Low Affinity Interactions of GDPβS and Ribose- or Phosphoryl-substituted GTP Analogues with the Heterotrimeric G Protein, Transducin*

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We have examined the effects of three commonly used classes of guanine nucleotide analogues on the retinal G protein, transducin (Gt), and found them to be quite different from those that might be expected from results with other GTP-binding proteins. The most surprising results were with guanosine 5'-O-(2-thiodiphosphate) (GDPβS) rather than inhibiting activation of Gt, GDPβS addition activated Gt as a result of a trace contaminant. Even when the contaminant levels were reduced 5-fold by chromatography, its effects dominated those of GDPβS, which binds Gt at least 1500-fold more weakly than guanosine 5'-O-(3-thiotriphosphate) (GTPγS). The affinity of Gt for GDP was found to be at least 300-fold weaker than for GTPγS, while the affinities of GTP and GDPβS were similar. Ribose-modified GTP analogues, including 2'(3')-O-(N-methylanthraniloyl) GTP (mant-GTP), 2'(3')-O-(2-aminoethyl)carbamyl] GTP (edGTP), and adducts of fluorescein 5-isothiocyanate and rhodamine B-isothiocyanate with edGTP, interacted extremely weakly, if at all, with the GTP binding site of the α subunit of Gt. They were neither effective activators of Gt nor effective inhibitors of activation by GTP or GDPβS. A γ-phosphoryl-modified analogue, an adduct of GTPγS and (5-(2(iodoacetyl)aminoethyl)amino)naphthalene-1-sulfonic acid (dnGTP), also activated Gt weakly, if at all, and did not inhibit its activation. The exclusion of these analogues points to the highly restrictive and specific nature of the GTP binding site of Gt, in contrast to those of numerous other GTP-binding proteins which are potently activated or inhibited by these analogues.

Structural analogues of nucleotides have played an important role in developing our understanding of protein-nucleotide interactions that govern the functions of nucleotide-binding proteins. The large class of proteins containing GTP binding sites homologous to those of signal-transducing G proteins has been extensively explored by the use of hydrolysis-resistant analogues of GTP and GDP, as well as by use of analogues modified at various positions to probe the structural and chemical characteristics of the binding sites. The G protein of retinal rod outer segments, Gt, has been probed by several series of such analogues (1–3).

For heterotrimeric G proteins, including Gt, a particularly large number of studies have been carried out using GDPβS as a GDP analogue resistant to conversion to an activating nucleotide and to other metabolic reactions (4). In the case of Gt, this analogue has been used in studies of Gt binding to R* (metarhodopsin II, the light-activated active form of rhodopsin) by light scattering and biochemical techniques (5) and in electrophysiological experiments where it was introduced into functional rod outer segments (6, 7). There have been very few studies aimed at directly determining whether GDPβS actually blocks GTP activation of heterotrimeric G proteins or not. In one study of Gt (2), essentially no effect of GDPβS on GTP binding was observed, although GDPβS was found to inhibit weakly binding of GTP. The often cited study that first demonstrated inhibitory activity of GDPβS in some cells (4) also reported hormone-dependent stimulation of adenylyl cyclase by GDPβS in liver and parotid membranes. Paris and Pouysségur (8) found that GTPγS effects on G protein pathways in fibroblast cells were mimicked, rather than antagonized, by addition of GDPβS.

When we attempted to use GDPβS to inhibit activation of Gt, we were surprised to observe activation by this analogue rather than inhibition of activation. This unexpected result, together with the finding of very high affinity interactions between Gt and GTPγS (see our companion study, Ref. 9) raised the possibility that trace contaminants may have contributed to some of the results previously observed with GDPβS, and led us to re-examinate its interactions with Gt.

Another potentially useful class of GTP and GDP analogues are those modified with fluorescent groups. While this class of analogues, particularly those modified at the 2' or 3' positions, have been very successfully used for studying small GTP binding proteins such as p21ras (e.g. see Refs. 10–13) and elongation factor EF-Tu (e.g. see Refs. 14 and 15), it is not clear whether

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1 The abbreviations used are: Gt, transducin, the α subunit of transducin; GTPγS, guanosine 5'-O-(3-thiotriphosphate); GDPβS, guanosine 5'-O-(2-thiodiphosphate); mant-GTP, 2'(3')-O-(N-methylanthraniloyl) GTP; edGTP, 2'(3')-O-(2-aminoethyl)carbamyl] GTP; dnsGTP, adduct of GTPγS and (5-(2(iodoacetyl)aminoethyl)amino)naphthalene-1-sulfonic acid; PDE γ, the inhibitory γ subunit of PDE; PEI, polyethyleneimine; ROS, rod outer segments; R*, the photoactivated form of rhodopsin; R, total rhodopsin regardless of its form; HPLC, high performance liquid chromatography; AA-GTP, P(4-azidoanilido)-P(8)-guanosine triphosphate.
these or any other fluorescent GTP analogues have sufficient affinity for the GTP binding sites of heterotrimeric G proteins in general, or of Gα in particular, to be useful for functional studies. Because of the structural conservation of GTP binding sites between Gα and proteins known to bind ribose-modified GTP, or any other fluorescent GTP analogues have sufficient affinity for the Gα in particular, to be useful for functional

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Analyses of Analogue Binding by Competition with GTPφS—For GDP and GdPS, equilibrium binding experiments were carried out using bleached ROS as described (9), with sufficient GDP or GdPS added to give the indicated final concentrations, just before addition of GTPφS. For the 2-GTP analogues and for GTP itself, transient experiments rather than equilibrium ones were carried out to minimize hydrolysis of the β-γ-phosphohydride bonds, which occurs with a rate constant of ∼1/(30 s) for GTP bound to GαS. (29) GTPφS uptake assays were carried out by timed vacuum filtration through nitrocellulose as described (23). Inhibition of GTPφS binding was measured by incubating purified transducin with urea-stripped ROS membranes, GTPφS, and varying concentrations of competing analogues for one minute before nitrocellulose filtration. GTPφS and the competing nucleotides were premixed before addition to the sample containing protein and membranes. Apparent Kα values were estimated by nonlinear least squares fitting of the data to the expression B = Bαα(βαα − Bα)GαS + GαS, where BαS is the bound GTPφS, Bα is the amount bound in the absence of inhibitor, I is the concentration of inhibitor (GTP, mantGTP, or edGTP), GαS is the concentration of GTPφS, Kαα is the dissociation constant for GTPφS, determined independently to be 50 pM (9), and Kα is the inhibition constant characteristic of each inhibitor. The parameter BαS corresponding to the amount of GTPφS bound at infinite inhibitor, was determined to be 11% of BαS by nonlinear least squares fitting to the GTPφS data with both BαS and Kα allowed to vary, while Kα values for edGTP and mantGTP were determined by fitting with BαS held constant at 11% and only Kα allowed to vary. It is possible that this 11% residual binding may represent the covalent radiolabeling by GTPφS previously reported to occur at high [GTPφS] (30, 31), because, in contrast to conditions of the equilibrium studies (9), the concentrations of protein and GTPφS in these kinetic experiments are sufficiently high for some thio phosphorylation to occur.

RESULTS

GαS Interactions with GDPφPS and GDP—Our experiments with GDPφPS reported here were motivated by the outcome of experiments in which we attempted to inhibit GαS activation by GTP in bleached ROS. The key observations made in these initial experiments, for which data are not shown, were as follows. 1) No inhibition was observed at any concentration tested. 2) As we increased the GDPφPS concentration in an attempt to force inhibition, we found that GDPφPS induced activation of PDE (and therefore, presumably of GαS) although such an action was not observed at much higher concentrations of 5′-GMP, the principal contaminant of commercial GDPφPS. 3) No effect of GDPφPS was observed when it was tested with purified PDE, implying its PDE effects were mediated by GαS. 4) The activation turned off much more slowly (koff < 1/500 s) than activation by low concentrations of GTP (koff < 1/30 s) (29), suggesting a nonhydrolyzable or slowly hydrolyzed contaminant was responsible. 5) The activation of PDE was a small fraction (<10%) of that observed with trypsin activation (i.e. complete activation by removal of PDE γ), but did not increase after the first several seconds, indicating that activa-
tion was not due to degradation of the inhibitory PDEγ subunit by contaminating proteases. 6) Prolonged incubation of GDPbS with ROS did not give rise to increased activation; rather, activation was decreased slightly by such incubation, including ATP prevented the decrease, possibly by replacing a phosphate group slowly released by hydrolysis.

Because of these unexpected results, we carried out equilibrium competition experiments using GTPγS as shown in Fig. 1A. These indicated that Gb's apparent Kd for GDPbS (assuming the inhibition was due to GDPbS and not a contaminant) was approximately 350-fold higher than that for GTPγS. However, at ratios of GDPbS to activating nucleotide sufficient to give measurable inhibition of GTPγS binding in the equilibrium studies (e.g. 100 μM GDPbS and 76 nM GTP), no inhibition of GTP-stimulated PDE activation was observed, but 100 μM GDPbS did give rise to easily measurable activation of PDE, about 40% of the level stimulated by 1 μM GTP (data not shown).

We compared these results to those obtained with GDP. As reported previously (32) GDP also activated PDE in ROS, even when highly purified (data not shown). In equilibrium binding experiments (Fig. 1A) GDP behaved similarly to GDPbS. Assuming, probably incorrectly, that GDP itself was entirely responsible for the inhibition of GTPγS binding, its Kd for Gb, appeared to be about 300-fold higher than Kd for GTPγS. Two successive rounds of purification of GDP by DE-52 anion exchange chromatography reduced contaminating GTP below levels detectable by our HPLC assay, and by calibration with radiolabeled GTP it was estimated to remove ~85% of any contaminating GTP each round. However, these procedures did not greatly reduce the ability of GDP to activate PDE in ROS (data not shown). The levels of PDE activity observed were much too high, given the concentration of Gb present in the ROS, to be accounted for by the weak activation of PDE by Gb, -GDP reported by Kutuzov and Pfister (33). Because results with GDP are complicated by a GDP phosphotransferase activity in ROS that is difficult to eliminate (32) and leads to GTP production from added GDP, we did not pursue further the reason for its ability to stimulate PDE activity. From assays of [32P]GMP production from (c-[32P]GDP, we estimate that, under the conditions of Fig. 1A, GDP conversion to GTP should occur at a rate of approximately 0.03% min-1. Conversion of as much as 5% of the GDP to GTP could have occurred over the 3 h time course of Fig. 1A, but the effect of this conversion would be greatly reduced by hydrolysis of GTP bound to Gb.

GDPbS is presumably resistant to the same reaction (4) and even if phosphorylated yields a product, GTPbS, that is not an efficient activator of Gb (2). Therefore we focused on GDPbS and attempted to purify away the activating contaminant(s) present in commercial stocks by ion exchange chromatography (Fig. 1B). It is clear, from comparing the profiles of GDPbS elution and PDE activation, that it is a contaminant, rather than GDPbS itself, that is responsible for PDE activation. It can also be seen from Fig. 1B that a distinct peak of 254-nm absorbance can be observed for GMP, the major contaminant of GDPbS, but that the activating contaminant is either present in much lower amounts than GMP, or absorbs at 254 nm much more weakly, as there is no identifiable peak of absorbance corresponding to it. Because the leading edge of the GDPbS peak had the lowest PDE activation potency, only these early fractions from the chromatogram shown, or from a similar one (i.e. those corresponding to pool i, Fig. 1B), were used for further studies. GDPbS prepared in this way showed a ~5-fold lower apparent potency in competition studies with GTPγS

2 J. K. Angleson, unpublished results.
Transducin Binding of Nucleotide Analogues

Gt Interactions with 2′(3′) Analogue—Because PDE activation in ROS is an easy, sensitive, and robust assay for Gt activation by nucleotides, we initially used this assay to assess the effects of the modified GTP analogues. Fig. 2 shows results typical of numerous attempts to detect either activation of Gt or inhibition of its activation by these analogues. When 1.3 μM to 15 μM mantGTP (Fig. 2A) was added to bleached ROS, very little activation of PDE was observed. In the experiments depicted in trace i of Fig. 2A, addition of 1.3 μM mantGTP appeared to stimulate a less than 60% increase over basal activity, to a final activity that was somewhat less than the basal activity in the subsequent control trace (ii), and that represented an activity increase only 6% of that stimulated by GTP in the control trace (ii). Tripling the concentration, to 4 μM total mantGTP, did not measurably increase the PDE activity. The lower mantGTP trace (Fig. 2A, iii) shows that the increase in PDE activity when 15 μM mantGTP was added was less than 5% of the increase induced by only 100 nM GTP. At 15 μM, mantGTP was also a poor inhibitor of transducin activation; in its presence, 100 nM GTP elicited an increase in PDE activity only 13% lower than that elicited by the same concentration of GTP in the absence of this 150-fold excess of mantGTP (Fig. 2A, trace iii). The high affinity of GTP for Gt, Kd = 50 nM (9), implies that in this experiment, with 5 μM R and −200 nM Gt, free GTP must be only about 13 nM, the amount prevented from binding by mantGTP. Therefore, this result implies that mantGTP must be competing for the GTP binding site at least 1000-fold less potently than GTP.

Fig. 2D shows that at very high concentrations of the 2′(3′) modified GTP analogues, significant PDE activation could be observed. In the case of edGTP, however, the 3% contamination with GTP would be expected to give rise to even greater PDE activation than observed (Fig. 2D, dashed line), consistent with no activation by edGTP, but a slight (<20%) inhibition of GTP-dependent activation by edGTP in >30-fold molar excess. In the case of mantGTP, the weak activation observed could be explained by as little as 0.1% contamination with GTP, an amount of contamination that could not be ruled out because it was at the level of noise in the chromatogram. Thus it remains unclear whether mantGTP can activate transducin at all.

As the effects on PDE activity caused by the analogues were so weak as to be barely detectable, we decided to use a more direct GTP-Y-S binding assay to detect competition for the GTP
binding site. This assay is done on a somewhat faster time scale than the PDE assays, so breakdown of the analogues is also less of a concern. Fig. 3 shows that GTP competes very effectively with GTP-S in this assay, yielding an apparent K_i of 93 pM, comparable to the GTP-S K_i of 50 pM. In contrast, mant-GTP and edGTP gave rise to marginal inhibition at the highest concentrations tested. If the results are taken at face value, K_i values are obtained that are approximately 2600-fold higher (mantGTP) or 156-fold higher (edGTP) than the K_i for GTP-S.

Even with such low affinity, we thought it might be possible to detect mantGTP binding to G_tα by following the distinctive fluorescence of the N-methylanthraniloyl group using an affinity column procedure which purifies G_tα with GTP-S or GDP still tightly bound. This procedure does not suffer from ambiguities due to hydrolytic release of GTP from the analogue discussed below. Fig. 4 shows the elution profiles of such Cibacron Blue affinity columns. GTP-S eluted with G_tα irrespective of the presence or absence of a 6-fold excess of mantGTP, confirming the results of Figs. 3 and 4 and verifying that the column procedure works as expected. Incubation with mantGTP in the absence of any competing nucleotide, other than a stoichiometric amount of endogenous GDP, did not result in the detection of a peak of mantGTP fluorescence corresponding to G_tα. Subsequent experiments have demonstrated that even GDP, which binds G_tα much more weakly than GTP or GTP-S (see Fig. 1A) remains tightly bound to G_tα on this type of column.2 While we cannot rule out the possibility that mantGTP fluorescence is quenched when bound to G_tα, such quenching would be surprising given the enhancement of mantGTP (or mantGTP-S) fluorescence reported upon binding to G_tα (16, 17).

The results of these different assays, taken together, make it clear that, if these analogues bind G_tα at the GTP-binding site at all, they do so with very low affinities compared to GTP-S or GTP and do not induce a conformation of G_tα that resembles that induced by GTP with respect to its ability to activate PDE. Ambiguity arises over whether they bind G_tα at all, because in the assays shown in Figs. 3 and 4, production of GTP from analogues might account for some or all of the inhibition or activation observed. We verified repeatedly that such hydrolysis does occur, as illustrated in Fig. 5 for edGTP, which is expected to be more resistant to hydrolytic GTP release than mantGTP. Fig. 5 shows the results obtained upon chromatographic analysis of a sample of edGTP that initially appeared pure and unable to activate transducin, but gradually acquired unusually high transducin activating activity upon use and several freeze-thaw cycles. Readily detectable levels of GTP had been released, presumably by spontaneous hydrolysis of the 2′(3′)-carbamoyl group. From the average of hydrolysis rates observed in samples from two different preparations stored at 4 °C, the apparent first order rate constant for hydrolysis was estimated to be 4.65 (± 1.1) × 10^{-5} s^{-1}. While this represents an extremely slow reaction (half-life of 173 days), consistent with the known stability of this class of compounds, it presents a serious problem for conducting comparisons with GTP, due to the extreme sensitivity to traces of contaminating GTP. Similar results have been observed with mantGTP (data not shown), and may account for some of the variability in the results observed at high [mantGTP] in Fig. 3. For the nucleotide stocks used for the experiments of Fig. 2D, a GTP contamination of ~3% was found for edGTP, while for mantGTP, it was possible to establish only an upper limit of ~0.1% contamination due to baseline noise at the GTP elution position. These results illustrate a fundamental problem in working with GTP analogues (such as mantGTP and edGTP) which interact very weakly (if at all) with transducin but which can generate traces of GTP (and possibly other active species) upon spontaneous hydrolysis. If these modified nucleotides even remotely resembled good analogues in their interactions with transducin’s GTP binding site, such low levels of contamination would be of little concern. However, the presence of traces of GTP, which binds G_tα with picomolar affinity, in solutions of analogues that must be used at micromolar levels for any effects to be observed, renders these analogues virtually useless for most purposes.

DISCUSSION

Structural Constraints of the Nucleotide Binding Site—The exquisite specificity of transducin’s nucleotide binding site may be understood in terms of its highly restrictive geometry and...
In the same series of experiments, a decrease in sensitivity and slowing of the rising phase of the light response were also observed, but these inhibitory effects were seen consistently only when the pipette contained higher GDP/PS (11 mM), and these effects developed much sooner after GDP/PS introduction than the prolongation of activation. Together with our results, these earlier studies suggest that there is a very weak inhibition of Gαs activation observable at high GDP/PS concentrations, but that it is generally accompanied by persistent (but also weak) activation due to a contaminant of GDP/PS or to a metabolic product of a contaminant.

Because radiolabeled GDP/PS was not available, and because of our failure to obtain GDP/PS completely free from contamination, we are not able to say accurately how tightly this nucleotide analogue binds Gαs. Results have been published demonstrating competition for GDP binding by GDP/PS (2, 5), although in spectroscopic and light scattering experiments, GDP/PS was shown not to mimic the effects of GDP on Gα, binding to metarhodopsin II (5) and was found to inhibit the rate of GTP binding very weakly (apparent IC50 of 500 μM). What we can say unequivocally is that GDP/PS is not an efficient inhibitor of Gαs activation. This conclusion is in stark contrast to the well known effects of GDP/PS on Gα, which is half-maximally inhibited at 1000-fold lower GDP/PS concentrations, 400 nM (4). Our results also suggest rather strongly that some previous studies with GDP/PS may need to be reinterpreted in light of its failure to inhibit Gαs activation of PDE in direct measurements, and in light of the likely presence in most GDP/PS preparations of contaminant(s) that give rise to long-lived Gαs activation.

2′(3′) Analogues—It is somewhat disappointing that mantGTP and related 2′(3′)-acyl GTP analogues are not functional GDP analogues for transducin; to date no fluorescent nucleotides have been found which activate this G protein. The apparent inability of these analogues to form productive complexes with Gαs is not surprising, given the structural constraints discussed above. While some of these constraints are also present in p21ras and EF-Tu, for which these analogues have high affinity, both the structural data (34) and our present results indicate that there are additional constraints in Gαs. Because even the relatively small and flexible 2-aminoethyl group of mantGTP seems to preclude productive interactions, it is likely that the hydrogen bonds to both the 2′ and 3′ positions of GTP play a critical role in binding. There is apparently less flexibility in this part of the nucleotide binding site in transducin than in EF-Tu which lacks analogous hydrogen bonds (37, 38) and binds mantGTP (15), as well as a rhodamine B sulfonochloride adduct of edGTP (39), and also less flexibility than in p21ras, which forms a hydrogen bond to 2′ OH (40), and binds the 2′ form of mantGTP with ~6-fold lower affinity than the 3′ form (10, 41).

Gαs has been reported to bind mantGTP and mantGDP as well as mantGTPγS (16). This binding appears to be dramatically different from that of GTP or GDP; dissociation was reported to be extremely rapid for both mantGTP (0.4 s−1) and mantGTP (0.03 s−1) as compared to the parent nucleotides. In addition, it was reported that mantGTP does not efficiently induce activation of Gαs. Thus, the most important conclusions that can be drawn from the results with Gαs that the mant nucleotides are poor analogues for GTP and GDP, and that the binding mode of mantGTP must differ greatly from the binding of GTP that induces the activated Gαs conformation, are consistent with our observations for Gα.

A previous study (3) of a GTP analogue acylated at the 2′(3′) position with a 3-[N-(4-azido-2-nitrophenyl)-aminolpropionyl] group also provided evidence that substitutions at these posi-
tions abolish GTP’s ability to activate G\(_{\alpha}\), or bind it with high affinity. Inhibition of GTP hydrolysis was half-maximal at a 1000-fold excess of analogue over GTP, and the analytical procedures described could not rule out a 0.1% contamination with GTP. PDE activation was half-maximal at 10 \(\mu\)M analogue, implying a 200,000-fold lower efficiency of activation by the analogue than by GTP-\(\gamma\)S, and it was not possible to induce photoreaction of G\(_{\alpha}\) with the highly reactive 4-azido-2-nitrophenyl group.

Substitution at the \(\gamma\)-Phosphoryl—The failure of dnsGTP either to activate G\(_{\alpha}\) or to block its activation by GTP is also somewhat disappointing, as the numerous studies (see references cited by Fields et al. (19) with AA-GTP (20)), including one demonstrating potent activation of G\(_{\alpha}\), and PDE (3), had suggested that GTP analogues substituted at the \(\gamma\)-phosphoryl or thio phosphoryl positions with fluorescent groups might be very useful for studying G\(_{\alpha}\). However, the results observed for this analogue are not too surprising given the great differences observed for different G\(_{\alpha}\) binding AA-GTP as compared to GTP-\(\gamma\)S; G\(_{\alpha}\) bound AA-GTP very weakly if at all, and G\(_{\alpha}\), G\(_{\alpha}\), and G\(_{\alpha}\) required much higher concentrations of Mg\(^{2+}\) for binding AA-GTP than for binding GTP-\(\gamma\)S (19). Even at high Mg\(^{2+}\) concentrations, dissociation of AA-GTP from G\(_{\alpha}\) was orders of magnitude faster than dissociation of GTP-\(\gamma\)S. It remains to be determined what substitutions to the \(\gamma\)-phosphoryl are compatible with high affinity binding and activation.

REFERENCES
1. Kelleher, D. J., Dudycz, L. W., Wright, G. E., and Johnson, G. L. (1986) Mol. Pharmacol. 30, 603–608
2. Yamanaka, G., Eckstein, F., and Stryer, L. (1985) Biochemistry 24, 8094–8101
3. Hirigoyen, V. N., Chang, L.-F. H., and Ho, Y.-K. (1989) Biochemistry 28, 7424–7432
4. Eckstein, F., Cassel, D., Levkovitz, H., Lowe, M. and Selinger, Z. (1979) J. Biol. Chem. 254, 9829–9834
5. Kahler, M., Koenig, B., and Hofmann, K. P. (1990) J. Biol. Chem. 265, 18928–18932
6. Sather, W. A., and Detwiler, P. B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 9290–9294
7. Lamb, T. D., and Matthews, H. R. (1988) J. Physiol. 407, 463–487
8. Paris, S. and Pouysségur, J. (1990) J. Biol. Chem. 265, 11567–11575
9. Malinski, J. A., Zera, E. M., Angleson, J. K., and Wensel, T. G. (1996) J. Biol. Chem. 271, 12919–12924
10. Rensland, H., Lautwein, A., Wittinghofer, A. and Goody, R. S. (1991) Biochemistry 30, 11181–11185
11. Hazlett, T. L., Moore, K. J. M., Lowe, P. N., Jameson, D. M., and Eccleston, J. F. (1993) Biochemistry 32, 13575–13583
12. Moore, K. J. M., Webb, M. R. and Eccleston, J. F. (1993) Biochemistry 32, 7451–7459
13. J ohn, J., Suhren, R., Feuerstein, J., Linke, R., Wittinghofer, A., and Goody, R. S. (1990) Biochemistry 29, 6058–6065
14. Molloy, D. P. (1990) Spectroscopic Studies on Elongation Factor Tu: An Approach to the Characterization of the Kinetic Events During the Elongation Cycle of Protein Biosynthesis. Ph.D. thesis, C.N.A., National Institute for Medical Research, Bethesda, MD
15. Kahlert, M., Konig, B., and Hofmann, K. P. (1990) J. Biol. Chem. 265, 11567–11575
16. Rensland, H., Lautwein, A., Wittinghofer, A. and Goody, R. S. (1993) J. Biol. Chem. 268, 13771–13778
17. Malinski, J. A., and Wensel, T. G. (1992) Biochemistry 31, 9502–9512
18. Malinski, J. A., and Wensel, T. G. (1992) Biochemistry 31, 939–949
19. Wieland, T., Ulillari, I., Gierschik, P., and Jakobs, K. H. (1991) Eur. J. Biochem. 199, 707–716
20. Wieland, T., Ronzani, M., and Jakobs, K. H. (1992) J. Biol. Chem. 267, 20791–20797
21. Panico, J., Parkes, J. H., and Liebman, P. A. (1990) J. Biol. Chem. 265, 18922–18927
22. Kutuzov, M. and Pfister, C. (1994) Eur. J. Biochem. 219, 367–371
23. Fields, T. A., Linder, M. E., and Casey, P. J. (1994) J. Biol. Chem. 269, 355–359
24. Cremo, C. R., Neuron, J. M., and Yount, R. G. (1990) J. Biol. Chem. 265, 7424–7432
25. Logsdon, N., Lee, C. G. L., and Harper, J. W. (1992) Anal. Biochem. 205, 36–41
26. M. E. and Feneley, R. E. (1971) Chemical Modification of Proteins, p. 217, Holden-Day, San Francisco
27. Papermaster, D. S., and Dreyer, W. J. (1974) Biochemistry 13, 2438–2444
28. Malinski, J. A., and Wensel, T. G. (1992) Biochemistry 31, 939–949
29. Angle, J. K., and Wensel, T. G. (1993) Neuron 29, 3309–3319
30. Logsdon, N., Lee, C. G. L., and Harper, J. W. (1992) Anal. Biochem. 205, 36–41
31. Means, G. E. and Feneley, R. E. (1971) Chemical Modification of Proteins, p. 217, Holden-Day, San Francisco
32. Papamitros, D. S., and Dreyer, W. J. (1974) Biochemistry 13, 2438–2444
33. Malinski, J. A., and Wensel, T. G. (1992) Biochemistry 31, 9502–9512
34. Angle, J. K., and Wensel, T. G. (1993) Neuron 29, 3309–3319
35. Wieland, T., Ulillari, I., Gierschik, P., and Jakobs, K. H. (1991) Eur. J. Biochem. 199, 707–716
36. Wieland, T., Ronzani, M., and Jakobs, K. H. (1992) J. Biol. Chem. 267, 20791–20797
37. Panico, J., Parkes, J. H., and Liebman, P. A. (1990) J. Biol. Chem. 265, 18922–18927
38. Kutuzov, M. and Pfister, C. (1994) Eur. J. Biochem. 219, 367–371
39. Noel, J. P., Hamm, H. E. and Sigler, P. B. (1993) Nature 366, 654–663
40. Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) Nature 369, 621–628
41. Fawzi, A. B., and Northup, H. J. K. (1990) Biochemistry 29, 3804–3812
42. IaCour, T. F. M., Nyborg, J., Thirup, S., and Clark, B. F. C. (1985) EMBO J. 4, 2385–2388
43. Kjeldgaard, M., and Nyborg, J. (1992) J. Mol. Biol. 223, 721–742
44. Watson, B. S., Hazlett, T. L., Eccleston, J. F., Davis, C., Jameson, D. M., and Johnson, A. E. (1995) Biochemistry 34, 7904–7912
45. Pai, E. F., Krenseng, U., Petsko, G. A., Goody, R. S., Kabsch, W., and Wittinghofer, A. (1990) EMBO J. 9, 2351–2359
46. Eccleston, J. F., Moore, K. J. M., Brownbridge, G. G., Webb, M. R., and Lown, P. N. (1991) Biochem. Soc. Trans. 19, 432–436
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