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Ability of Guanine Nucleotide Derivatives to Bind and Activate Bovine Transducin

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SUMMARY

Several guanine nucleotide analogs, in one series of which a hydrogen on the 2-amino group is replaced with the *p-n*-butylphenyl group (BuPGNP derivatives), were used to probe the GTP binding domain of bovine transducin. The order of apparent binding affinities in a series of nucleoside 5'-triphosphates was GTPγS > GTP ~ BuPGTP > dGTP ~ ITP >>> ATP, values which were 30–100 times higher than affinities of the corresponding 5'-diphosphates. A derivative bearing a 6-aminohexylamino group on the γ-phosphate, BuPGTP·C_6, had a 60-fold lower affinity compared to BuPGTP. In contrast, the *p-n*-butylphenyl substituent on the 2-amino group had little effect on the binding affinity relative to GTP. Substitutions at the 2-amino group had little effect on either the hydrolysis of the derivatives by the GTPase activity associated with the α-subunit of transducin or the activation of cGMP phosphodesterase. The results indicate that the GTP binding domain of transducin is similar in tertiary structure to the corresponding domain of EF-Tu. The 5'-phosphates of GTP are oriented in the binding site of transducin so that the bulky C_6 group of BuPGTP·C6 dramatically interferes with binding. The 2-amino group on the guanine ring is probably located at the periphery of the binding site, with the *p-n*-butylphenyl substituent of BuPGTP facing outward and only weakly interacting with the protein. BuPGTP should be an excellent parent compound for development of novel probes of G-protein interactions with other cellular proteins involved in receptor signal transduction.

Transducin is the GTP-binding regulatory protein which couples the photoexcitation of rhodopsin with the activation of cGMP phosphodiesterase (1, 2). The activation process involves the rhodopsin-catalyzed release of GDP from Tα and the subsequent binding of GTP. Recent advances allow us to begin to understand the molecular interactions of guanine nucleotides with the binding site on Tα. The elucidation of the primary amino acid sequence for Tα has identified four domains involved in binding of guanine nucleotides and GTPase activity (3, 4). These sequences are highly conserved in the family of proteins that utilize GDP-GTP exchange in their regulation and function (5). In addition, the recent crystallographic analysis of the GDP-binding site in EF-Tu has defined the three-dimensional relationship of these domains at the 2.5 Å level of resolution and has identified several key residues involved in binding guanine nucleotides (6, 7). We have used a series of guanine nucleotide analogs to probe the GTP binding domain of bovine Tα. The findings indicate that the GTP binding domain of Tα is very similar in functional properties to that predicted for the corresponding binding site in EF-Tu. This suggests that the tertiary structure comprising the GTP-binding site in transducin and EF-Tu is highly conserved.

Materials and Methods

Commercial nucleotides were obtained from Sigma Chemical Co. and PL Biochemicals. Thin layer chromatographic analyses on aluminum-backed silica gel plates (Merck) were done to assure purity of all nucleotides. UV spectra were recorded on a Gilford Response spectrophotometer. ^{31}P NMR spectra of synthetic nucleotides were determined with a Bruker WM250 instrument at Clark University; spectra were obtained in solutions containing equal volumes of H_2O and D_2O, and chemical shifts are referenced to external phosphoric acid.

Synthesis of Butylphenyl Guanine Nucleotide Derivatives

N^2-(*p-n*-Butylphenyl)guanosine 5'-phosphate (BuPGMP). N^2-(*p-n*-Butylphenyl)guanosine (8) (200 mg, 0.5 mmol) was dissolved in trimethyl phosphate (3 ml), and the solution was cooled to approximately −4°. Phosphoryl chloride (150 μl, 1.64 mmol) was added and the reaction was maintained at 0° for 4 hr. The product was precipitated by the addition of water (20 ml) and then dissolved by the addition of

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ABBREVIATIONS: Tα, α-subunit of transducin; BuPGMP, N^2-(*p-n*-butylphenyl)guanosine 5'-phosphate; BuPGTP, N^2-(*p*-n-butylphenyl)guanosine 5'-triphosphate; BuPGDP, N^2-(*p*-n-butylphenyl)guanosine 5'-diphosphate; BuPGTP·C_6, N^2-(*p*-n-butylphenyl)guanosine 5'-*O*-[γ-(*N*-6-aminohexyl)triphosphoramidate]; ROS, rod outer segment; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTPγS, guanosine 5'-*O*-(3-thiotriphosphate).

1.0 N sodium hydroxide solution. The resulting solution (pH 10) was filtered through an 0.5 μM Millex filter to remove particulate matter. The filtrate was loaded on a 27 × 4 cm DEAE-Sephadex A-25 column (HCO_3^- form) and eluted with a linear gradient of triethylammonium bicarbonate (0.2 M–2.0 M). Fractions containing the BuPGMP were evaporated under vacuum, with the addition of *n*-butanol to prevent foaming. The BuPGMP as the triethylammonium salt was dissolved in water and lyophilized, resulting in 270 mg (77%) of the product. The nucleotide was stored under vacuum over P_2O_5.

This procedure was performed several times, including scaled-up versions, with yields of ~90%. The largest scale reaction gave ~2 g of the pure BuPGMP as the bis(triethylammonium) salt. UV(H_2O): λ_{max} 288 nm (ε 17,800). ^{31}P NMR: 3.63 δ (t, $J_{P,H5'}$ = 4.3 Hz).

N^2-(*p*-*n*-Butylphenyl)guanosine 5′-triphosphate (BuPGTP). BuPGMP (2.0 g, 2.9 mmol) was dissolved in distilled water (100 ml), and the solution was poured over a Dowex 50Wx8 column (pyridinium form). The resultant filtrate was evaporated under vacuum with the addition of pyridine and tri-*n*-butylamine (700 μl, 5.8 mmol), and the syrup was lyophilized. To a solution of monophosphate (2.5 mmol) in hexamethyltriphosphoramide (15 ml), 1,1-carbonyldiimidazole (2.05 g, 12.5 mmol) was added. After 6 hr the reaction was quenched by the addition of methanol (2 ml). A solution of tetrasodium pyrophosphate (5.58 g, 12.5 mmol) in hexamethyltriphosphoramide (100 ml) was added to the reaction mixture and it stood overnight. The mixture was loaded on a 50×5 cm DEAE-Sephadex A-25 column (HCO_3^- form) and eluted with a linear gradient (0.2 M–2.0 M) of triethylammonium bicarbonate. Fractions containing the BuPGTP were evaporated under vacuum, with the addition of *n*-butanol to prevent foaming. The product was dissolved in water and lyophilized, resulting in 2.15 g of BuPGTP (70% yield). The nucleotide was stored under vacuum over P_2O_5. UV(H_2O): λ_{max} 288 nm (ε 17,000). ^{31}P NMR: −8.34 δ (P_γ, d, $J_{\beta,\gamma}$ = 20.3 Hz), −11.29 δ (P_α, d of t, $J_{\alpha,\beta}$ = 20.3 Hz, $J_{\alpha,H5'}$ = 4.4 Hz), −22.7 δ (P_β, t, $J_{\alpha\beta}$, = $J_{\beta,\gamma}$ = 20.3 Hz).

N^2-(*p*-*n*-Butylphenyl)guanosine 5′-diphosphate (BuPGDP). This nucleotide was isolated as a byproduct from the synthesis of BuPGTP. The fractions on the leading edge of the peak from the BuPGTP purification were combined and evaporated under vacuum, adding *n*-butanol to prevent foaming. The resultant syrup was passed through a 0.5 μM Millex filter and diluted with distilled water (10 ml). This solution was then reloaded onto a 27×4 cm DEAE-Sephadex A-25 column (HCO_3^- form) with a linear gradient of tetraethylammonium bicarbonate (0.2 M–2.0 M). The diphosphate was eluted with ~1.6 M buffer. The fractions containing BuPGDP were evaporated as previously described and lyophilized, leaving 228 mg of BuPGDP as the Tris(triethylammonium) salt (7% yield based on the BuPGTP reaction). UV(H_2O): λ_{max} 287.5 nm (ε 16800). ^{31}P NMR: −9.90 δ (P_β, d, $J_{\alpha,\beta}$ = 20.8 Hz), −11.13 δ (P_α, d of t, $J_{\alpha,\beta}$ = 20.8 Hz, $J_{\alpha,H5'}$ = 4.4 Hz).

N^2-(*p*-*n*-Butylphenyl)guanosine 5′-*O*-[γ-(*N*-6-aminohexyl)-triphosphoramidate] (BuPGTP·C_6). BuPGTP (728 mg, 0.69 mmol) was dissolved in dimethyl sulfoxide (9 ml) that had been dried over 4A molecular sieves. After 1 hr, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (450 mg, 2.3 mmol) was added. After 1 hr, 1,6-diaminohexane (3.6 g, 30 mmol) in dry dimethyl sulfoxide (50 ml) was added to the solution. The reaction was stirred for 3 hr and then stored overnight in the refrigerator. The solution was diluted with water (150 ml) and loaded in a 25×4 cm DEAE-Sephadex A-25 column (HCO_3^- form). Elution was done with a linear gradient of tetraethylammonium bicarbonate (0.2 M–2.0 M) during 24 hr, and the product appeared in approximately 15 hr.

The yield of γ-(6-aminohexylamino) derivative of BuPGTP was 453 mg (69%). (This compound in aqueous solution slowly undergoes hydrolysis to BuPGTP as shown by ^{31}P NMR spectroscopy.) UV(H_2O): λ_{max} 287.5 nm (ε 16800). ^{31}P NMR: −0.77 (P_γ, m, $J_{\beta,\gamma}$ = 20.7 Hz, J_{γ,NCH_2} = 8.9 Hz), −11.31 δ (P_α, d of t, $J_{\alpha,\beta}$ = 20.7 Hz, $P_{\alpha,H5'}$ = 4.2 Hz), −22.56 δ (P_β, t, $J_{\alpha,\beta}$ = $J_{\beta,\gamma}$ 20.7 Hz).

Preparation of ROS Membranes and Transducin Purification

Bovine ROS membranes were prepared under dim red light as previously described (9). Isolated membranes were stored in 20 mM Hepes, pH 8.0, 150 mM KCl, 2 mM $MgCl_2$, and 2 mM dithiotrhreitol. Stripped ROS membranes depleted of extrinsic membrane proteins were prepared and characterized as described elsewhere (10).

[^{35}S]GTPγS Binding to Transducin

Competition for binding of [^{35}S]GTPγS to transducin with unlabeled nucleotides was assayed using a modification of the procedure described by Baehr *et al.* (11). Briefly, transducin was preincubated with an excess of bleached rhodopsin for 10 min at 23° to release bound GDP from the nucleotide-binding site on Tα. The reaction mixture was rapidly chilled to 0°. [^{35}S]GTPγS and varying concentrations of the appropriate unlabeled nucleotide were then added and the mixture was incubated for 10 min at 0°. Samples were diluted 30-fold in ice-cold buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$) and rapidly filtered through a BA85 nitrocellulose filter. The filters were washed in ice-cold buffer and filters were counted in a liquid scintillation counter.

Measurement of NTPase Activity

The procedure of Yamanaka *et al.* (12) was adopted to measure the ability of transducin to hydrolyze nonradioactive guanosine triphosphates. The assay utilizes the fact that guanosine diphosphates are readily exchanged in the presence of bleached rhodopsin, allowing the binding of [^{35}S]GTPγS, whereas guanosine triphosphates bound to transducin exchange very slowly. Transducin, in the presence of an excess of bleached rhodopsin, was incubated for varying times in the presence of GTP or various analogs such as BuPGTP. After the appropriate time, [^{35}S]GTPγS was added to give a 5 μM concentration and the mixture was incubated for 30 sec at 23°. The sample was diluted in ice-cold wash buffer and filtered on BA85 filters as described above. The hydrolysis rate of unlabeled nucleoside triphosphates, whose K_d for binding to transducin had been previously determined, can be compared relative to that for GTP by measuring the time required for recovery of [^{35}S]GTPγS binding.

Chemical Analysis of GTP and BuPGTP Hydrolysis Products

Transducin in the presence of bleached rhodopsin was incubated with 60 μM GTP or BuPGTP for various times at 23°. Aliquots were removed and 720 pmol of the appropriate nucleotide were spotted on silica gel thin layer plates and chromatographed in 2-propanol:H_2O:NH_4OH (6:3:1). Hydrolysis products in the reaction mixtures were identified by their mobility relative to standards whose R_f values were GTP (0.12), GDP (0.21), GMP (0.34), BuPGTP (0.31), BuPGDP (0.50), and BuPGMP (0.66).

cGMP Phosphodiesterase Assay

The method of Yamanaka *et al.* (12) was used to monitor the generation of protons using a pH electrode. Complete hydrolysis of 2 mM cGMP resulted in a pH change from 8.00 to 7.74.

Results and Discussion

Fig. 1 shows the structures of some of the GTP analogs used in a competition binding assay with [^{35}S]GTPγS to measure their apparent binding affinities for transducin. Substitutions were made at the 2-amino group on the guanine ring, terminal γ-phosphate at the 5′-position, and by deletion of one or both hydroxyls at the 2′- and 3′-positions of the ribose moiety. By measuring the apparent affinity of a series of GTP analogs modified at these positions, the relative importance of different chemical groups of the guanine nucleotide for interaction with specific amino acids in the transducin α-subunit sequence can be elucidated.

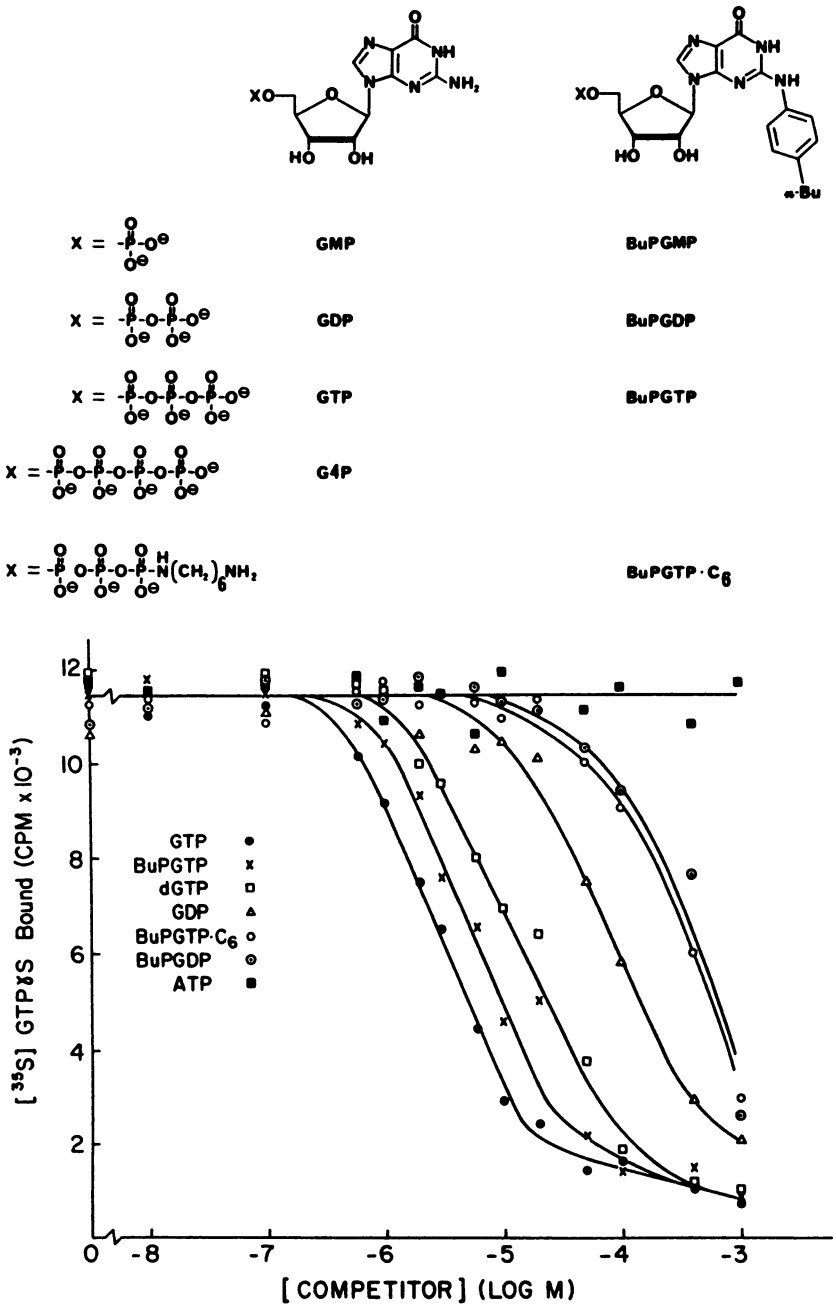

Fig. 1. Structure of the guanine nucleotides used to probe the GTP-binding site of transducin. See text for discussion.

Fig. 2. Competitive displacement of [^{35}S]GTPγS binding to transducin by GTP analogs. Purified transducin (80 nM) was incubated with bleached rhodopsin (800 nM) in stripped ROS membranes for 10 min at 23°. The reaction mixture was then chilled on ice for 5 min. Fifty-μl aliquots were mixed with an equal volume of [^{35}S]GTPγS (3400 cpm/pmol, 500 nM final concentration = 0.9 fractional saturation) and the appropriate concentration of unlabeled competing nucleotide. Samples were incubated for 10 min at 0° and then rapidly filtered on BA85 filters as described in Materials and Methods.

Fig. 2 shows the competition binding curves for six of the GTP analogs used in this study. The normal steepness of the competition curves (Hill coefficients equal to 1) indicates a single affinity class of binding sites for all of the compounds tested. This suggests a simple bimolecular binding reaction for the guanine nucleotide and transducin. As predicted, there is remarkable specificity for GTP relative to ATP, and the GTPs have 30–100-fold higher affinities than the corresponding diphosphates.

Table 1 summarizes the apparent K_d values for all of the GTP analogs tested. The high potency of GTPγS for binding to various G-proteins, including transducin, is well documented (12–14). The γ-thio analog of GTP has approximately 7 times higher affinity for transducin than GTP. Removal of the 2′-hydroxyl group on the sugar ring of GTP to give 2′-deoxy GTP reduces the affinity 4.4-fold, whereas the 2′,3′-dideoxy compound has 175-fold lower affinity than GTP. The hydroxyl groups may be critical in maintenance of a proper ribofuranosyl conformation or in hydrogen bonding to specific amino acids in the transducin-binding pocket. Removal of the 2′-hydroxyl had only a small effect on the binding affinity, an observation consistent with the ability of dGTP to bind G-proteins with reasonably high affinity.

ITP differs from GTP only in the replacement of the 2-amino group of the guanine ring by a hydrogen. The 10-fold decrease in affinity of ITP relative to GTP is consistent with the 2-amino group being involved in hydrogen bonding to a specific amino acid in the binding site. In two related GTP-binding proteins, EF-Tu and Ha-*ras*-p21, this amino acid corresponds to Asp-138 (6, 7) and Asp-119 (15), respectively. In Tα this residue is most likely Asp-268, within the highly conserved guanine recognition sequence.

TABLE 1

Apparent binding affinity of nucleotides to rhodopsin-activated transducin

Competitive displacement curves were generated using rhodopsin-activated transducin and 500 nM [^{35}S]GTPγS as described in the legend of Fig. 2. Apparent binding affinities (K_d') were corrected for the fractional saturation of transducin-binding sites (0.9) estimated from the binding isotherm for [^{35}S]GTPγS in the absence of unlabeled nucleotides.

Competing nucleotide	K_d'
	μM
GTPγS	0.05
GTP	0.34
BuPGTP	0.65
dGTP	1.5
ITP	3.3
GDP	10
BuPGTP·C_6	39
BuPGDP	47
ddGTP	54
dGDP	153
G4P[a]	165
GMP	>400
BuPGMP	>450
ATP	>450

[a]G4P, guanosine 5′-tetraphosphate.

Addition of a bulky butylphenyl group at the N^2 position of GTP (BuPGTP) had little effect on the K_d' for binding to transducin. BuPGTP had a K_d' that was approximately twice that for GTP. The modest decrease in affinity for BuPGTP relative to the 10-fold decreased affinity for ITP compared to GTP suggests that the NH group at the 2-position to which the butylphenyl group is attached is still capable of hydrogen bonding to Asp-268. This butylphenyl residue has little effect on the apparent interaction of this portion of the guanine ring with Tα.

In marked contrast to the relatively small effects observed with substitutions at the 2-amino group, a dramatic decrease in affinity was observed with substitution at the 5′-γ-phosphate. When a 6-aminohexylamino group was added to the γ-phosphate of BuPGTP, the K_d' was reduced approximately 60-fold relative to BuPGTP. These results would be predicted if the tertiary structure of Tα were similar to that proposed for EF-Tu (6, 7). The 5′-phosphates of GTP would be oriented in the binding site of transducin so that the bulky aminohexylamino group dramatically interferes with binding. This might occur as a consequence of a steric repulsion or loss of a critical negative change at the γ-phosphate. Interestingly, guanosine 5′-tetraphosphate, in which an extra phosphate group is placed on the γ-phosphate of GTP, is significantly weaker in affinity for Tα compared with BuPGTP·C_6. This observation suggests that steric repulsion by a substituent is less important than the change in charge interactions with the protein. In contrast, the 2-amino group on the purine ring is most probably located at the periphery of the binding site as in the structure of EF-Tu. This would allow the bulky *p-n*-butylphenyl group to face outward and only weakly influence binding of the GTP moiety to transducin.

Fig. 3 shows results using a kinetic assay developed by Yamanaka *et al.* (12) to measure GTPase activity of transducin in the presence of BuPGTP or GTP. This assay is based on the finding that triphosphates of guanosine analogs do not readily exchange when bound to Tα, but hydrolysis to the diphosphate allows rapid exchange. Thus, concentrations of GTP or BuPGTP severalfold above their respective K_d values will inhibit 80–90% of the [^{35}S]GTPγS binding to transducin compared to the binding measured in their absence. Binding of [^{35}S]GTPγS increases during the time-dependent hydrolysis of GTP or BuPGTP to the appropriate diphosphate. Clearly, both GTP and BuPGTP are hydrolyzed in a time-dependent manner. The 2-fold longer time required for BuPGTP hydrolysis compared to GTP is due to the 2-fold higher initial concentration of BuPGTP used to achieve the same initial inhibition of [^{35}S]GTPγS binding as that for GTP, requiring twice as many BuPGTP molecules to be hydrolyzed relative to GTP. Nonetheless, bulky side groups like *p-n*-butylphenyl have little or no effect on the hydrolysis rate of triphosphates bound to transducin. The kinetic determination of BuPGTP hydrolysis was confirmed by direct chemical analysis by thin layer chromatography. Transducin (1.5 μM) was activated in the presence of bleached rhodopsin (2.5 μM) at 23° in the presence of 60 μM GTP or BuPGTP. At appropriate times aliquots were removed and guanine nucleotides were identified by their R_f value relative to standards as described in Materials and Methods. BuPGTP and GTP were converted to their corresponding diphosphates in a time-dependent manner which required the presence of both transducin and bleached rhodopsin (not shown).

Fig. 4 demonstrates that BuPGTP was also capable of supporting the transducin-dependent, light-activated cGMP phosphodiesterase activity in ROS membrane preparations, similar to that observed with GTP. The V_{max} for activation was the same for GTP and BuPGTP, whereas the apparent K_m was 0.27 μM for GTP compared to 0.44 μM for BuPGTP. These findings are consistent with the differerences observed in K_d' for binding of GTP (0.34 μM) and BuPGTP (0.65 μM) to transducin. Interestingly, the ability of ITP to activate phosphodiesterase activity (K_m = 1.6 μM) was also similar to its K_d' (3.3 μM) for binding to transducin (see Fig. 4). Since ITP lacks the 2-amino group, this indicates that the hydrogen bonding to Asp-268 at this position on the guanine ring plays a minor role in mediating the conformational changes in Tα induced by the binding of guanosine triphosphates. Rather, it appears that the 2-amino group is involved primarily in hydrogen bonding for positioning the 5′-triphosphates in an orientation in the binding site required for transducin activation.

Interestingly, Sigal *et al.* (16) recently reported that replacement of Asp-119 in Ha-*ras*-p21 with an alanine residue reduced the affinity of GTP and GDP by a factor of 20. However, the ability of [Ala^{119}] Ha-*ras*-p21 to induce transformation of NIH 3T3 cells was similar to that of the oncogenic [Val^{12},Thr^{59}] Ha-*ras*-p21. The increased transformation potential observed with the reduced affinity for guanine nucleotides in [Ala^{119}] Ha-*ras*-p21 was attributed to an increased dissociation rate for bound GDP. Asp-119 in *ras* proteins is apparently equivalent to Asp-268 in Tα. The dramatic change in biological function of the mutated [Ala^{119}] Ha-*ras*-p21 suggests that guanine nucleotide analogs with modifications at the 2-amino group on the guanine ring, which have correspondingly lower binding affinities than GTP or GDP, might induce similar alterations in the properties of the normal cellular GTP-binding regulatory protein.

Our findings show that substitutions at the 2-amino group of GTP have little effect on the ability of the nucleoside triphosphate to induce Tα stimulation of cGMP phosphodiesterase activity and to be hydrolyzed by the GTPase activity associated with Tα. At equal fractional occupancy of the Tα-

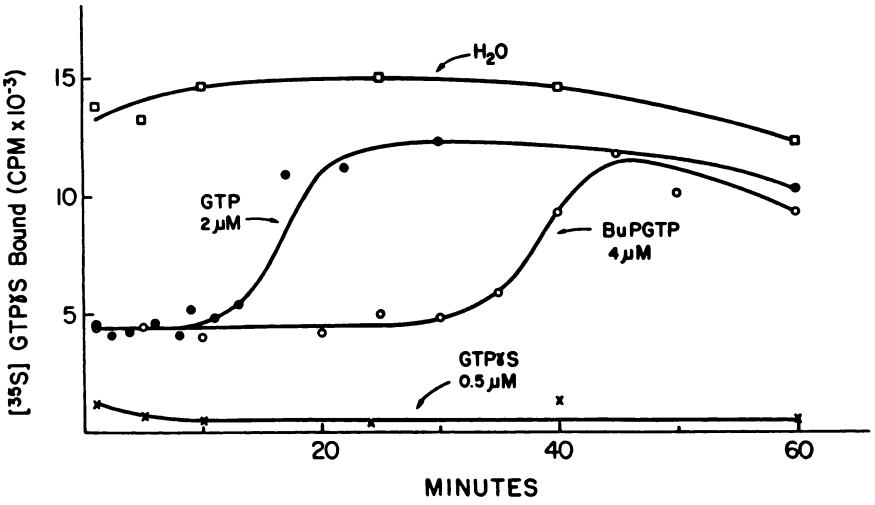

Fig. 3. Hydrolysis of GTP and BuPGTP by activated transducin. One hundred fifty nM transducin was incubated with 500 nM illuminated rhodopsin in the presence of 2 μM GTP, 4 μM BuPGTP, 0.5 μM GTPγS, or H_2O. At the indicated times 80-μl aliquots were removed and mixed with 20μl of [^{35}S]GTPγS (1220 cpm/pmol, 5 μM final concentration). After 30 sec at 23° the samples were filtered as described in Materials and Methods. The presence of 0.5 μM unlabeled GTPγS, a nonhydrolyzable GTP analog, for 1 min prevented subsequent binding of 5 μM [^{35}S]GTPγS binding throughout the time course of the experiment, but the time- and transducin-dependent hydrolysis of GTP and BuPGTP allowed a recovery of [^{35}S]GTPγS binding with a $t_{½}$ of approximately 16 and 38 min, respectively, for the two nucleotides. The relative hydrolysis rates for GTP and BuPGTP were 0.9 and 0.8 min^{-1}, respectively.

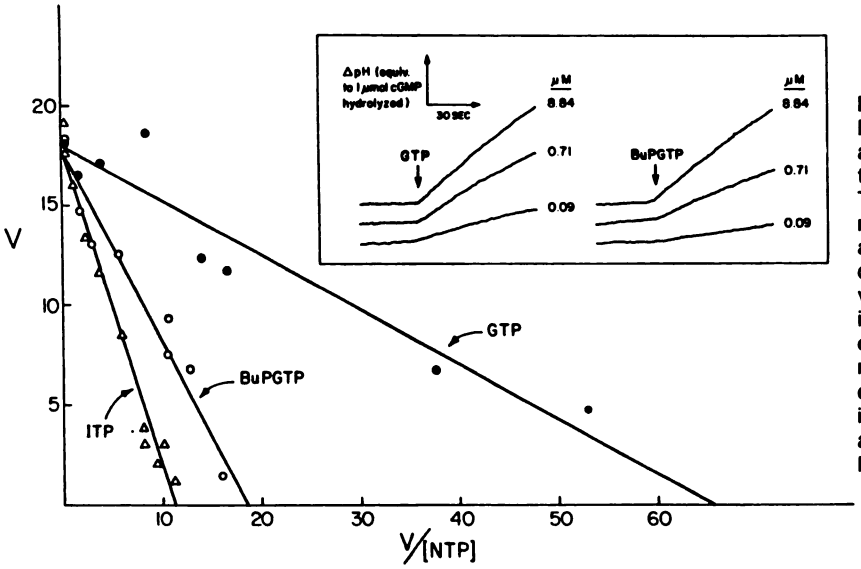

Fig. 4. Activation of cGMP phosphidesterase by GTP, BuPGTP, and ITP. Initial rates of cGMP phosphodiesterase activation in ROS membranes was determined in the presence of GTP, BuPGTP, or ITP (0.09–89 μM). The change in pH due to hydrolysis of cyclic GMP was monitored using a pH electrode as described in Materials and Methods. ROS membranes containing 5.2 μM rhodopsin were incubated with 1.77 mM cGMP in a total volume of 4.5 ml. The basal cGMP hydrolysis was monitored by recording the pH for 2–3 min before the addition of GTP, BuPGTP, or ITP. Hydrolysis of 1 μmol of cGMP resulted in a pH change of 0.03 unit. The *inset* shows examples of typical recordings. Data are expressed using a Woolf plot where velocity (V) is in arbitrary units and concentration is in μM for GTP (●), BuPGTP (○), and ITP (△).

binding site, BuPGTP and ITP gave similar Tα activation states as compared to GTP. Thus, the only measurable difference with BuPGTP and ITP relative to GTP is their K_d for binding to Tα. This is consistent with the hypothesis of Sigal *et al.* (16) that the [Ala^{119}] *ras* mutation, which has lowered GTP and GDP affinity, probably leads to increased transformation as a result of an increased fraction of GTP-liganded *ras*-p21 molecules. This could result from an increased dissociation of GDP and subsequent binding of GTP. The cellular concentration of GTP is greater than the K_d of GTP for the [Ala^{119}] *ras*-p21, which would allow rapid binding and activation of the mutant. The [Ala^{119}] mutation would then result in an increased fraction of activated *ras* molecules relative to the normal *ras*-p21 having a higher affinity for GDP.

The demonstrated ability to derivatize the 2-amino group of GTP with retention of functional Tα activation indicates it is feasible to synthesize GTP analogs with different chemically reactive substituents to probe domains of Tα. These domains might include sequences that interact with Tβγ as well as other proteins such as cGMP phosphodiesterase and rhodopsin. The extreme conservation in primary amino acid sequence of G-protein α-subunits (17), and the apparent importance of interactions beween the 2-NH_2 group of the guanine ring with specific aspartic acid residues in the primary sequence of *ras*-p21, suggests that similar studies may be useful in defining effector protein interactions in other G-protein systems.

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References

1. Fung, B. K. K., and L. Stryer. Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc. Natl. Acad. Sci. USA* **77**:2500–2504 (1980).
2. Fung, B. K. K., and L. Stryer. Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. USA* **78**:152–156 (1981).
3. Medynski, D. C., K. Sullivan, D. Smith, C. Van Dop, F.-H. Chang, B. K. K. Fung, P. H. Seeburg, and H. R. Bourne. Amino acid sequence of the α subunit of transducin deduced from the cDNA sequence. *Proc. Natl. Acad. Sci. USA* **82**:4311–4315 (1985).
4. Tanabe, T., T. Nukada, Y. Nishikawa, K. Sugimoto, H. Suzuki, H. Takahaski, M. Noda, T. Haga, A. Ichiyama, K. Kangawa, N. Minamino, H. Matsuo, and S. Numa. Primary structure of the α-subunit of transducin and its relationship to *ras* proteins. *Nature (Lond.)* **315**:242–245 (1985).
5. Halliday, K. R. Regional homology in GTP-binding proto-oncogene products and elongation factors. *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9**:435–448 (1984).
6. Jurnak, F. Structure of the GDP domain of EF-Tu and location of the amino acids homologous to *ras* oncogene proteins. *Science (Wash. D. C.)* **230**:32–36 (1985).
7. la Cour, T. F., J. Nyborg, S. Thirup, and B. F. C. Clark. Structural details of the binding of guanosine diphosphate to elongation factor Tu from *E. coli* as studied by X-ray crystallography. *EMBO (Eur. Mol. Biol. Organ.) J.* **4**:2385–2388 (1985).

8. Dudycz, L. W., and G. E. Wright. A simple one-pot method for 6-oxopurine ribonucleoside synthesis: control and mechanism of isomer distribution. *Nucleosides Nucleotides* **3**:33–44 (1984).
9. Kelleher, D. J., and G. L. Johnson. Phosphorylation of rhodopsin by protein kinase C *in vitro*. *J. Biol. Chem.* **261**:4749–4757 (1986).
10. Yamazaki, A., F. Bartucca, A. Ting, and M. W. Bitensky. Reciprocal effects of an inhibitory factor on catalytic activity and noncatalytic cGMP binding sites of rod phosphodiesterase. *Proc. Natl. Acad. Sci. USA* **79**:3702–3706 (1982).
11. Baehr, W., E. Morita, R. J. Swanson, and M. L. Applebury. Characterization of bovine rod outer segment G-protein. *J. Biol. Chem.* **257**:6452–6460 (1982).
12. Yamanaka, G., F. Eckstein, and L. Stryer. Stereochemistry of the guanyl nucleotide binding site of transducin probed by phosphorothioate analogs of GTP and GDP. *Biochemistry* **24**:8094–8101 (1985).
13. Brandt, D. R., and E. M. Ross. Catecholamine-stimulated GTPase cycle: Multiple sites of regulation by β-adrenergic receptor and Mg^{2+} studied in reconstituted receptor-G_s vesicles. *J. Biol. Chem.* **261**:1656–1664 (1986).
14. Huff, R. M., and E. J. Neer. Subunit interactions of native and ADP-ribosylated α_{39} and α_{41}, two guanine nucleotide-binding proteins from bovine cerebral cortex. *J. Biol. Chem.* **261**:1105–1110 (1986).
15. McCormick, F., B. F. Clark, T. F. M. la Cour, M. Kjeldgaard, L. Norshov-Lauritsen, and J. Nyborg. A model for the tertiary structure of p21, the product of the *ras* oncogene. *Science (Wash. D. C.)* **230**:78–82 (1985).
16. Sigal, I. S., J. B. Gibbs, J. D' Alonzo, G. L. Temeles, B. S. Wolanski, S. H. Socher, and E. M. Scolnick. Mutant *ras*-encoded proteins with altered nucleotide binding exert dominant biological effects. *Proc. Natl. Acad. Sci. USA* **83**:952–955 (1986).
17. Itoh, H., T. Kozasa, S. Nagata, S. Nakamura, T. Katada, M. Ui, S. Swai, E. Ohtsuka, H. Kawasaki, K. Suzuki, and Y. Kaziro. Molecular cloning and sequence determination of cDNAs for α subunits of the guanine nucleotide-binding proteins G_s, G_i, and G_o from rat brain. *Proc. Natl. Acad. Sci. USA* **83**:3776–3780 (1986).

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