G Protein $\beta\gamma$ Subunits from Bovine Brain and Retina: Equivalent Catalytic Support of ADP-ribosylation of α Subunits by Pertussis Toxin but Differential Interactions with $G_{s\alpha}$[†]

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ABSTRACT: We have examined the ability of the $\beta\gamma$ subunits of guanine nucleotide binding regulatory proteins (G proteins) to support the pertussis toxin (PT) catalyzed ADP-ribosylation of G protein α subunits. Substoichiometric amounts of the $\beta\gamma$ complex purified from either bovine brain G proteins or the bovine retinal G protein, G_t, are sufficient to support the ADP-ribosylation of the α subunits of G_i (the G protein that mediates inhibition of adenylyl cyclase) and G_o (a G protein of unknown function) by PT. This observation indicates that ADP-ribosylated G protein oligomers can dissociate into their respective α and $\beta\gamma$ subunits in the absence of activating regulatory ligands, i.e., nonhydrolyzable GTP analogues or fluoride. Additionally, the catalytic support of ADP-ribosylation by bovine brain $\beta\gamma$ does not require Mg^{2+}. Although the $\beta\gamma$ subunit complexes purified from bovine brain G proteins and the $\beta\gamma$ complex of G_t support equally the ADP-ribosylation of α subunits by PT, there is a marked difference in their abilities to interact with $G_{s\alpha}$. The enhancement of deactivation of fluoride-activated $G_{s\alpha}$ requires 25-fold more $\beta\gamma$ from G_t than from brain G proteins to produce a similar response. This difference in potency of $\beta\gamma$ complexes from the two sources was also observed in the ability of $\beta\gamma$ to produce an increase in the activity of recombinant $G_{s\alpha}$ produced in *Escherichia coli*.

G_s,[1] G_i, G_o, and G_t are members of a family of guanine nucleotide binding regulatory proteins (G proteins). These proteins function as transducers in pathways of transmembrane signaling by coupling receptors to their respective effector proteins [for a review, see Gilman (1987)]. G_s and G_i mediate the hormonal stimulation and inhibition of adenylyl cyclase, respectively. G_t activates a retinal cGMP-specific phosphodiesterase in response to activation of rhodopsin by light. The function of G_o is not entirely clear, although it interacts with muscarinic receptors (Florio & Sternweis, 1985) and Ca^{2+} channels (Hescheler et al., 1987).

G proteins are heterotrimers, with subunits designated α, β, and γ in order of decreasing mass. The α subunits contain a high-affinity guanine nucleotide binding site; differences in this subunit among the members of the G protein family define the individual. The β and γ subunits remain tightly associated with each other under all but denaturing conditions; this $\beta\gamma$ complex is thought to be functionally interchangeable among several members of the G protein family (Gilman, 1987).

The α subunits of the characterized G proteins are substrates for ADP-ribosylation catalyzed by bacterial toxins. Thus, $G_{s\alpha}$ can be ADP-ribosylated by cholera toxin, while $G_{i\alpha}$ and $G_{o\alpha}$ are substrates for ADP-ribosylation by pertussis toxin (PT, also known as islet activating protein); $G_{t\alpha}$ can be ADP-ribosylated by both toxins (Cassel & Pfeuffer, 1978; Katada & Ui, 1982; Abood et al., 1982; Van Dop et al., 1984). ADP-ribosylation of $G_{i\alpha}$ and $G_{o\alpha}$ by PT results in an impaired ability of these G proteins to interact with receptors (Ui, 1984). This covalent modification is dependent on the presence of the $\beta\gamma$ subunit complex (Katada et al., 1986; Huff & Neer, 1986) and guanine nucleotides (Katada et al., 1986).

Despite the fact that $\beta\gamma$ subunit complexes can be exchanged among different α subunits, there are multiple forms of these polypeptides. Although the β subunit of G_t can be visualized on sodium dodecyl sulfate–polyacrylamide gels as a single band of 36 kDa (β_1), the β subunit of the other G proteins is a doublet of proteins with apparent masses of 36 and 35 kDa (β_2). cDNAs that encode two distinct forms of the β subunit have been isolated (Suigmoto et al., 1985; Fong et al., 1987; Gao et al., 1987a); the deduced amino acid sequences are 90% identical. Immunological evidence and studies involving expression of these cDNAs have revealed that one encodes β_1 and the other encodes β_2 (Gao et al., 1987b). Evidence for heterogeneity of γ subunits is based on analysis of the proteins, since only $G_{t\gamma}$ has been cloned to date (Hurley et al., 1984). Comparison of peptide maps of $G_{t\gamma}$ with those of human erythrocyte γ indicates that these proteins are distinct (Hildebrandt et al., 1985). Thus, the $\beta\gamma$ subunit complex of G_t differs from that associated with at least some other G proteins. Additionally, electrophoretic and immunological evidence suggests that there is more than one non-$G_{t\gamma}$ subunit (Sternweis & Robishaw, 1984; Evans et al., 1987); thus, functional differences may exist between the $\beta\gamma$ subunits of other G proteins as well.

In this paper we have examined the abilities of $\beta\gamma$ subunits and regulatory ligands to support the ADP-ribosylation of bovine brain $G_{o\alpha}$ and $G_{i\alpha}$ by PT. Also included is a comparison

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[1] Abbreviations: G proteins, guanine nucleotide binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylyl cyclase; G_t, major G protein of retinal rods; G_o, G protein of unknown function purified from bovine brain; $G_{o\alpha}$, example of nomenclature specifying the α subunit of G_o; PT, pertussis toxin; GTPγS, guanosine 5′-(γ-thiotriphosphate); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol.

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of the ability of bovine brain $\beta\gamma$ and $G_{t\beta\gamma}$ to interact with $G_{s\alpha}$. Since the dissociation of α from $\beta\gamma$ is believed to be an important component of G protein action (Gilman, 1987; Fung, 1983), information on the interactions of these subunits will help to clarify the mechanisms of G protein mediated events.

Experimental Procedures

Preparation of Membranes and G Proteins. Membranes from bovine brain (Sternweis & Robishaw, 1984), the $G_{s\alpha}$-deficient (cyc^-) variant of the S49 lymphoma cell (Ross et al., 1977), and bovine retinal rod outer segments (Kuhn, 1980) were prepared as described. The two major G proteins from bovine brain were purified by the method of Sternweis and Robishaw (1984). The resolved α subunit of G_o and the $\beta\gamma$ subunit complex of G_o and G_i were prepared by chromatography of a mixture of oligomeric G_o and G_i on heptylamine–Sepharose in the presence of 20 μM $AlCl_3$, 6 mM $MgCl_2$, and 10 mM NaF (AMF) as reported (Roof et al., 1985), except that the buffers contained 20 μM GDP and the sodium cholate gradient used was 5–20 mM. The subunits were concentrated by pressure filtration through an Amicon PM-10 membrane or by centrifugation through a Centricon 10 concentrator. The resolved subunits were filtered free of AMF by use of centrifuge G-50 gel filtration columns (Penefsky, 1979) equilibrated in 50 mM Tris-HCl, pH 8.0, 1 mM DTT, 1 mM EDTA, and 0.05% Lubrol (buffer A). For experiments involving the effect of $\beta\gamma$ subunits on G_s, the $\beta\gamma$ subunits were further purified to remove trace (<0.1%) amounts of G_s. The $\beta\gamma$ subunits were injected onto a 0.5 × 5 cm FPLC Mono Q column equilibrated with buffer A and eluted with a 20-mL linear gradient of 0–200 mM NaCl in buffer A; 1-mL fractions were collected. $\beta\gamma$ eluted from this column in a broad peak centered at 170 mM NaCl. Fractions containing $\beta\gamma$ devoid of G_s activity were pooled and concentrated with a Centricon 10.

The resolved α subunit of G_i was purified from fractions that eluted from the heptylamine–Sepharose column noted above between $G_{o\alpha}$ and $\beta\gamma$. Fractions from this column that contained a mixture of $G_{i\alpha}$ and $\beta\gamma$ were injected onto the 0.5 cm × 5.0 cm FPLC Mono Q column equilibrated with 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 1 mM EDTA, 1.0% sodium cholate, AMF, and 10 μM GDP. The column was eluted with a 30-mL gradient of 0–250 mM NaCl in the same buffer, and fractions of 1 mL were collected. $G_{i\alpha}$ eluted from this column at ~50 mM NaCl, while the $\beta\gamma$ subunit complex eluted at ~170 mM NaCl. Purified $G_{i\alpha}$ was concentrated and gel filtered as described above for $G_{o\alpha}$ and $\beta\gamma$.

G_s was purified from rabbit liver by the method of Sternweis et al. (1981). For experiments that involved the deactivation of Al^{3+}- plus F^--activated G_s as a measure of $\beta\gamma$ subunit activity, the G_s was further purified to remove excess $\beta\gamma$; this extends the half-time of deactivation. G_s that eluted from the hydroxylapatite column described by Sternweis et al. was diluted 7-fold with buffer A containing AMF (except the Tris-HCl concentration was 20 mM and the Lubrol concentration was 0.10%) and injected onto the FPLC Mono Q column. The column was washed with 5 mL of buffer A containing AMF, and G_s was eluted with a 25-mL linear gradient of 0–200 mM NaCl in the same buffer. G_s activity eluted in a broad peak centered at 175 mM NaCl. Fractions that exhibited a half-time of deactivation of >10 min in the absence of added $\beta\gamma$ (those eluting at ~150–170 mM NaCl) were used for the G_s deactivation assay. The 45 000-Da form of the $G_{s\alpha}$ subunit, produced in *Escherichia coli*, was partially purified from a high-speed supernatant of an *E. coli* lysate as described (Graziano et al., 1987).

G_t was purified from rod outer segment membranes by the method of Fung (1983). The $\beta\gamma$ subunit complex of G_t was prepared by chromatography of the G_t oligomer on heptylamine–Sepharose in the presence of Gpp(NH)p (Mumby et al., 1986).

ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ by PT. The procedure used was a modification of that described for the ADP-ribosylation of G_i by PT (Bokoch et al., 1983). Purified $G_{o\alpha}$ or $G_{i\alpha}$ was ADP-ribosylated in a total volume of 40 μL containing 75 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM DTT, 2.5 μM NAD, [^{32}P]NAD (~15 000 cpm/pmol), 0.5 mM dimyristoylphosphatidylcholine, and 5 μg/mL PT. Purified $\beta\gamma$ subunits, $MgCl_2$, and guanine nucleotide were varied in the reaction mixture as indicated in the figure legends. The reaction was allowed to proceed at 30 °C for 20 min unless otherwise indicated. Under these conditions, the reaction was linear with time for concentrations of $\beta\gamma$ of ≤0.5 pmol/40 μL. The reaction was stopped by the addition of 0.5 mL of a solution containing 2% SDS and 50 μM NAD, and the protein was precipitated by the addition of 0.5 mL of 30% TCA. The precipitated protein was collected on BA 85 nitrocellulose filters and washed with a total of 16 mL of 6% TCA. The filters were dissolved in Liquiscint and analyzed for radioactivity.

Assays. G_s activity was measured by its ability to reconstitute adenylyl cyclase activity in membranes of the cyc^- S49 lymphoma cell. The activated form of G_s was produced by incubation of the protein at 30 °C for 60 min in buffer A containing 10 μM GTPγS and 5 mM $MgCl_2$. After the activation period, the mixture (20 μL) was added to 80 μL of a solution containing cyc^- membranes and other assay components as described by Sternweis et al. (1981) (assay II). Samples were incubated for 30 min at 30 °C. Reactions were terminated, and [^{32}P]cAMP was quantitated by the method of Salomon et al. (1974).

$G_{o\alpha}$ and $G_{i\alpha}$ were assayed by the binding of [^{35}S]GTPγS as described by Sternweis and Robishaw (1984); protein was also determined. We generally find that 80% to >95% of the protein is capable of binding GTPγS. The $\beta\gamma$ subunits were assayed by their ability to support the ADP-ribosylation of $G_{o\alpha}$ by PT (see Results) and by their ability to deactivate Al^{3+}-plus F^--activated G_s (Northup et al., 1983a). $\beta\gamma$ concentration was determined by two separate protein assays; a molecular mass of 45 000 Da was assumed. The quantity of G_s present in the deactivation assay was determined from activity measurements, a specific activity of 1.6 μmol·min^{-1}·mg^{-1} being used for the purified protein (Sternweis et al., 1981).

Protein concentrations were determined by staining with Amido black (Schaffner & Weissmann, 1973) and by the Coomassie blue dye binding method of Bradford (1976). Bovine serum albumin was used as the standard for both assays.

Materials. GTPγS was obtained from Boehringer Mannheim. [^{35}S]GTPγS, [α-^{32}P]ATP, and [^{32}P]NAD were obtained from New England Nuclear. PT was obtained from List Biological Laboratories. The toxin was reconstituted at 100 μg/mL with a solution containing 2 M urea and 100 mM potassium phosphate, pH 7.0, and stored at 4 °C. The FPLC chromatography system was from Pharmacia. Lubrol 12A9 was obtained from ICI and deionized prior to use.

Results

ADP-ribosylation of $G_{o\alpha}$: Effect of $\beta\gamma$ and Phospholipid. The ADP-ribosylation of $G_{o\alpha}$ by PT is dependent on the presence of $\beta\gamma$ subunits (Katada et al., 1986; Huff & Neer, 1986). In these reports, stoichiometric or greater amounts of

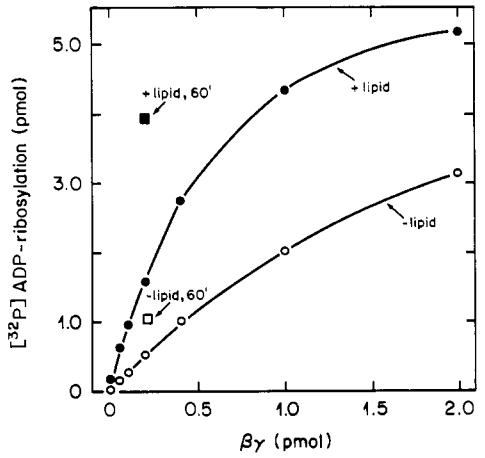

FIGURE 1: Effect of brain $\beta\gamma$ on ADP-ribosylation of $G_{o\alpha}$ by PT. $G_{o\alpha}$ (14 pmol) in buffer A containing 0.025% Lubrol was mixed with the indicated amount of brain $\beta\gamma$ in the same buffer, such that the final volume was 25 μL. ADP-ribosylation was initiated by the addition of 15 μL of a reaction mixture containing the additional components to bring their final concentrations to those noted under Experimental Procedures. The assay mixture also contained 2 mM $MgCl_2$ and 100 μM GDP. The reactions were carried out in the presence (●, ■) or absence (○, □) of 0.5 mM dimyristoylphosphatidylcholine. For two points (■, □), the reaction was allowed to proceed for 60 min, rather than for 20 min.

$\beta\gamma$ were required for optimal ADP-ribosylation. By contrast, we observed that substoichiometric amounts of $\beta\gamma$ could support the ADP-ribosylation of $G_{o\alpha}$. Figure 1 (closed symbols) shows the dependence of ADP-ribosylation of $G_{o\alpha}$ on $\beta\gamma$ in the presence of GDP, Mg^{2+}, and dimyristoylphosphatidylcholine. At all concentrations of $\beta\gamma$, the amount of ADP-ribose incorporated into $G_{o\alpha}$ exceeded the amount of $\beta\gamma$ present; the highest ratio observed of [^{32}P]ADP-ribose incorporation to $\beta\gamma$ was 20:1. Thus, $\beta\gamma$ can act catalytically in support of the ADP-ribosylation of $G_{o\alpha}$. Consistent with previous observations, the inclusion of GDP (or GTP) to the assay mixture increased the level of ADP-ribosylation of $G_{o\alpha}$ (results not shown). This effect is apparently due to an increased stability of the α subunit in the presence of guanine nucleotide, since the addition of 100 μM GDP to reaction mixtures incubated for 20 min in the absence of guanine nucleotide could not restore the levels of ADP-ribosylation to those obtained when guanine nucleotide was present throughout the assay (results not shown).

In some laboratories, ADP-ribosylation of G proteins by PT is carried out in the absence of phospholipid (Huff & Neer, 1986). Although the levels of ADP-ribosylation of $G_{o\alpha}$ are lower in the absence of dimyristoylphosphatidylcholine (Figure 1, open symbols), $\beta\gamma$ can still act catalytically to support the ADP-ribosylation of $G_{o\alpha}$ when phospholipid is absent. Thus, the inclusion of 0.2 pmol of $\beta\gamma$ in the assay mixture without phospholipid supported the ADP-ribosylation of ~1 pmol of $G_{o\alpha}$ over 60 min.

A more striking demonstration of the catalytic support of ADP-ribosylation of $G_{o\alpha}$ by PT can be seen in Figure 2, in which a time course of modification was carried out at three concentrations of $\beta\gamma$. The same final level of ADP-ribosylation was achieved at ratios of $\alpha/\beta\gamma$ of 50:1 and 1.5:1; with the latter condition, the reaction was essentially complete by 10 min.

The dependence of the ADP-ribosylation on the concentration of $G_{o\alpha}$ in the assay mixture is shown in Figure 3. Increasing the amount of $\beta\gamma$ in the assay mixture from 0.2 to 1.0 pmol resulted in a 4-fold increase in the amount of ADP-ribosylation observed at high concentrations of $G_{o\alpha}$. More interestingly, both curves saturate at readily achievable

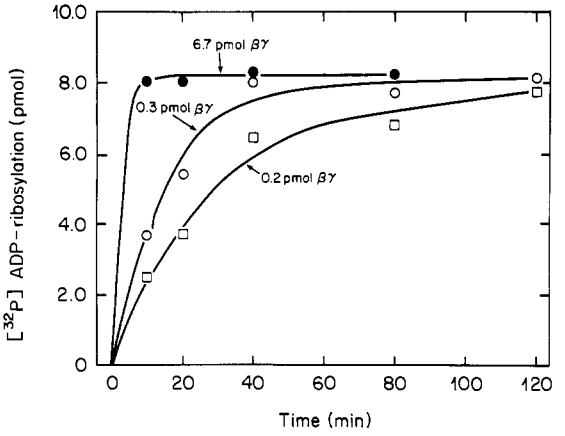

FIGURE 2: Time course of ADP-ribosylation of $G_{o\alpha}$ at increasing levels of $\beta\gamma$. Conditions were as described in the legend to Figure 1 for the assays containing phospholipid, except that the reactions were terminated at the indicated times. The assay mixtures contained 10 pmol of $G_{o\alpha}$ and either 0.2 (□), 0.3 (○), or 6.7 pmol (●) of brain $\beta\gamma$.

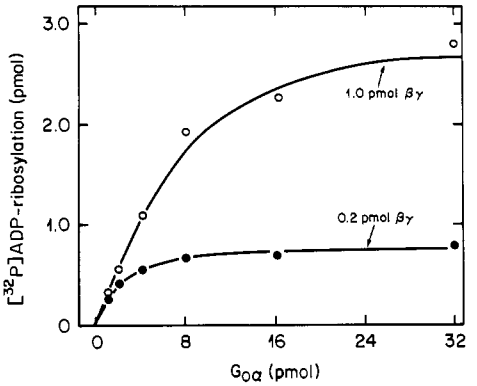

FIGURE 3: Effect of $G_{o\alpha}$ concentration on its ADP-ribosylation by PT. The indicated amounts of $G_{o\alpha}$ and either 0.2 pmol (●) or 1.0 pmol (○) of brain $\beta\gamma$ (all in buffer A containing 0.025% Lubrol) were mixed in a final volume of 25 μL, and ADP-ribosylation was initiated with 15 μL of the reaction mixture described in the legend to Figure 1. All points were corrected for the amount of ADP-ribosylation observed in the absence of $\beta\gamma$, which increased linearly to 0.3-pmol incorporation at 32 pmol of $G_{o\alpha}$.

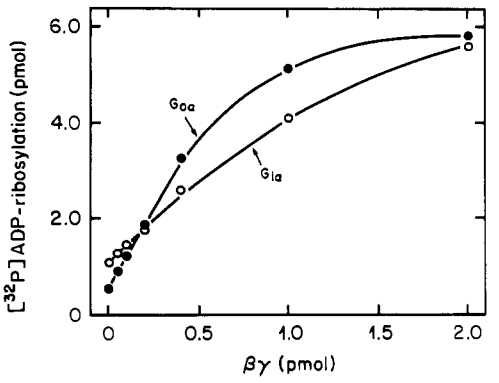

FIGURE 4: Effect of $\beta\gamma$ on the ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ by PT. Conditions were as described in the legend to Figure 1 for the assays containing phospholipid, except that the GDP concentration was 10 μM and the assay mixture contained 12 pmol of either $G_{o\alpha}$ (●) or $G_{i\alpha}$ (○).

concentrations of $G_{o\alpha}$. This observation suggests conditions for performance of a very sensitive assay for $\beta\gamma$ in partially purified extracts of cell membranes. If one conducts the ADP-ribosylation reaction in the presence of added $G_{o\alpha}$ (≥20 pmol), it is possible to measure subpicomole amounts of functional $\beta\gamma$ in samples that contain picomole amounts of α subunits.[2]

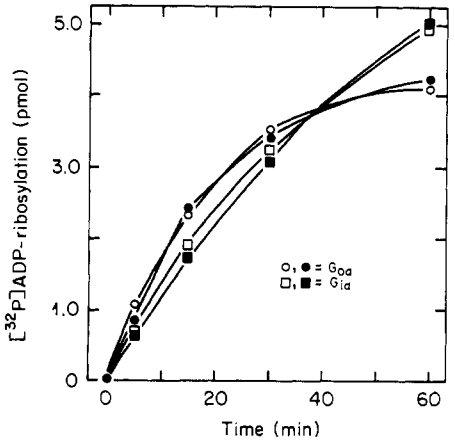

FIGURE 5: Time course of the ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ in the presence and absence of Mg^{2+}. Conditions were as described in the legend to Figure 1 for the assays containing phospholipid, except that the reactions were terminated at the indicated times and the concentration of GDP was 10 μM. The assay mixtures contained 12 pmol of either $G_{o\alpha}$ (●, ○) or $G_{i\alpha}$ (■, □) and 0.5 pmol of bovine brain $\beta\gamma$. For two sets of reactions (○, □), $MgCl_2$ was omitted and EDTA was added to a final concentration of 10 mM.

ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$: Comparison of the Effects of $\beta\gamma$ and Mg^{2+}. The observation that $\beta\gamma$ could catalytically support the ADP-ribosylation of $G_{o\alpha}$ by PT prompted us to test whether this ability also extended to the analogous interactions required for ADP-ribosylation of $G_{i\alpha}$. Our studies indicate this is indeed the case. Figure 4 shows that similar concentrations of $\beta\gamma$ are required for ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ over the range tested. As with $G_{o\alpha}$, the amount of [^{32}P]ADP-ribose incorporation into $G_{i\alpha}$ was consistently much greater than the amount of $\beta\gamma$ present in the assay. The significant level of ADP-ribosylation of $G_{i\alpha}$ observed in the absence of added $\beta\gamma$ is ascribed to contamination of the preparation of $G_{i\alpha}$ with a small (~2%) amount of $\beta\gamma$.

The ability of the $\beta\gamma$ complex to act catalytically to support the ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ indicates that the G protein oligomers, at least once they are ADP-ribosylated, dissociate into their respective ADP-ribosyl-α and $\beta\gamma$ constituents and that the $\beta\gamma$ complex released upon dissociation can combine with another unmodified α subunit to support its ADP-ribosylation. Furthermore, this dissociation can occur in the absence of activating ligands (GTP analogues, F^-). Another requirement for G protein dissociation frequently mentioned in the literature is Mg^{2+} (Northup et al., 1983b; Birnbaumer et al., 1985). The dependence of $\beta\gamma$-supported ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ on Mg^{2+} was examined by conducting a time course of the reactions with 0.5 pmol of $\beta\gamma$ in the presence of either 2 mM Mg^{2+} or 10 mM EDTA. Figure 5 reveals that there is no significant difference between these conditions. In both cases, 0.5 pmol of the $\beta\gamma$ complex supported the ADP-ribosylation of 4–5 pmol of α subunit over the time period of the assay. Thus, neither the ADP-ribosylation of G_α by PT nor the ability of the ADP-ribosylated $\alpha\cdot\beta\gamma$ complex to dissociate requires Mg^{2+}.

Comparison of $\beta\gamma$ Subunits from Bovine Brain G Proteins with Those from G_t. The $\beta\gamma$ subunit complex of G_t can inhibit G_s-mediated stimulation of adenylyl cyclase in phospholipid vesicles (Cerione et al., 1986) and can inhibit hormonally stimulated adenylyl cyclase activity in intact membranes (Bockaert et al., 1985). These studies suggest that the $\beta\gamma$ complex of G_t is functionally similar to those of other identified G proteins. This functional similarity extends to interactions between $G_{o\alpha}$ and these $\beta\gamma$ subunit complexes. The $\beta\gamma$ complex resolved from G_t and that resolved from brain G proteins are equally efficacious in their support of the ADP-ribosylation of $G_{o\alpha}$ by PT (Figure 6). Similar results have been obtained in comparisons of the abilities of these $\beta\gamma$ subunits to support the ADP-ribosylation of $G_{i\alpha}$ (results not shown).

[2] I.-H. Pang, unpublished observations.

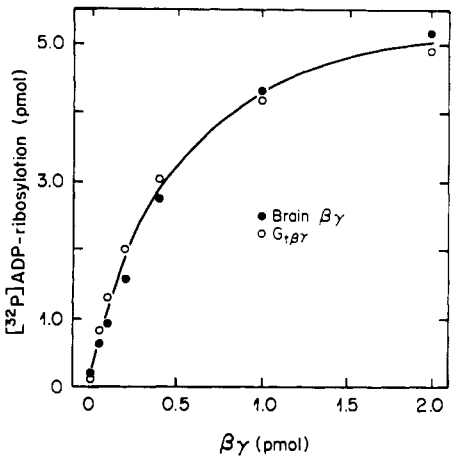

FIGURE 6: Effect of brain $\beta\gamma$ and $G_{t\beta\gamma}$ on the ADP-ribosylation of $G_{o\alpha}$ by PT. The conditions were as described in Figure 1 for the assays containing phospholipid. The $\beta\gamma$ used was isolated either from bovine brain G proteins (●) or from G_t (○).

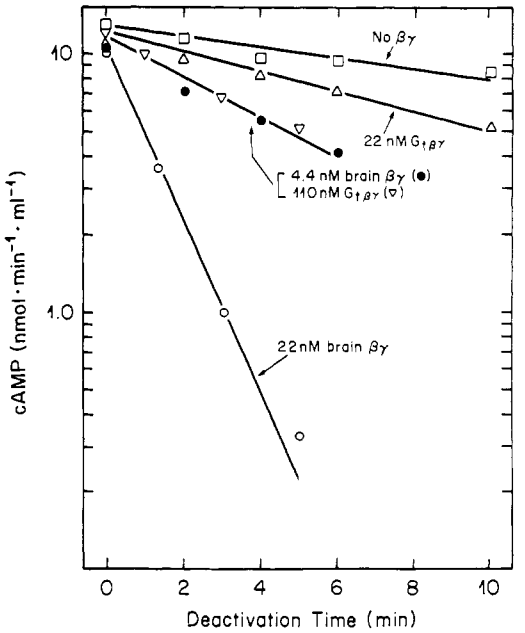

FIGURE 7: Effect of brain $\beta\gamma$ and $G_{t\beta\gamma}$ on the deactivation of $G_{s\alpha}$. G_s was activated by incubation in 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, 0.05% Lubrol, 10 μM $AlCl_3$, 6 mM $MgCl_2$, and 10 mM NaF for 20 min at 30 °C. Aliquots were then withdrawn and diluted 10-fold into 50 mM Hepes, pH 8.0, 5 mM EDTA, 1 mM DTT, 0.1% Lubrol, and either no $\beta\gamma$ (□), 4.4 nM brain $\beta\gamma$ (●), 22 nM brain $\beta\gamma$ (○), 22 nM $G_{t\beta\gamma}$ (△), or 110 nM $G_{t\beta\gamma}$ (▽). The concentration of G_s in the deactivation mixture was 8 nM. Deactivation was allowed to proceed for the indicated times at 22 °C, whereupon 10-μL aliquots were withdrawn and added to 90 μL of a solution containing cyc^- membranes and the adenylyl cyclase assay components described under Experimental Procedures. This initiated the adenylyl cyclase assay, which was terminated after 20 min at 30 °C.

The ability of bovine brain $\beta\gamma$ and $G_{t\beta\gamma}$ to deactivate Al^{3+}-plus F^--activated $G_{s\alpha}$ was also probed (Figure 7). In contrast to the observed similarity of these two $\beta\gamma$ complexes in supporting the ADP-ribosylation of $G_{o\alpha}$, we observed a marked difference in thier respective abilities to interact with (deac-

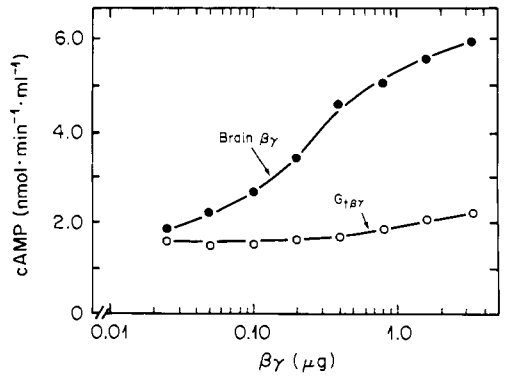

FIGURE 8: Effect of brain $\beta\gamma$ and $G_{t\beta\gamma}$ on the activity of recombinant $G_{s\alpha}$. Partially purified recombinant $G_{s\alpha}$ was activated by incubation in 20 μL of 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, 0.025% Lubrol, 5 mM $MgCl_2$, 10 μM GTPγS, and the indicated amounts of $\beta\gamma$ from brain G proteins (●) or G_t (○). After 60 min at 30 °C, the mixture was added to 80 μL of a solution containing cyc$^-$ membranes and the adenylyl cyclase assay components described under Experimental Procedures. This initiated the adenylyl cyclase assay, which was terminated after 30 min at 30 °C. The activity of G_s in the absence of added $\beta\gamma$ was 1.50 nmol·min^{-1}·mL^{-1}.

tivate) G_s. While the $\beta\gamma$ complexes isolated from both sources are capable of enhancing the rate of deactivation of $G_{s\alpha}$, the $\beta\gamma$ from bovine brain is considerably more potent in this regard. Thus, the inclusion of 4.4 nM bovine brain $\beta\gamma$ decreased the half-time of deactivation of G_s from ~15 to ~3.5 min, while a 25-fold greater amount of $G_{t\beta\gamma}$ (110 nM) was required to produce a similar effect.

We have recently described the expression in *E. coli* of active $G_{s\alpha}$ subunits ($rG_{s\alpha}$) (Graziano et al., 1987). The protein has been partially purified from extracts of the bacteria and is capable of reconstituting hormone plus GTP-dependent stimulation of adenylyl cyclase in cyc$^-$ ($G_{s\alpha}$–deficient) S49 cell membranes. However, on the basis of comparison of this activity with immunoreactivity, it appears that the specific activity of the expressed protein is significantly decreased compared to that of the wild-type protein. We have also observed that incubation of $rG_{s\alpha}$ with the brain G protein $\beta\gamma$ complex and GTPγS results in a $\beta\gamma$-dependent increase in $rG_{s\alpha}$ activity (Graziano et al., 1987). Figure 8 compares the ability of bovine brain $\beta\gamma$ and $G_{t\beta\gamma}$ to activate the 45 000-Da form of $rG_{s\alpha}$. Although incubation with the $\beta\gamma$ complex from brain G proteins results in up to a 4-fold increase in the activity of $rG_{s\alpha}$, the $\beta\gamma$ complex of G_t is considerably less effective. In other experiments, we have achieved increases in $G_{s\alpha}$ activity of >10-fold by incubating the recombinant protein with brain $\beta\gamma$ (Graziano et al., 1987), yet $G_{t\beta\gamma}$ is consistently 25–100-fold less potent in this regard.

Discussion

The data presented demonstrate that the $\beta\gamma$ subunit complex isolated from brain G proteins can act catalytically to support the ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ by PT. Thus, it appears that these ADP-ribosylated α subunits possess a relatively low affinity for $\beta\gamma$, even in the absence of an activating ligand. Hydrodynamic studies have shown that ADP-ribosylated $G_{o\alpha}$ and $G_{i\alpha}$, when recombined with $\beta\gamma$, sediment as the oligomer, although GTPγS does cause dissociation of the subunits (Huff & Neer, 1986). Therefore, although the catalytic support by $\beta\gamma$ of ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ demonstrates that these ADP-ribosylated G protein oligomers can dissociate into their respective α and $\beta\gamma$ components, the equilibrium for this dissociation (under the conditions in which the hydrodynamic studies were performed) may lie toward the oligomeric species. One important point to note about these studies is that they were conducted at 4 °C, whereas the ADP-ribosylation reactions were carried out at 30 °C. Subunit dissociation induced by activating ligands is temperature dependent; much less dissociation is apparent when hydrodynamic studies are performed at 4 °C than 32 °C (Codina et al., 1984). Alternatively, it is possible that the dissociation observed in the present study is mediated or promoted in some way by the interaction of PT with the oligomeric G proteins. If ADP-ribosylation of $G_{i\alpha}$ and $G_{o\alpha}$ does, in fact, promote subunit dissociation, this phenomenon could contribute to the inability of these ADP-ribosylated G proteins to interact with receptors.

It should be noted that even under optimal conditions for ADP-ribosylation we have been unable to incorporate more than 0.5–0.8 mol of [^{32}P]ADP-ribose per mole of functional G_α (as determined by [^{35}S]GTPγS binding). This inability to achieve stoichiometric ADP-ribosylation of bovine brain $G_{o\alpha}$ and $G_{i\alpha}$ has also been noted by others (Neer et al., 1984; Huff & Neer, 1986). Interestingly, G_o and G_i purified from rat brain can be stoichiometrically modified by PT (Katada et al., 1986). It is now known that $G_{i\alpha}$ purified from bovine brain is composed of more than one species of GTP binding protein (Mumby et al., 1988); this might also be true for $G_{o\alpha}$. Accordingly, some of these entities may not be substrates for ADP-ribosylation by PT. This could be due either to differences in primary amino acid sequence or, possibly, to a covalent modification that impairs substrate activity. By analogy with G_t, a cysteine residue near the carboxy terminus of $G_{i\alpha}$ and $G_{o\alpha}$ is presumed to be the site of ADP-ribosylation by PT (West et al., 1985). Noteworthy in this regard is that the analogous cysteine residue in the related GTP binding protein, *ras*, is a site of palmitylation (Chen et al., 1985). However, chemical analysis of purified $G_{o\alpha}$ and $G_{i\alpha}$ for base-labile (such as thioester linked) fatty acids did not reveal such fatty acids on these polypeptides (Buss et al., 1987). Alternatively, the domain containing the site of ADP-ribosylation in these proteins may be more labile than that for guanine nucleotide binding; a fraction of these proteins could lose the ability to be ADP-ribosylated during purification.

Previous studies have shown that the $\beta\gamma$ complexes from G_i or G_t are interchangeable in their ability to interact with $G_{i\alpha}$ or $G_{t\alpha}$ in reconstituting rhodopsin-stimulated GTPase activity (Kanaho et al., 1984). Our finding that the $\beta\gamma$ complex from G_t can substitute for $\beta\gamma$ from brain G proteins in supporting the ADP-ribosylation of $G_{o\alpha}$ and $G_{i\alpha}$ by PT provides further evidence of the functional similarity of $\beta\gamma$ subunits isolated from G proteins engaged in diverse roles [see also Heckman et al. (1987)]. We were thus surprised to discover that the $\beta\gamma$ complex from G_t was ~25-fold less potent than brain $\beta\gamma$ in its ability to enhance the deactivation of fluoride-activated G_s. While this paper was in preparation, reports concerning similar disparities in the abilities of brain $\beta\gamma$ and $G_{t\beta\gamma}$ to inhibit G_s-stimulated adenylyl cyclase activity in reconstituted phospholipid vesicle systems (Cerione et al., 1987) and in the deactivation of fluoride-activated G_s (Heckman et al., 1987) have appeared. In addition, the ability of the $\beta\gamma$ complex from G_t to increase the activity of $rG_{s\alpha}$ expressed in *E. coli* was markedly less than that of bovine brain $\beta\gamma$. We presume that the sites on Gα subunits responsible for interaction with $\beta\gamma$ differ significantly between $G_{s\alpha}$ and the other α subunits. This difference apparently permits $G_{s\alpha}$ to discriminate between brain $\beta\gamma$ and $G_{t\beta\gamma}$.

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