Membranes from bovine brain bind relatively large quantities of guanosine 5′-3(O-thio)triphosphate (GTPγS) with high affinity. The two proteins responsible for most of this activity were purified; they account for 1.5% of the membrane protein. The two proteins contain α subunits of either 39,000 or 41,000 Da, β subunits of 36,000 or 35,000 Da, and a potential γ subunit (11,000 Da). These structures are the same as a family of proteins that includes transducin and the regulatory proteins, Gs and Gi1, of adenylate cyclase.

The 41,000- and 39,000-Da polypeptides can be ADP-ribosylated with islet-activating protein from Bordetella pertussis, bind guanine nucleotides specifically, and migrate through polyacrylamide gels with rates similar to the α subunits of Gi and Gs. The γ subunit is found whenever β subunits are present. The 41,000- and 39,000-Da polypeptides (with β and γ) are designated, respectively, Gα and Gγ from brain.

The α subunit of Gs was isolated without the use of ligands known to dissociate other G proteins. Gα binds GTPγS reversely in the absence of Mg2+ and is relatively stable in cholate. This isolated α subunit should be of great utility in elucidating the mechanism of action of this family of GTP-binding proteins.

The stimulation or inhibition of adenylate cyclase by hormones is mediated by two regulatory proteins, Gα1 and Gα2, respectively. T is a regulatory protein of the retina that participates by the payment of $5'-(3-O-thio)triphosphate: Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; SDS, sodium dodecyl sulfate.

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Isolation of Two Proteins with High Affinity for Guanine Nucleotides from Membranes of Bovine Brain*

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The mechanisms of action of Gα1, Gγ, and T are also quite similar. GTP is required for the activation of all three proteins in membranes, and GDPase activities have been implicated in their function (1–3). The α subunits contain the binding site for guanine nucleotides (8, 13, 23, 24), and activation of the purified proteins in vitro coincides with dissociation of the subunits (8, 10, 11, 13, 24). These and other experiments with purified subunits (8, 25, 26) have implicated the α subunits of these proteins as the active species in the regulation of adenylate cyclase by Gα1 and Gγ and cGMP-dependent phosphodiesterase by T. Additional experiments suggest that the β subunit may be a key element in the inhibition of adenylate cyclase by hormones (26) and may be involved in the deactivation of dissociated α subunits (27, 28).

Thus, Gα1, Gγ, and T comprise a family of homologous proteins with diverse functions. We now report the purification and partial characterization of a potential fourth member in this family. Purification of the high-affinity GTP-binding proteins from membranes of bovine brain yielded two proteins that, together, account for about 1.5% of the membrane protein. The α subunit of one protein has a molecular weight of 41,000 and is believed to be Gα2. The α subunit of the other G protein is 39,000 Da, is similar to but probably distinct from bovine rod cells. The purified protein contains two larger subunits (α, β) with molecular weights of 39,000 and 36,000, respectively (4–8). It also contains a smaller γ subunit of about 10,000 Da (6, 8). Gα1 has been purified from rabbit liver (9, 10) and turkey and human erythrocytes (11, 12); it was reported to have a composition consisting of two major subunits (α, β) with molecular weights of 45,000 and 35,000. A second α subunit of 52,000 Da was also obtained in lower quantities from the liver. Procedures developed for the purification of Gα1 from liver and erythrocytes have been slightly modified to purify Gγ from rabbit liver (13, 14) and human erythrocytes (15). Gγ contains two major subunits (α, β) with molecular weights of about 41,000 and 35,000. The presence of a potential γ subunit of smaller size has been observed in Gγ from liver (14) and human erythrocytes (16). A γ subunit is also reported to be present in Gs from human erythrocytes (16).

The structural homology of these three proteins is striking. Furthermore, the β subunits from Gα1, Gγ, and T have the same electrophoretic mobility in SDS gels, yield similar peptides upon proteolysis, and have identical amino acid compositions (17). The α subunits were also similar with respect to amino acid composition and proteolysis (17).

All three proteins can be modified by ADP-ribosylation. Thus, the α subunits of Gα1 can be specifically modified by cholera toxin (9, 18, 19), and the α subunit of Gγ can be modified by IAP, the ADP-ribosylating toxin of Bordetella pertussis (13, 20). Transducin α can be modified by both IAP (21) and cholera toxin (22).

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from the other G proteins, and can be isolated from its other subunits in a stable and unliganded form. The function of this protein is not yet clear.

EXPERIMENTAL PROCEDURES

Membrane Preparations—Bovine brains were obtained from the heads of freshly slaughtered cattle and placed in ice-cold 10 mM Tris-Cl, pH 7.5 (about 1 h). All further procedures were carried out at 0-4 °C. Cerebra were dissected crudely to remove any remaining brainstem and to excise large portions of white matter. The remaining cerebral tissue (~150-200 g/brain) was homogenized in a blender (medium speed) with 4 volumes of 10 mM Tris-Cl, pH 7.5, 10% sucrose. The homogenate was filtered through 4 layers of cheesecloth, and the membranes were collected by centrifugation at 20,000 × g for 30 min. The membrane pellet was suspended in 5 volumes of 10 mM Tris-Cl, pH 7.5, 10% sucrose with a Potter-Elvehjem homogenizer and collected by centrifugation at 20,000 × g for 60 min. The membranes were subjected to a second identical wash and then resuspended with the same solution to a protein concentration of about 20 mg/ml for storage at ~80 °C.

This procedure was also utilized to prepare membranes from brains of decapitated rats with the following changes: excision and cooling of brains was rapid (within 5 min after death) and buffers contained 0.5 mM phenylmethylsulfonyl fluoride.

Membranes from bovine heart (both atrial and ventricular muscle) were prepared at 0-4 °C as follows. Muscle tissue (250 g) was homogenized with 1,400 ml of 10 mM Tris·Cl, pH 7.5, 0.7 M KCl in a blender (high speed) and passed through 2 layers of cheesecloth. After centrifugation at 8,000 × g for 30 min, the pelleted membranes were suspended in 800 ml of 10 mM Tris·Cl, pH 7.5, and homogenized with a Polytron homogenizer, model PT-20, at a setting of 7 for 30 s. More dense membranes were separated from a lighter fraction by centrifugation at 8,000 × g for 20 min. This procedure was repeated three times, and the supernatants were collected each time. Supernatants from the three slow spins were centrifuged at 20,000 × g for 60 min to collect a fraction of less-dense membranes. These membranes were resuspended in the same buffer and stored at ~80 °C; the membranes were enriched 4- to 15-fold for muscarinic receptor-binding activity.

GTP-binding proteins were then eluted from the DEAE with a linear gradient of NaCl (2 liters; 0-225 mM) in TED/1% cholate. The eluate was collected in fractions of 23 ml. The gradient was followed by further elution with 1 liter of 500 mM NaCl in TED/1% cholate. Fig. 1 details the activities measured in the eluted fractions and will be discussed further under “Results.”

Fractions containing the major peak of GTP’S-binding activity (fractions 64-74) were pooled and concentrated to 28 ml by pressure filtration through an Amicon PM-30 membrane. The material was applied to a 1.2-liter column of Ultrogel AcA 34 (LKB) and eluted overnight with TED/1% cholate (400 mM NaCl). Fractions of 14.5 ml were collected. The major peak of GTP’S-binding activity (fractions 51-56; 87 ml) was diluted with 270 ml of TED/100 mM NaCl. This diluted pool was applied to a 100-ml column of heptylamine-Sepharose. The column was then washed with 100 ml of TED/0.25% cholate/300 mM NaCl. Elution of GTP’S-binding proteins was accomplished with a linear gradient (800 ml total) of TED/0.25% cholate/200 mM NaCl to TED/1.3% cholate/50 mM NaCl. Fractions of about 8 ml were collected in tubes that had been siliconized with Aquasil (Pierce Chemical Co.). Figs. 2 and 3 show the profiles of the activities that were eluted from these two columns.

Purified proteins from the heptylamine-Sepharose column were utilized directly for experiments or pooled appropriately and concentrated by filtration on an Amicon PM-30 membrane to about 1 mg/ml. The proteins could then be stored at ~80 °C or on ice for several weeks with little or no loss of binding activity.

Subunits of the G protein from brain were obtained by further treatment of fractions eluted from heptylamine-Sepharose that contained Gs activity; these procedures were described by Norup et al. (24).

Assays—G proteins were identified by their ability to bind GTP’S. Samples were diluted into 10 mM NaHepes, pH 8, 1 mM EDTA, 1 mM dithiothreitol, 0.1% w/v Lubrol 12A9. In the standard assay, 20 µl of diluted sample were mixed with 20 µl of 50 mM NaHepes, pH 8, 40 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 200 mM NaCl, 2 µM of 50 mM NaHepes, pH 8, 40 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 200 mM NaCl, 2 µM

FIG. 1. Chromatography of GTP’S-binding proteins through DEAE-Sepharose. See “Experimental Procedures” for explanation.

FIG. 2. Chromatography of GTP’S-binding proteins through Ultrogel AcA 34. See “Experimental Procedures” for explanation.

FIG. 3. Chromatography of GTP’S-binding proteins through heptylamine-Sepharose. See “Experimental Procedures” for explanation.
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GTPyS-S, and [35S]GTPyS (~10^6 cpm). The samples were incubated at 30 °C for 40–60 min. Alterations in these conditions are indicated in the descriptions of specific experiments. GTPyS-S bound to protein was determined by dilution of the samples with ice-cold filtration buffer (20 mM Tris-Cl, pH 8, 100 mM NaCl, 25 mM MgCl2) followed by rapid filtration of the samples through BA85 nitrocellulose filters (Schleicher and Schuell). The filters were then washed 4 times with 2 ml of the same buffer (25). Filters were dried and dissolved in 10 ml of Liquiscint (National Diagnostics) for analysis of retained radioactivity.

Gs and the / subunits were assayed as described previously (10, 27). One unit of Gs activity is defined as the amount of Gs that will release 1 nmol of cAMP/min in cys membranes. Proteins were determined by staining with Amido Black as described by Schaffner and Weissman (30) with bovine serum albumin as the standard.

ADP-ribosylation of G Proteins by IAP—The procedure used for labeling was the same as that described (19). Purified G proteins were ADP-ribosylated in a total volume of 80 ml containing 75 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 2.7 mM MgCl2, 10 mM thymidine, 1 mM ATP, 5 /LM NAD, [35S]NAD (10,000 cpm/pmol), 1 mg/ml dimyristoyl phosphatidylcholine, and 25 pg/ml IAP. In some cases, 100 /LM GTP was included; residual detergent from G protein samples did not exceed 0.1% Lubrol and 0.02% sodium cholate. The reaction was allowed to proceed for 60 min at 30 °C. At 60 min, an aliquot of 10 /LM was diluted into 0.5 ml of 2% SDS, and the protein was precipitated by the addition of 0.5 ml of 30% trichloroacetic acid. The precipitate was washed with a total of 20 ml of 6% trichloroacetic acid. The filters were then washed with Liquiscint and analyzed for radioactivity. The remainder of the sample (70 /LM) was treated with 20 /LM of 10% SDS to halt further reaction. Samples were then treated with N-ethylmaleimide and sample buffer and subjected to analysis on SDS gels as described below.

SDS-Polyacrylamide Gels—Electrophoresis of polypeptides through 15 or 20% polyacrylamide gels was accomplished with the discontinuous system described by Laemmli (31). Samples were prepared as follows. Proteins in 20 /LM of TED/1% cholate were mixed with 5 /LM of 1 mM dithiothreitol and 5 /LM of 10% SDS. After warming to 90 °C for 2–5 min, the samples were cooled, mixed with 10 /LM of 10 mM N-ethylmaleimide, and incubated at room temperature for 15 min. Sample buffer (160 /LM of 40 mM Tris-Cl, pH 6.8, 1% SDS, 4% /LM-mercaptoethanol, and 40% glycerol) was then added, and samples were heated at 100 °C for 5 min. The treatment with N-ethylmaleimide results in polypeptide bands of sharper clarity and, thus, better resolution; this is presumably due to prevention of the formation of interchain disulfide bonds during electrophoresis for long periods of time at room temperature. Molecular weight standards were obtained from Bio-Rad. Proteins were visualized with either Coomassie Blue or silver (32).

Materials—GTPyS and [35S]GTPyS were obtained from Boehringer Mannheim and New England Nuclear, respectively. [35S]NAD and [35S]GTP were synthesized as described by Johnson and Walsh (33); [35S]NAD was synthesized by the method of Cassel and Pfeuffer (19). Other nucleotides were obtained from a variety of sources and checked for purity by thin-layer chromatography. Lubrol 12A9 was purchased from ICI and deionized prior to use. Heptylamine-Sepharose was prepared by the method of Shaltiel (34) with modifications (8). IAP was the most generous gift of Toshiaki Katada and Michio Ui, Hokkaido University, Sapporo, Japan.

RESULTS

We have used GTPyS-binding activity as an assay to identify and purify GTP-dependent regulatory proteins in membranes from bovine brain. The low concentration of GTPyS (1 /LM) and the filtration method used limit the assay to detection of proteins with a relatively high affinity (or slow dissociation rate) for the nucleotide. This assay has been used to characterize Gs (25) and G1 (14, 28), two proteins that mediate stimulation and inhibition of adenylate cyclase, respectively.

Table I compares the amount of binding of GTPyS in cholate extracts of crude membranes from rat and bovine brain with extracts of membranes from other tissues and S49 lymphoma cells. A striking feature is the high level of binding in the two preparations from brain. Much less binding was observed in crude membranes from rabbit liver, a tissue used for preparation of purified Gs and Gt. Somewhat higher quantities of binding were observed in extracts from partially purified plasma membranes from bovine heart and S49 lymphoma cells; these levels are still considerably less than those seen in crude membranes from brain. This result contrasts with the amounts of Gs extracted from these membranes. The highest Gs activity was found in partially purified plasma membranes from bovine heart. The crude membrane preparations from rabbit liver and bovine brain yielded lower but probably comparable amounts of Gs. The significance of the low yield of Gs from membranes of rat liver and rat brain is not known.

The significance of the high quantity of binding of GTPyS in membranes of bovine brain became more apparent when this activity was purified. Fig. 1 demonstrates that the majority of the extracted binding activity was bound to DEAE-Sepharose and eluted as a single peak (solid circles) that was partially resolved from the peak of Gs activity. The behavior of this peak of GTPyS-binding activity is similar to that reported for liver Gt (assayed by ADP-ribosylation with IAP) by Bokoch and co-workers (13, 14). The remainder of the binding activity eluted with higher salt concentrations but without any other obvious peaks to indicate the presence of another protein species in high quantity.

The peak of GTPyS-binding activity was pooled, concentrated, and subjected to filtration through Ultrogel AcA 34 (Fig. 2). Again, one major peak of GTPyS-binding activity was eluted at about the same position as residual Gs in the preparation. However, the broadened descending portion of the binding peak suggests some heterogeneity of the proteins responsible for this binding activity; the material that eluted more slowly was not included in the pool of the binding peak (see “Experimental Procedures”) and has not been characterized further at this time.

The result of chromatography of the pool of GTPyS-binding activity through heptylamine-Sepharose is shown in Fig. 3. Two peaks of GTPyS-binding activity were obtained, and both were resolved from Gs activity. Analysis of the polypeptide composition of fractions that contain GTPyS-binding activity is shown in Fig. 4. The first peak (fractions 34–39) was composed to a large degree of one major polypeptide with a molecular weight of 39,000. The specific activity of ~30 nmol/mg of protein across this peak (Fig. 3) suggests that 1 mol of nucleotide binds/mol of polypeptide. Essentially pure 39,000-Da polypeptide could be obtained by passing this front peak through the heptylamine-Sepharose column a second time.

The second peak of GTPyS-binding activity had a lower
specific activity (~15 nmol/mg of protein) and contained three major polypeptides with molecular weights of 41,000, 39,000, and 36,000. A smaller polypeptide of 35,000 Da was also observed; the amount of this polypeptide was variable; some preparations yielded more equivalent amounts of the 35,000- and 36,000-Da polypeptides. The specific activity of this second peak is consistent with the presence of heterodimers of α and β subunits as has been observed for purified Gs (10) and Gi (13). The existence of larger complexes with multiple binding sites is unlikely in view of the dimeric size observed during gel filtration (Fig. 2).

A summary of the purification of these proteins is shown in Table II. About 8% of the total binding activity in membranes was obtained in the two peaks obtained from heptylamine-Sepharose. Purifications of 80- and 40-fold were obtained for the two respective peaks. While a yield of 8% may seem low for such a simple purification procedure, it should be noted that 14% of the extracted activity was recovered as purified protein and that the peaks of GTPγS-binding activity have been deliberately pooled to eliminate some of the fractions containing Gs. The total yield of 19 mg of purified G-protein was still possible due to the high concentration of purified protein and that the peaks of GTPγS-binding activity appear as a single peak. The results of the purification and the initial binding activity suggest that the α subunits of these two GTP-binding proteins constitute about 1% of the total protein in the crude membranes from bovine brain.

How do these purified proteins relate to similar GTP-binding proteins? Fig. 5 compares the polypeptide structure of the proteins purified from brain with Gα purified from rabbit liver and transducin purified from bovine rod outer segments. The 41,000-Da polypeptide from brain has the same mobility as Gα from rabbit liver; the 39,000-Da polypeptide has a mobility similar to the α subunit of transducin. The major β subunit (36,000 Da) in the brain preparations shows a similar mobility to the β subunit of transducin and the larger β subunit of liver Gβ. The existence of a β subunit doublet in preparations of Gβ from liver has been observed previously (10); the current method of sample preparation allows the consistent resolution of these two polypeptides, and both are observed routinely in preparations of Gβ and Gγ from this laboratory. The significance of the two β subunits is not clear, and any specific segregation with specific α subunits has not been determined. For purposes of discussion, we will refer to the separate β subunits as βα and ββ. Further experimentation will be required to determine whether βα and ββ are products of the same gene, whether they differ functionally, or whether both polypeptides actually possess the activities defined for the β subunit (27).

These preparations were also examined for the presence of a γ subunit. Fig. 6 shows a 15% acrylamide gel, which allows
As described under "Experimental Procedures" and subjected to electrophoresis through 15% polyacrylamide. The gels were stained with silver. Lane A, 100 ng of α-39,000-Da subunit (see Fig. 5); lane B, 300 ng of 41,000-Da-enriched G protein; lane C, 250 ng of a fraction that contains equivalent amounts of the 41,000- and 39,000-Da polypeptides and an excess of the β subunits; lane D, 350 ng of purified β subunits (a different preparation than shown in Fig. 5). All preparations were derived from bovine brain.

The significant of multiple polypeptides in the γ region in some preparations of the β subunit (Fig. 6) is not known.

The comparative analysis of these purified proteins on SDS gels has led us to identify, tentatively, the 41,000-Da polypeptide as Gα with the protein content and suggests that both polypeptides in brain are labeled equally well. The exclusion of GTP during the reaction had no effect on the ADP-ribosylation of Gα by IAP; this behavior is similar to the modification of Gα from brain in rabbit liver and transducin labeled less well, with stoichiometries of only 0.3 and 0.6 mol of ADP-ribose/mol of α subunit, respectively. The brain preparation that contained both the 41,000- and 39,000-Da subunits (lanes E and F) is shown with Coomassie blue stain in the outer lanes of Fig. 7. The extent of labeling of Gα and Gα in this preparation was consistent with the protein content and suggests that both polypeptides in brain are labeled equally well. The exclusion of GTP during the reaction had no effect on the ADP-ribosylation of Gα by IAP; this behavior is similar to the modification of Gα from brain in rabbit liver and transducin labeled less well, with stoichiometries of only 0.3 and 0.6 mol of ADP-ribose/mol of α subunit, respectively.

The behavior of Gα through purification and its ADP-ribosylation by IAP but not cholera toxin closely resembles Gα. Therefore, one explanation for the existence of Gα (39,000) in brain membranes could be proteolytic cleavage of Gα (41,000). Analysis of the products and time course of digestion of the two polypeptides with trypsin (Fig. 8) suggests that this is not the case. Gα, Gα (enriched in the 41,000-Da polypeptide) from bovine brain, β from bovine brain, and Gα from rabbit liver were exposed to trypsin for varying lengths of time under identical conditions (in the presence of MgCl2 and GTPγS). Gα was digested rapidly to a polypeptide of 38,000 Da; this polypeptide was remarkably stable as shown by its continued presence even after 6 h of exposure to trypsin.

G. M. Bokoch, and A. G. Gilman, personal communication.
Enriched in the component of higher mobility trypsin inhibitor (8 times the amount of trypsin by weight). Aliquots same as those shown in Fig. 5. Purified digestion allowed to proceed at 30 °C. At the times indicated, aliquots phenylalanyl chloromethyl ketone trypsin (0.3 mg/ml) was added and electrophoresis and silver stain as described under "Experimental of these samples were then processed and analyzed by SDS-gel electrophoresis and silver stain as described under "Experimental Procedures." Samples of brain Goa, brain α, and liver G1 were the same as those shown in Fig. 5. Purified β subunit from brain was the same sample as shown in Fig. 6; this preparation of β is actually enriched in the component of higher mobility (βm).

Goα from liver was also digested rapidly to polypeptides of 39,000 and 38,000 Da. However, these polypeptides did not display the same stability as the 38,000-Da fragment from Goα and were digested to polypeptides of about 30,000 Da; total digestion occurred within 6 h. To eliminate the possibility that G1 from brain and G1 from liver have different susceptibilities to trypsin, a preparation of brain G1 was digested. Goα (41,000 Da) from brain appeared to be digested with a time course similar to that of Goα from liver. Again, intermediate digestion products were observed in the 30,000-Da region; digestion is almost complete at 6 h. The small amount of 38,000-Da product observed at 6 h was consistent with the digestion of Goα that was present in this preparation. Under these same conditions, the β subunits are digested with extreme rapidity, and no visible products were detected except near the dye front.

The different susceptibility of the two α subunits to digestion with trypsin strongly suggests that the two polypeptides are distinct entities. It seems unlikely, although not impossible, that a proteolytic cleavage of 41,000 to 39,000 Da could be vice versa, producing a random proteolytic fragment that is the explanation for Goa, the event would have to be reversible. 5) In the presence of Mg2+, the protein has a high affinity for GTPγS either in the presence or absence of the β subunit. These and other properties require more extensive study.

Initial experiments that examined the specificity of binding to Goα in the absence of Mg2+ indicate that GTPγS > GTP,

\[ \text{FIG. 8. Sensitivity of G proteins to digestion by trypsin.} \]

Samples at 0.3 mg/ml protein in 25 mM Tris-C1, pH 8, 1 mM EDTA, 1 mM dithiothreitol, 50 mM NaCl, 30 mM MgSO4, 10 μM GTPγS, and 1% sodium cholate (liver G1 also contained 0.04% Lubrol) were incubated for 1 h at 30 °C. At zero time, an equal volume of tosyl-phenylalanyl chloromethyl ketone trypsin (0.3 mg/ml) was added and digestion allowed to proceed at 30 °C. At the times indicated, aliquots of the digestion mixtures were removed and mixed with soybean trypsin inhibitor (8 times the amount of trypsin by weight). Aliquots of these samples were then processed and analyzed by SDS-gel electrophoresis and silver stain as described under "Experimental Procedures." Samples of brain Goα, brain α, and liver G1 were the same as those shown in Fig. 5. Purified β subunit from brain was the same sample as shown in Fig. 6; this preparation of β is actually enriched in the component of higher mobility (βm).

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Initial experiments that examined the specificity of binding to Goα in the absence of Mg2+ indicate that GTPγS > GTP,
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GDP > ITP >> GMP, ATP, CTP, UTP. At 10 nM GTP-γS ($K_D = 6$ nM for these conditions), the concentrations of nucleotides required for half-maximal inhibition were: GDP, 0.5 mM; CDP, 0.5 μM; ITP, 8.0 μM; GMP, ATP, CTP, UTP, >1 mM. Thus, Gα is a binding protein specific for guanine nucleotides. The affinity of Gα for GTP-γS is variable with conditions; the apparent binding constant in the presence of Mg²⁺ appears to be less than 1 nM, and this would be a higher affinity than has been observed for the dimeric forms of Gα (25) or Gγ (14). Finally, a GTPase activity has been observed with this subunit; the rate is low (0.1-0.3 mol of P, produced/min/mol of Gα), but the reaction is catalytic.

**DISCUSSION**

We have reported the purification of two major GTP-binding proteins from membranes of bovine brain. The isolation procedures are essentially the same as those used to purify Gα and Gγ from rabbit liver (9, 10, 13, 14). This confirms further the utility of the procedures for the isolation of these GTP-binding proteins from other tissues. The ease of purification is the direct result of the high quantity of the two proteins in membranes from brain. The purification profiles indicate that about 50% of the GTP-γS-binding activity (0.2 nmol/mg) observed in these membranes is due to the two purified proteins (labeled Gα and Gγ). Therefore, with an assumed molecular weight of about 80,000, the two proteins together account for more than 1.6% of the total protein of the membrane. Twenty mg of purified protein was obtained from 12 g of membranes even with stringent pooling of peak activities. Other experiments suggest that a more generous collection of fractions containing binding activity will result in similar purification of the proteins with a greater yield. Thus, bovine brain is an excellent source for obtaining the large quantities of protein that will facilitate structural and biochemical studies of these and related G proteins.

The structure of the purified G proteins from bovine brain and their activity as substrates for ADP-ribosylation by IAP identify them as probable members of a family of membrane-associated proteins that bind GTP and mediate the regulation of intracellular functions by external stimuli. Like Gα, Gγ, and T, the G proteins from brain have at least two subunits (α, β) and possibly a third (γ). Direct comparison of these polypeptides on SDS gels suggests that the larger α subunit from brain is Gα; demonstration that this protein can act as an inhibitor of adenylate cyclase will be required to confirm this. The function of the smaller α subunit (Gβγ) remains unknown. Its migration in SDS gels with a mobility very similar to that of the α subunit of transducin is provocative. Initial experiments, however, indicate that the two proteins are different. Gα aggregates in the absence of detergent, and attempts to remove Gα from membranes with GTP-γS have failed (the 3-5% release of guanine nucleotide binding activity that was observed may indicate that some solubilization of the protein occurs under the conditions utilized for chromatography through heptylamine-Sepharose. The 41,000-Da polypeptide (presumed Gα from brain) does run as a dimer with β under these same conditions; this parallels the behavior of liver Gα (13, 14).

The ability to obtain Gα from without the intercession of small regulatory ligands provides us with the opportunity to study this protein in a native state. We can study the interaction of the α subunit of this G protein with guanine nucleotides in the absence of other subunits and without having to depend on reversing the effects of activating ligands to which the protein was exposed previously. Thus, initial kinetics can be studied readily. We have already observed several properties of Gα that relate to the mechanism of G-protein activation. One of these is the association of GTP-γS with Gα in the absence of Mg²⁺. The binding is readily reversible and can be influenced by the simple addition of β subunit. This experiment suggests that a real equilibrium is being observed directly (this has not been the case for preparations of Gα and Gγ). Addition of β subunit then appears to reduce the affinity of the α subunit for GTP-γS in the absence of Mg²⁺. Mg²⁺ prevents the effects of the β-subunit and apparently causes
Goα to have a very high affinity for the nucleotide. These observations coincide with those of the dimeric form of Gs and Gt (10, 14, 25). While confirming these data, we have also demonstrated how well this protein lends itself to experimental manipulation. We expect that more extensive studies will yield new and useful information on the mechanisms utilized by Go and other members of this family of GTP-binding proteins.

At this time, we can only speculate on the function of Go. Its relationship to Go, Gt, and transducin suggests that it is involved in the conversion of extracellular signals to intracellular regulation. Further experiments are required to determine the prevalence of Go in other tissues, the exact relationship of Go to Gt and transducin, and the potential function of Go as a regulator of adenylate cyclase or as a modulator of some other cellular process under hormonal control.

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REFERENCES

1. Ross, E. M., and Gilman, A. G. (1980) Annu. Rev. Biochem. 49, 533-564
2. Stryer, L., Hurley, J. B., and Fung, B. K. K. (1981) Curr. Top. Membr. Transp. 15, 93-106
3. Gilman, A. G. (1984) J. Clin. Invest. 73, 1-4
4. Godchaux, W., III, and Zimmerman, W. F. (1979) J. Biol. Chem. 254, 7874-7884
5. Baehr, W., Devlin, M. J., and Applebury, M. L. (1979) J. Biol. Chem. 254, 11669-11677
6. Kuhn, H. (1980) Nature (Lond.) 283, 587-588
7. Hurley, J. B. (1980) Biochem. Biophys. Res. Commun. 92, 505-510
8. Fung, B. K. K., Hurley, J. B., and Stryer, L. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 153-158
9. Norup, J. K., Sternweis, P. C., Smigel, M. D., Schleifer, L. S., Ross, E. M., and Gilman, A. G. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6516-6520
10. Sternweis, P. C., Norup, J. K., Smigel, M. D., and Gilman, A. G. (1981) J. Biol. Chem. 256, 11617-11620
11. Hanski, E., Sternweis, P. C., Norup, J. K., Dromerick, A. W., and Gilman, A. G. (1981) J. Biol. Chem. 256, 12911-12919
12. Hanski, E., and Gilman, A. G. (1982) J. Cyclic Nucleotide Res. 8, 323-335
13. Bokoch, G. M., Katada, T., Norup, J. K., Hewlett, E. L., and Gilman, A. G. (1983) J. Biol. Chem. 258, 2072-2075
14. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M., and Gilman, A. G. (1984) J. Biol. Chem. 259, 3560-3567
15. Codina, J., Hildebrandt, J., Jenyng, R., Birnbaumer, L., Sekura, R. D., and Manclark, C. R. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4276-4280
16. Hildebrandt, J. D., Codina, J., Rüsinger, R., and Birnbaumer, L. (1984) J. Biol. Chem. 259, 2039-2042
17. Manning, D. R., and Gilman, A. G. (1983) J. Biol. Chem. 258, 7059-7063
18. Gill, D. M., and Meren, R. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3050-3054
19. Cassel, D., and Pfeuffer, T. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2669-2673
20. Katada, T., and Ui, M. (1982) J. Biol. Chem. 257, 7210-7216
21. Manning, D. R., Fraser, B. A., Kahn, R. A., and Gilman, A. G. (1984) J. Biol. Chem. 259, 749-756
22. Abood, M. E., Hurley, J. B., Pappone, M.-C., Bourne, H. R., and Stryer, L. (1982) J. Biol. Chem. 257, 10540-10543
23. Pfeuffer, T. (1977) J. Biol. Chem. 252, 7224-7234
24. Northup, J. K., Smigel, M. D., Sternweis, P. C., and Gilman, A. G. (1983) J. Biol. Chem. 258, 11369-11376
25. Northup, J. K., Smigel, M. D., and Gilman, A. G. (1982) J. Biol. Chem. 257, 1140-11423
26. Katada, T., Bokoch, G. M., Smigel, M. D., Ui, M., and Gilman, A. G. (1984) J. Biol. Chem. 259, 3568-3575
27. Northup, J. K., Sternweis, P. C., and Gilman, A. G. (1983) J. Biol. Chem. 258, 11361-11366
28. Katada, T., Bokoch, G. M., Northup, J. K., Ui, M., and Gilman, A. G. (1984) J. Biol. Chem. 259, 3568-3577
29. Ross, E. M., Maguire, M. E., Sturgill, T. W., Biltonen, R. L., and Gilman, A. G. (1977) J. Biol. Chem. 252, 5761-5775
30. Schaffner, W., and Weissman, C. (1973) Anal. Biochem. 56, 502-504
31. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
32. Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 197-203
33. Johnson, R. A., and Walsh, T. F. (1979) Adv. Cyclic Nucleotide Res. 10, 135-168
34. Shattil, S. (1974) Methods Enzymol. 34, 126-140
35. Schleifer, L. S., Garrison, J. C., Sternweis, P. C., Northup, J. K., and Gilman, A. G. (1980) J. Biol. Chem. 255, 2641-2644
36. Schleifer, L. S., Kahn, R. A., Hanski, E., Northup, J. K., Sternweis, P. C., and Gilman, A. G. (1982) J. Biol. Chem. 257, 23-30
37. Kahn, R. A., and Gilman, A. G. (1984) J. Biol. Chem. 259, 6228-6234
38. Smigel, M. D., Northup, J. K., and Gilman, A. G. (1982) Recent Prog. Horm. Res. 38, 601-624