphocytes (29), and rat C6 glioma cells (32). We have obtained the genomic sequences for human $G_{s\alpha}$ (33) as well as human $G_{i1\alpha}$, $G_{i2\alpha}$, and $G_{i3\alpha}$ (32).

Abbreviations: G protein, guanine nucleotide-binding regulatory protein; G_α, α subunit of G protein; G_s, G_i, G_o, G_x, and G_t, stimulatory, inhibitory, other, tentative designation, and transducin G proteins, respectively; $G_{s\alpha}$, $G_{i\alpha}$, $G_{o\alpha}$, etc., α subunit of G_s, G_i, G_o, etc., respectively.

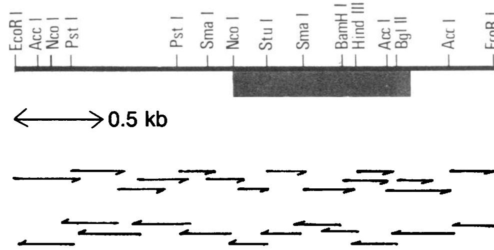

FIG. 2. Restriction map of the λRGx2 insert and strategy of sequence determination. Closed box, coding region; arrows, sequence strategy.

We have obtained a human genomic clone whose partial sequence was similar to, but distinct from, any published G_α sequence. We have isolated and sequenced a cDNA clone from rat brain corresponding to this G_α species, and we have tentatively designated this species $G_{x\alpha}$.* By RNA blot hybridization, we found that the $G_{x\alpha}$ gene is expressed mainly in neuronal tissues. The deduced amino acid sequence of $G_{x\alpha}$ suggests that this protein may represent the putative pertussis toxin-resistant G_α species.

METHODS AND MATERIALS

Human Genomic and Rat cDNA Libraries. A human genomic library (34) was kindly provided by T. Maniatis (Harvard University, Cambridge, MA). A rat brain cDNA library was constructed as described (17), except for the use of random hexamer primers.

Library Screening. The genomic and cDNA libraries were screened by the plaque-hybridization method (35). Hybridization was performed at 42°C (a high-stringency condition) or at 28°C (a low-stringency condition) overnight in a solution containing 50% (vol/vol) formamide, 5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate, pH 7.0), 1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll), 20 mM sodium phosphate (pH 7.0), heat-denatured calf thymus DNA (100 μg/ml), 0.1% $NaDodSO_4$, and 10% (wt/vol) dextran sulfate. Filters were washed at room temperature in 0.1× SSC/0.1% $NaDodSO_4$ for high stringency or in 1× SSC/0.1% $NaDodSO_4$ for low stringency. ^{32}P-labeled probes were prepared by nick-translation of DNA fragments purified from low-temperature-melting agarose gels.

DNA Sequence Analysis. DNA sequencing was performed by the dideoxynucleotide chain-termination method (36) with [α-^{32}P]dCTP. 2'-Deoxy-7-deazaguanosine 5'-triphosphate was used in place of dGTP (37).

RNA Blot Hybridization Analysis. Total RNA was extracted by the guanidinium thiocyanate method (38). RNA was denatured by heating at 60°C for 5 min in 2.2 M formaldehyde/50% (vol/vol) formamide and subjected to electrophoresis in a 1.2% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to nitrocellulose filters. Hybridization was carried out under the high-stringency conditions described above. Filters were washed at 65°C in 0.1× SSC/0.1% $NaDodSO_4$.

RESULTS

Isolation and Partial Characterization of a Human Genomic Clone Encoding $G_{x\alpha}$. We screened a human genomic library (3×10^5 phage plaques) with the 1.7-kilobase (kb) *Eco*RI fragment of λGX13 (17), which contains the entire coding sequence of rat $G_{i2\alpha}$. Eighteen clones were isolated under low-stringency conditions. Among them, a clone, designated λHGi62, that hybridized weakly with the $G_{i2\alpha}$ cDNA probe was purified and characterized, and the G_α species that it encodes was tentatively named $G_{x\alpha}$.

As shown in Fig. 1 (*Upper*), the *Eco*RI insert of phage λHGi62 possessed seven *Bgl* II sites. On digestion with *Bgl* II, two fragments (1.4 kb and 1.6 kb) were found to hybridize with

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03773).

FIG. 3. Nucleotide sequence and deduced amino acid alignment of the λRGx2 insert. Numbers indicate the position of nucleotides or amino acid residues starting at the initiator codon. The 5'-flanking region is shown up to position −14.

$G_{i2\alpha}$ cDNA. The 1.4-kb and 1.6-kb *Bgl* II fragments were subcloned to the *Bam*HI site of pBR327, yielding pHGx62S and pHGx62L, respectively, and subjected to DNA sequence analysis.

The results of the sequence determination for the $G_{x\alpha}$ sequence revealed that there is an open reading frame of 114 amino acid codons, encompassing the junction of two *Bgl* II fragments (Fig. 1). Comparison of the deduced amino acid sequence of the $G_{x\alpha}$ open reading frame with the amino acid sequences encoded by human genes for $G_{s\alpha}$ (33) and $G_{i2\alpha}$ and $G_{i3\alpha}$ (32) indicated that it is similar to the C-terminal region corresponding to the sequences encoded by exons 7 and 8 of $G_{i\alpha}$ genes. However, the $G_{x\alpha}$ sequence is clearly distinct from characterized G_α sequences since the corresponding regions of the amino acid sequences for human $G_{s\alpha}$, $G_{i1\alpha}$, $G_{i2\alpha}$, and $G_{i3\alpha}$ were 41%, 77%, 75%, and 78% identical, respectively.

The open reading frame of the $G_{x\alpha}$ gene is preceded by the consensus sequence for the 3′ end of the introns (AG) and succeeded by a termination codon (TGA). Apparently, the sequences corresponding to exons 7 and 8 of the G_i subfamily are connected in $G_{x\alpha}$, eliminating intron 7.

Isolation of a Rat cDNA Clone Coding for $G_{x\alpha}$ and Its Nucleotide Sequence. To determine which cells and tissues express the $G_{x\alpha}$ gene, we carried out RNA blot hybridization analysis of various rat tissues. When hybridization was performed under high-stringency conditions with a labeled probe (the 0.36-kb *Sph* I–*Sma* I fragment of pHGx62L) containing 123 nucleotides of the 3′ coding region of human $G_{x\alpha}$, an ≈3.2-kb mRNA was detected mainly in brain (data not shown). We, therefore, constructed a rat brain cDNA library for the isolation of rat $G_{x\alpha}$ cDNA.

A rat brain cDNA library (6×10^5 phage plaques) was screened with the same probe under high-stringency conditions. Two clones, λRGx1 and λRGx2, were obtained containing inserts of 0.6 kb and 2.8 kb, respectively. The cDNA insert of λRGx2 was subcloned to the *Eco*RI site of pUC119. Fig. 2 shows the restriction map of the λRGx2 insert and the strategy for sequence determination.

Nucleotide and deduced amino acid sequences are shown in Fig. 3. The λRGx2 cDNA contained an open reading frame coding for 355 amino acids, assuming that the initiator methionine codon is at positions 1–3 and the termination codon TGA is at positions 1066–1068. (The nucleotide position assignments were based on the alignment with the amino acid sequence of $G_{i2\alpha}$, which also consists of 355 amino acids.) The nucleotide sequence surrounding the first methionine codon agreed fairly well with the consensus sequence that is characteristic for the initiation codon of many eukaryotic mRNAs (39). The calculated molecular weight of the polypeptide specified by this open reading frame (1065 base pairs) is 40,879.

Fig. 4 compares the amino acid sequence of rat $G_{x\alpha}$ with those of rat $G_{i1\alpha}$, $G_{i2\alpha}$, $G_{i3\alpha}$, $G_{o\alpha}$, and $G_{s\alpha}$ as well as with those of bovine $G_{t1\alpha}$ and $G_{t2\alpha}$. Strong similarities were observed over the entire sequence. However, the similarity of $G_{x\alpha}$ to the $G_{i\alpha}$ subtypes, $G_{o\alpha}$, $G_{t\alpha}$ subtypes, or $G_{s\alpha}$ is not as great as the similarity between the $G_{i\alpha}$ subtypes and $G_{o\alpha}$.

The 3′-flanking sequence of rat $G_{x\alpha}$ cDNA was determined down to position 1515; however, no polyadenylylation signal of mammalian mRNA (AATAAA) was found.

Expression of the $G_{x\alpha}$ Gene. We have carried out the RNA blot hybridization analysis of several rat tissues. The 1515-

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Gxα : MGCRQSS-------EEKEAARRSRRIDRHLRSESQRQRREIKLLLLGTSNSGKSTIVKQM  53
Gi1α: MGCTLSA-------EDKAAVERSKMIDRNLREDGEKAAREVKLLLLGAGESGKSTIVKQM  53
Gi2α: MGCTVSA-------EDKAAAERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQM  53
Gi3α: MGCTLSA-------EDKAAVERSKMIDRNLREDGEKAAKEVKLLLLGAGESGKSTIVKQM  53
Goα : MGCTLSA-------EERAALERSKAIEKNLKEDGISAAKDVKLLLLGAGESGKSTIVKQM  53
Gt1α: MGAGASA-------EEK---HSRELEKKLKEDAEKDARTVKLLLLGAGESGKSTIVKQM  49
Gt2α: MGSGASA-------EDKELAKRSKELEKKLQEDADKEAKTVKLLLLGAGESGKSTIVKQM  53
Gsα : MGCLGNSKTEDQRNEEKAQREANKKIEKQLQKDKQVYRATHRLLLLGAGESGKSTIVKQM  60

Gxα : KIIHSGGFNLE---------------ACKEYKPLIIYNAIDSLTRIIRALAALKIDFHNP  98
Gi1α: KIIHEAGYSEE---------------ECKQYKAVVYSNTIQSIIAIIRAMGRLKIDFGDA  98
Gi2α: KIIHEDGYSEE---------------ECRQYRAVVYSNTIQSIMAIVKAMGNLQIDFADP  98
Gi3α: KIIHEDGYSED---------------ECKQYKVVVYSNTIQSIIAIIRAMGRLKIDFGEA  98
Goα : KIIHEDGFSGE---------------DVKQYKPVVYSNTIQSLAAIVRAMDTLGVEYGDK  98
Gt1α: KIIHQDGYSLE---------------ECLEFIAIIYGNTLQSILAIVRAMTTLNIQYGDS  94
Gt2α: KIIHQDGYSPE---------------ECLEYKAIIYGNVLQSILAIIRAMPTLGIDYAEV  98
Gsα : RILHVNGFNGEGGEEDPQAARSNSDGEKATKVQDIKNNLKEAIETIVAAMSNLVPPVELA 120

Gxα : DRAYDAVQLFALTGPAESKGEITPELLGVMRRLWADPGAQACFGRSSEYHLEDNAAYYLN 158
Gi1α: ARADDARQLFVLAGAAEE-GFMTAELAGVIKRLWKDSGVQACFNRSREYQLNDSAAYYLN 157
Gi2α: QRADDARQLFALSCAAEEQGMLPEDLSGVIRRLWADHGVQACFGRSREYQLNDSAAYYLN 158
Gi3α: ARADDARQLFVLAGSAEE-GVMTSELAGVIKRLWRDGGVQACFSRSREYQLNDSASYYLN 157
Goα : ERKADSKMVCDVVSRMEDTEPFSAELLSAMMRLWGDSGIQECFNRSREYQLNDSAKYYLD 158
Gt1α: ARQDDARKLMHMADTIEE-GTMPKEMSDIIQRLWKDSGIQACFDRASEYQLNDSAGYYLS 153
Gt2α: SCVDNGRQLNNLADSIEE-GTMPPELVEVIRKLWKDGGVQACFDRAAEYQLNDSASYYLN 157
Gsα : NPENQFRVDYILSVMNVPNFDFPPEFYEHAKALWEDEGVRACYERSNEYQLIDCAQYFLD 180

Gxα : DLERIAAPDYIPTVEDILRSRDMTTGIVENKFTFKELTFKMVDVGGQRSERKKWIHCFEG 218
Gi1α: DLDRIAQPNYIPTQQDVLRTRVKTTGIVETHFTFKDLHFKMFDVGGQRSERKKWIHCFEG 217
Gi2α: DLERIAQSDYIPTQQDVLRTRVKTTGIVETHFTFKDLHFKMFDVGGQRSERKKWIHCFEG 218
Gi3α: DLDRISQTNYIPTQQDVLRTRVKTTGIVETHFTFKELYFKMFDVGGQRSERKKWIHCFEG 217
Goα : SLDRIGAADYQPTEQDILRTRVKTTGIVETHFTFKNLHFRLFDVGGQRSERKKWIHCFED 218
Gt1α: DLERLVTPGYVPTEQDVLRSRVKTTGIIETQFSFKDLNFRMFDVGGQRSERKKWIHCFEG 213
Gt2α: QLDRITAPDYLPNEQDVLRSRVKTTGIIETKFSVKDLNFRMFDVGGQRSERKKWIHCFEG 217
Gsα : KIDVIKQADYVPSDQDLLRCRVLTSGIFETKFQVDKVNFHMFDVGGQRDERRKWIQCFND 240

Gxα : VTAIIFCVELSGYDLKLYEDNQTSRMAESLRLFDSICNNNWFINTSLILFLNKKDLLSEK 278
Gi1α: VTAIIFCVALSDYDLVLAEDEEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEK 277
Gi2α: VTAIIFCVALSAYDLVLAEDEEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEK 278
Gi3α: VTAIIFCVALSDYDLVLAEDEEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEK 277
Goα : VTAIIFCVALSGYDQVLHEDETTNRMHESLMLFDSICNNKFFIDTSIILFLNKKDLFGEK 278
Gt1α: VTCIIFIAALSAYDMVLVEDDEVNRMHESLHLFNSICNHRYFATTSIVLFLNKKDVFSEK 273
Gt2α: VTCIIFCAALSAYDMVLVEDDEVNRMHESLHLFNSICNHKFFAATSIVLFLNKKDLFEEK 277
Gsα : VTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQDLLAEK 300

Gxα : IR--RIPLSVCFPEYKGQNTYEE-------------AAVYIQRQFEDLNRNKETKEIY-- 321
Gi1α: IK--KSPLTICYPEYAGSNTYEE-------------AAAYIQCQFEDLNKRKDTKEIY-- 320
Gi2α: IT--QSPLTICFPEYTGANKYDE-------------AASYIQSKFEDLNKRKDTKEIY-- 321
Gi3α: IK--RSPLTICYPEYTGSNTYEE-------------AAAYIQCQFEDLNRRKDTKEVY-- 320
Goα : IK--KSPLTICFPEYPGSNTYED-------------AAAYIQTQF-ESKNRSPNKEIY-- 320
Gt1α: IK--KAHLSICFPDYNGPNTYED-------------AGNYIKVQFLELNMRRDVKEIY-- 316
Gt2α: IK--KVHLSICFPEYDGNNSYED-------------AGNYIKSQFLDLNMRKDVKEIY-- 320
Gsα : VLAGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRDEFLRISTASGDGRHYCY 360

Gxα : SHFTCATDTSNIQFVFDAVTDVIIQNNLKYIGLC 355
Gi1α: THFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF 354
Gi2α: THFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF 355
Gi3α: THFTCATDTKNVQFVFDAVTDVIIKNNLKECGLY 354
Goα : CHMTCATDTNNIQVVFDAVTDIIIANNLRGCGLY 354
Gt1α: SHMTCATDTQNVKFVFDAVTDIIIKENLKDCGLF 350
Gt2α: SHMTCATDTQNVKFVFDAVTDIIIKENLKDCGLF 354
Gsα : PHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL 394
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FIG. 4. Amino acid alignment (standard one-letter abbreviations) of rat $G_{x\alpha}$ and the other mammalian G_α proteins [rat $G_{i1\alpha}$ (24), rat $G_{i2\alpha}$ (17), rat $G_{i3\alpha}$ (32), rat $G_{o\alpha}$ (24), bovine $G_{t1\alpha}$ (20), bovine $G_{t2\alpha}$ (23), and rat $G_{s\alpha}$ (17)]. Sets of identical or conservative residues are enclosed with solid lines. Conservative substitutions are grouped as follows: cysteine; serine, threonine, proline, alanine, and glycine; asparagine, aspartic acid, glutamic acid, and glutamine; histidine, arginine, and lysine; methionine, isoleucine, leucine, and valine; phenylalanine, tyrosine, and tryptophan. Hyphens indicate gaps introduced to obtain maximal identity.

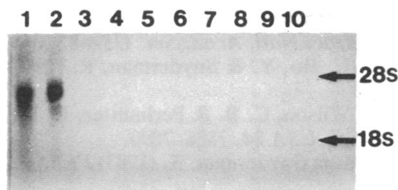

FIG. 5. RNA blot hybridization of various rat tissues. Twenty micrograms of total RNA from brain (lanes 1 and 2), fat cells (lane 3), heart (lane 4), kidney (lane 5), liver (lanes 6 and 7), lung (lane 8), or spleen (lanes 9 and 10) was applied per lane. For brain, liver, and spleen, total RNA of different preparations was used for two lanes. Positions of 18S and 28S rRNA are indicated. The probe was a ^{32}P-labeled 1515-base-pair *Nco* I–*Eco*RI fragment from λRGx2.

base-pair *Nco* I–*Eco*RI Fragment of λRGx2, containing the coding and the 3′-flanking regions of rat $G_{x\alpha}$ cDNA, was labeled by nick-translation and used as a probe. The results indicated that the 3.2-kb $G_{x\alpha}$ mRNA was expressed almost exclusively in brain (Fig. 5). It was not observed in other tissues such as fat, heart, kidney, liver, and lung. However, a faint band at the same position was detected in spleen by radioautography after a long exposure (data not shown).

DISCUSSION

In this paper, we have isolated a rat cDNA and part of a human gene encoding a G_α, termed $G_{x\alpha}$, that is similar to, but clearly distinct from, any characterized mammalian G_α. The predicted $G_{x\alpha}$ protein contains 355 amino acid residues with a calculated molecular weight of 40,879. The deduced amino acid sequence of the $G_{x\alpha}$ protein is 40%, 67%, 66%, 66%, 55%, 57%, and 59% identical, respectively, to $G_{s\alpha}$, $G_{i1\alpha}$, $G_{i2\alpha}$, $G_{i3\alpha}$, $G_{t1\alpha}$, $G_{t2\alpha}$, and $G_{o\alpha}$. Therefore, $G_{x\alpha}$ is most closely related to the $G_{i\alpha}$ subfamily, but distinct from it, since the similarity between $G_{x\alpha}$ and any of the $G_{i\alpha}$ subtypes is far less than that between the $G_{i\alpha}$ subtypes (>85%).

Comparison of the predicted amino acid sequences of known G_α proteins from mammalian cells indicates three highly conserved regions (Fig. 6). The region responsible for GTP hydrolysis is shown in Fig. 6*A*, and the site for interaction with the guanine ring is shown in Fig. 6*C*. Fig. 6*B* shows the region that is highly conserved among the various G_α species. The sequence of the $G_{x\alpha}$ protein is very similar to that of other G_α proteins, but three amino acid residues in the GTP-hydrolysis site of $G_{x\alpha}$ (Thr-Ser-Asn, at positions 41–43) are different from these residues in other G_α proteins (Ala-Gly-Glu).

The most remarkable feature of the primary structure of the $G_{x\alpha}$ is that it lacks the cysteine residue in the fourth position from the C terminus, having instead an isoleucine (Fig. 7). The cysteine at the fourth position from the C terminus is a feature common to all G proteins susceptible to modification by pertussis toxin, and the cysteine residue at this position of $G_{t\alpha}$ has been shown to be ADP-ribosylated by pertussis toxin (40). $G_{s\alpha}$, which is resistant to modification by pertussis toxin, has a tyrosine in the fourth position from the C terminus instead of cysteine.

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A     Gxα: 35-50  : K L L L L G T S N S G K S T I V
     Gi1α: 35-50  : K L L L L G A G E S G K S T I V
     Gi2α: 35-50  : K L L L L G A G E S G K S T I V
     Gi3α: 35-50  : K L L L L G A G E S G K S T I V
      Goα: 35-50  : K L L L L G A G E S G K S T I V
     Gt1α: 31-46  : K L L L L G A G E S G K S T I V
     Gt2α: 35-50  : K L L L L G A G E S G K S T I V
      Gsα: 42-57  : R L L L L G A G E S G K S T I V

B     Gxα: 201-218 : D V G G Q R S E R K K W I H C F E G
     Gi1α: 200-217 : D V G G Q R S E R K K W I H C F E G
     Gi2α: 201-218 : D V G G Q R S E R K K W I H C F E G
     Gi3α: 200-217 : D V G G Q R S E R K K W I H C F E G
      Goα: 201-218 : D V G G Q R S E R K K W I H C F E D
     Gt1α: 196-213 : D V G G Q R S E R K K W I H C F E G
     Gt2α: 200-217 : D V G G Q R S E R K K W I H C F E G
      Gsα: 223-240 : D V G G Q R D E R R K W I Q C F N D

C     Gxα: 263-274 : T S L I L F L N K K D L
     Gi1α: 263-274 : T S I I L F L N K K D L
     Gi2α: 262-273 : T S I I L F L N K K D L
     Gi3α: 262-273 : T S I I L F L N K K D L
      Goα: 263-274 : T S I I L F L N K K D L
     Gt1α: 258-269 : T S I V L F L N K K D V
     Gt2α: 263-273 : T S I V L F L N K K D L
      Gsα: 285-296 : I S V I L F L N K Q D L
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FIG. 6. Amino acid comparison (standard one-letter abbreviations) of mammalian G_α proteins in the three highly homologous regions (*A*, *B*, and *C*). Sets of identical amino acids are boxed. The sequences are shown in Fig. 4.

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 Gxα: 349-355 : L K Y I G L C
Gi1α: 348-354 : L K D C G L F
Gi2α: 349-355 : L K D C G L F
Gi3α: 348-354 : L K E C G L Y
 Goα: 348-354 : L R G C G L Y
Gt1α: 344-350 : L K D C G L F
Gt2α: 348-354 : L K D C G L F
 Gsα: 388-394 : L R Q Y E L L
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FIG. 7. Comparison of C-terminal amino acid sequence (standard one-letter abbreviations) of mammalian G_α proteins. The amino acid residues located at the fourth position from the C-terminal end that are not cysteine are shaded.

It has been suggested that a pertussis toxin- and cholera toxin-insensitive G protein is involved in activation of phospholipase C in a human astrocytoma cell line (41), a rat pituitary cell line (42), and rat liver plasma membrane (43). There are reports that a cholera toxin-sensitive and pertussis toxin-insensitive G protein is involved in the inositol phospholipid hydrolysis in a human pituitary cell line (44) and in a human T-lymphocyte line (45). It is not known whether $G_{x\alpha}$ protein is ADP-ribosylated in the presence of cholera toxin, although it possesses an arginine residue at amino acid position 179. The arginine residue at this position is ADP-ribosylated by cholera toxin in $G_{s\alpha}$ and $G_{t\alpha}$ (46), but not in $G_{i\alpha}$ and $G_{o\alpha}$. Thus $G_{x\alpha}$ could be a candidate for one of the proposed species of G_α.

RNA blot hybridization analysis indicated that the $G_{x\alpha}$ gene is expressed mainly in brain. Among the tissues examined, it is weakly expressed only in spleen. We observed $G_{x\alpha}$ mRNA also in the rat pituitary cell lines GH_3 and GH_4C_1 and in the rat pheochromocytoma cell line PC12 (unpublished observation). Therefore, $G_{x\alpha}$ protein may be present mainly in neuronal cells and to a small amount in lymphocytes. It will be of interest to examine the function of $G_{x\alpha}$ in the various cell lines above.

Note. Simon and colleagues (47) have found a human cDNA clone for the α subunit of another G protein, G_z, that corresponds to the human genomic clone and the rat cDNA clone for $G_{x\alpha}$.

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