0026-895X/92/050731-05$03.00/0

MOLECULAR PHARMACOLOGY, 42:731-735

ACCELERATED COMMUNICATION

Evidence for Nucleoside Diphosphokinase-Dependent Channeling of Guanosine 5′-(γ-Thio)triphosphate to Guanine Nucleotide-Binding Proteins

THOMAS WIELAND and KARL H. JAKOBS

Institut für Pharmakologie der Universität GH Essen, D-4300 Essen, FRG

Received May 29, 1992; Accepted August 10, 1992

SUMMARY

Agonist binding to guanine nucleotide-binding protein (G protein)-coupled receptors in membranes of myeloid differentiated human leukemia (HL-60) cells is inhibited by guanine nucleotides, most potently by the GTP analog guanosine 5′-(γ-thio)triphosphate (GTPγS). In order to study whether GTPγS formed locally from adenosine 5′-(γ-thio)triphosphate (ATPγS) and GDP by nucleoside diphosphokinase has any advantage over exogenously added GTPγS in binding to and activating G proteins, regulation of complement component 5a (C5a) binding to its receptors, as well as formation of GTPγS, was studied in membranes of HL-60 cells. GTPγS added to HL-60 membranes potently inhibited binding of ^{125}I-C5a (IC_{50} about 3 nM), an effect not influenced by addition of either GDP or ATPγS. When HL-60 membranes were incubated with the combination of ATPγS and GDP, a marked potentiation (up to 300-fold) of the inhibition caused by either GDP or ATPγS alone was observed. By measuring nucleoside diphosphokinase-catalyzed formation of GTPγS and inhibition of ^{125}I-C5a binding in the presence of GDP and ATPγS under identical assay conditions, it was found that formed GTPγS inhibited binding of ^{125}I-C5a with an IC_{50} value of about 0.3 nM, thus being about 10-fold more potent than exogenously added GTPγS. These data suggest that the GTPγS-forming nucleoside diphosphokinase is closely associated with the C5a receptor-G protein complex and channels the formed GTPγS into the G protein.

Membranes of myeloid differentiated human leukemia (HL-60) cells contain large numbers of receptors for different chemotactic stimuli such as formyl peptides and C5a. These peptide agonists induce chemotaxis and respiratory burst apparently through the same signal transduction cascade involving pertussis toxin-sensitive G proteins, of which the G_{i2} and G_{i3} subtypes interacting with formyl peptide receptors have been identified (1–4). Binding of agonists to these G_i protein-coupled receptors is inhibited by guanine nucleotides, most potently by the GTP analog GTPγS, as demonstrated for formyl peptide receptors in macrophage and HL-60 cell membranes (5, 6) and for C5a receptors in membranes of polymorphonuclear leukocytes (7). Recent cloning data confirmed that these two receptor types belong to the superfamily of seven-transmembrane region G protein-coupled receptors (8–10).

We have recently reported that in HL-60 membranes GTPγS can be formed from ATPγS and GDP by nucleoside diphosphokinase and that the GTPγS thus formed inhibits the binding of the agonist fMet-Leu-Phe to formyl peptide receptors (11, 12). If this transfer reaction is of any physiological relevance for G protein regulation, either G protein-bound GDP has to be thiophosphorylated to GTPγS *in situ* by nucleoside diphosphokinase or, alternatively, GTPγS formed by nucleoside diphosphokinase has to be channeled into the G protein, i.e., has an advantage over GTPγS just being present in the solution. In order to study the latter hypothesis, we measured the formation of GTPγS from ATPγS and GDP by nucleoside diphosphokinase in membranes of HL-60 cells and, under identical conditions, we compared the regulation of C5a receptor binding by nucleoside diphosphokinase-formed and exogenously added GTPγS. We report herein that formed GTPγS potently and efficiently regulates the binding of C5a to its receptors present in HL-60 membranes. Most importantly, GTPγS locally formed by nucleoside diphosphokinase was about 10-fold more potent in inhibition of C5a receptor binding than exogenously added GTPγS; these data suggest a close association of nucleoside diphosphokinase and receptor-G protein complex.

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

ABBREVIATIONS: C5a, complement component 5a; fMet-Leu-Phe, *N*-formylmethionyl-leucyl-phenylalanine; G protein, guanine nucleotide-binding protein; ATPγS, adenosine 5′-(γ-thio)triphosphate; GTPγS, guanosine 5′-(γ-thio)triphosphate.

Experimental Procedures

Materials. Recombinant human ^{125}I-C5a (2200 Ci/mmol) and [^{35}S]GTPγS (1200–1400 Ci/mmol) were obtained from DuPont New England Nuclear (Bad Homburg, FRG). [8-^{3}H]GDP (10 Ci/mmol) was from Amersham Buchler (Braunschweig, FRG). Unlabeled recombinant human C5a was from Sigma (Deisenhofen, FRG). Unlabeled guanine nucleotides were from Boehringer Mannheim (Mannheim, FRG). All other materials were from previously described sources (6, 11, 12).

Preparation of HL-60 membranes. HL-60 cells were grown in suspension culture and were induced to differentiate into mature myeloid forms by cultivation in the presence of 1.25% (by volume) dimethylsulfoxide for 5 days. Membranes were prepared by nitrogen cavitation and differential centrifugation, as described (6). Before assays, membrane aliquots were thawed, diluted with 10 mM triethanolamine-HCl, pH 7.4, containing 5 mM EDTA, centrifuged for 10 min at 30,000 × *g*, and resuspended in 10 mM triethanolamine-HCl, pH 7.4, at the appropriate membrane concentration.

C5a receptor binding assay. HL-60 membranes (1–3 μg of protein/tube) were incubated in a reaction mixture containing 50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, and the indicated nucleotide additions, in a total volume of 90 μl, for 60 min at 30°. Thereafter, 10 μl of ^{125}I-C5a (final concentration about 20 pM; dissolved in a buffer containing 10 mM triethanolamine-HCl, pH 7.4, and 25 mg/ml bovine serum albumin) were added to initiate the binding reaction. The incubation was continued for 30 min at 30°, under which conditions binding equilibrium was reached. The reaction was terminated by rapid filtration through glass fiber filters (Whatman GF/C) soaked in a buffer containing 0.33% (by volume) polyethyleneimine, 50 mM Tris·HCl, pH 7.5, and 5 mM $MgCl_2$. The filters were washed five times with 2.5 ml each of an ice-cold buffer (50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$) and were counted in a γ-radiation counter. Nonspecific binding was defined as the binding not competed for by 100 nM unlabeled C5a.

Measurement of GTPγS formation. Formation of [^{3}H]GTPγS was measured in a total volume of 50 μl of a reaction mixture containing 50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 0.1 μM [^{3}H]GDP, and the indicated concentrations of ATPγS. The reaction was started by addition of HL-60 membranes (about 3 μg of protein/tube) to the prewarmed reaction mixture and was conducted for 90 min at 30°. The enzymatic reactions were terminated by addition of 5 μl of a 33% (by mass) perchloric acid solution, leading to denaturation of proteins and release of protein-bound nucleotides. After 15 min at room temperature, the samples were neutralized with a solution (about 5 μl) containing 4.6 M KOH, 100 mM Tris·HCl, pH 7.5, and 100 mM EDTA and were centrifuged for 3 min at 8800 × *g*. Twenty microliters of the resulting supernatant were analyzed for [^{3}H][GTPγS formation by thin layer chromatography, as described (11).

Results

The binding of C5a to its receptors in HL-60 membranes was very sensitive to regulation by GTPγS. When HL-60 membranes were incubated with GTPγS at increasing concentrations, followed by measurement of binding of ^{125}I-C5a to its receptors, a concentration-dependent inhibition of agonist binding was observed (Figs. 1 and 2). Half-maximal and maximal inhibition was obtained at about 3 nM and 30–100 nM GTPγS, respectively. These numbers are about 3-fold lower than those reported for inhibition of fMet-Leu-Phe binding to HL-60 membranes (6) and of C5a binding to membranes of polymorphonuclear leukocytes by GTPγS (7).

We have recently reported that in HL-60 membranes nucleoside diphosphokinase can contribute to the regulation of fMet-Leu-Phe receptor binding by formation of GTPγS from ATPγS and GDP (12). Similarly, a marked potentiation of the inhibition of ^{125}I-C5a binding was observed when HL-60 membranes were incubated with combinations of ATPγS and GDP, compared with the inhibition caused by one of these nucleotides alone. Incubation of the membranes with GDP alone inhibited the binding of ^{125}I-C5a half-maximally at about 0.5 μM, similarly as described for inhibition of fMet-Leu-Phe binding (6, 12) (Fig. 3). In the additional presence of 0.1 μM ATPγS, which by itself caused no or only marginal inhibition, the potency of GDP to inhibit the binding of ^{125}I-C5a was enhanced about 10-fold. Half-maximal inhibition was observed at about 50 nM GDP. Conversely, the potency of ATPγS to inhibit binding of ^{125}I-C5a was increased by about 300-fold in the presence of 0.1 μM GDP (Fig. 2). In the absence and presence of GDP, half-

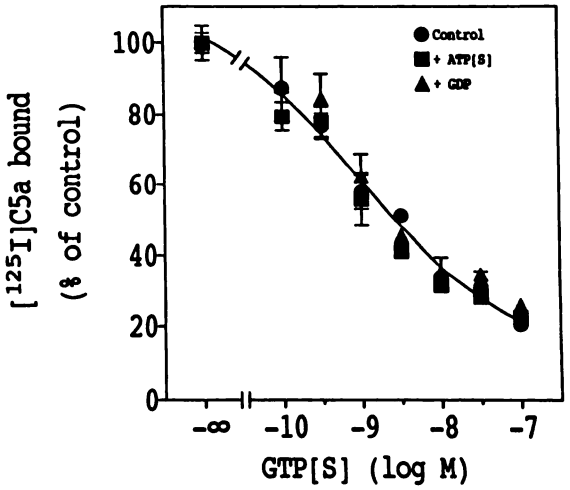

Fig. 1. GTPγS regulation of C5a binding in HL-60 membranes. HL-60 membranes (1.2 μg of protein/tube) were incubated for 1 hr at 30° with the indicated concentrations of GTPγS in the absence (●) or presence of either 10 μM ATPγS (■) or 0.1 μm GDP (▲). Then, ^{125}I-C5a (final concentration, 29 pM) was added and the incubation was continued for 30 min at 30°. The amount of specifically bound ^{125}I-C5a was determined as described in Experimental Procedures. Means ± standard deviations of assay triplicates are given.

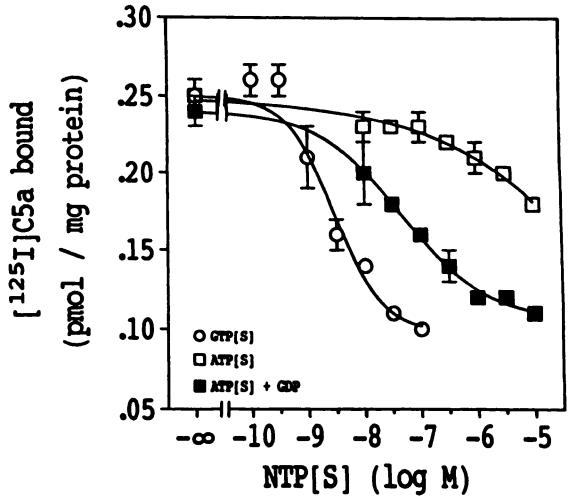

Fig. 2. Potentiation of ATPγS-induced inhibition of C5a binding by GDP and comparison with GTPγS-induced inhibition. HL-60 membranes (3.3 μg of protein/tube) were incubated for 1 hr at 30° with the indicated concentrations of GTPγS (○), ATPγS (□), or ATPγS plus 0.1 μM GDP (■). Then, ^{125}I-C5a (final concentration, 24 pM) was added and the incubation was continued for 30 min at 30°. Means ± standard deviations of specifically bound ^{125}I-C5a from assay triplicates are given.

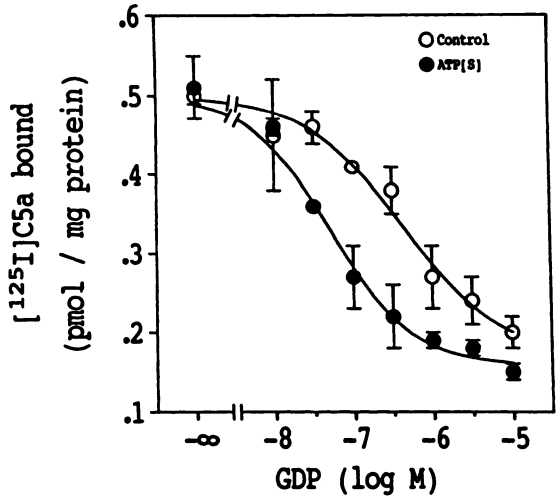

Fig. 3. Potentiation of GDP-induced inhibition of C5a binding by ATPγS. HL-60 membranes (2.4 μg of protein/tube) were incubated for 1 hr at 30° with the indicated concentrations of GDP in the absence (○) or presence (●) of 0.1 μM ATPγS. Then, ^{125}I-C5a (final concentration, 25 pM) was added and the incubation was continued for 30 min at 30°. Means ± standard deviations of specifically bound ^{125}I-C5a from assay triplicates are given.

maximal inhibition of ^{125}I-C5a binding was observed at about 30 μM and 0.1 μM ATPγS, respectively. These data are similar to those described before for inhibition of fMet-Leu-Phe receptor binding, with the exception that about 10-fold less ATPγS is required for potentiation of GDP-induced inhibition of C5a than of fMet-Leu-Phe receptor binding (12). On the other hand, the potencies of GDP and ATPγS added alone were similar with regard to regulation of C5a and fMet-Leu-Phe receptor binding (6, 12).

In order to compare the potency of GTPγS locally formed by nucleoside diphosphokinase with that of exogenously added GTPγS, we measured formation of GTPγS and inhibition of ^{125}I-C5a binding in parallel experiments using identical assay conditions. First, the efficiency of the elution procedure for membrane-bound nucleotides, as described in Experimental Procedures, and the stability of GTPγS during this treatment were analyzed. For this, [^{35}S]GTPγS (2.5 nM) was incubated for 90 min at 30° in a total volume of 50 μl of the reaction mixture used for GTPγS formation, with or without HL 60 membranes (5 μg of protein/tube). Thereafter, the samples were treated with or without perchloric acid and were analyzed by thin layer chromatography for [^{35}S]GTPγS. As shown in Table 1, treatment of [^{35}S]GTPγS with perchloric acid did not lower the amount of radioactivity detected in the GTPγS spot. Most importantly, the recovery of [^{35}S]GTPγS was not lowered by preincubation of the nucleotide with HL-60 membranes. Although 40–50% of the added [^{35}S]GTPγS was bound to membranes under this condition (data not shown), it was apparently completely released as intact nucleotide by the subsequent elution procedure. These data indicated that GTPγS is not hydrolyzed by the acid treatment and, most importantly, that all GTPγS formed, even when bound to membrane G proteins, can be detected by the method used.

Formation of [^{3}H]GTPγS by HL-60 membrane nucleoside diphosphokinase was measured at increasing concentrations of ATPγS with 0.1 μM [^{3}H]GDP as nucleoside diphosphate substrate (Fig. 4). [^{3}H]GTPγS formation was concentration-dependently increased by ATPγS. At the maximal concentration

TABLE 1

Recovery of GTPγS from HL-60 membranes

[^{35}S]GTPγS (2.5 nM) was incubated for 90 min at 30° in a reaction mixture containing 50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, and 1 mM EDTA in the absence and presence of 5 μg of HL-60 membranes, in a total volume of 50 μl. Then, to the samples containing no membranes either 10 μl of a buffer containing 50 mM Tris·HCl, pH 7.5, and 50 mM EDTA or 5 μl of perchloric acid (33% by mass) were added. Incubation of samples containing membranes was terminated by addition of 5 μl of perchloric acid. After 15 min at room temperature, samples treated with perchloric acid were neutralized with 5 μl of 4.6 M KOH, 100 mM Tris·HCl, pH 7.5, and 100 mM EDTA. After pelleting of the membranes, the supernatants were analyzed for [^{35}S]GTPγS by thin layer chromatography, as described in Experimental Procedures. Means ± standard deviations of triplicates are given.

Treatment	[^{35}S]GTPγS recovered
	cpm
No membranes, no perchloric acid	13,998 ± 2,738
No membranes, perchloric acid	14,555 ± 1,060
Membranes, perchloric acid	13,638 ± 317

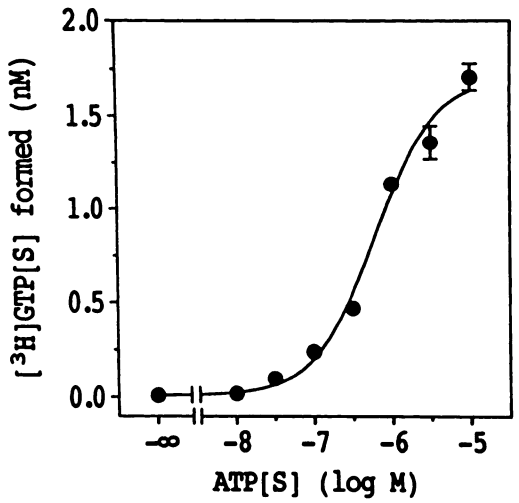

Fig. 4. Formation of GTPγS from ATPγS and GDP. HL-60 membranes (3.3 μg of protein/tube) were incubated with 0.1 μM [^{3}H]GDP and the indicated concentrations of ATPγS for 90 min at 30°. After acid denaturation and pelleting of the proteins, the supernatant was analyzed for [^{3}H]GTPγS by thin layer chromatography, as described in Experimental Procedures. Means ± standard deviations of assay triplicates are given.

of ATPγS studied (10 μM), a final concentration of about 2 nM [^{3}H]GTPγS formed was measured, corresponding to about 30 pmol of [^{3}H]GTPγS formed per mg of protein. In the parallel experiment, the inhibition of ^{125}I-C5a binding by increasing concentrations of exogenously added GTPγS was compared with the inhibition caused by increasing concentrations of ATPγS in the absence and presence of 0.1 μM GDP (Fig. 2). As mentioned above, exogenously added GTPγS was about 30- and 10,000-fold more potent than ATPγS in the presence and absence of GDP, respectively, in inhibiting ^{125}I-C5a receptor binding. However, when the binding data obtained from the combination of ATPγS and GDP were plotted against the GTPγS concentration measured in the GTPγS formation experiment and compared with the binding data obtained with exogenously added GTPγS, it was evident that GTPγS locally formed from ATPγS and GDP was about 10-fold more potent in the regulation of ^{125}I-C5a binding than was the exogenously added nucleotide (Fig. 5). The half-maximally inhibitory concentration was estimated to be about 0.3 nM and 3 nM for formed and added GTPγS, respectively. This higher potency of formed GTPγS was not due to the additional presence of GDP and ATPγS. First, at the concentrations studied, GDP and ATPγS had only minor effects by themselves and, second,

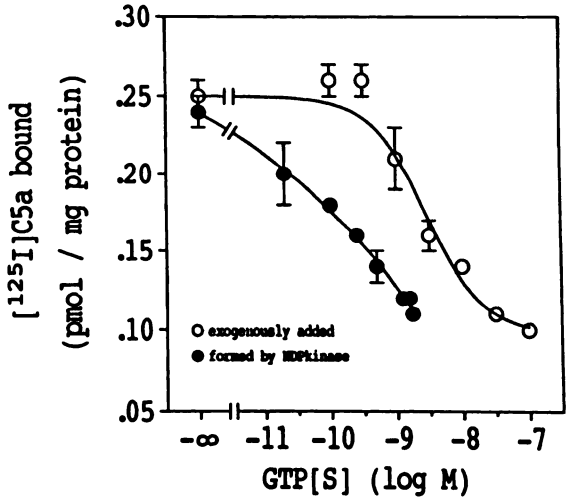

Fig. 5. Comparison of the potencies of exogenously added and nucleoside diphosphokinase-formed GTPγS to inhibit C5a receptor binding. Plotted are the ^{125}I-C5a binding data obtained from the combination of ATPγS (various concentrations) and GDP (0.1 μM) against the GTPγS concentration measured in the parallel GTPγS formation experiment (●) and compared with the binding data obtained with exogenously added GTPγS (○). Data are from Figs. 2 and 4.

TABLE 2

Binding to HL-60 membranes of GTPγS formed from ATPγS and GDP

HL-60 membranes (3.2 μg of protein/tube) were incubated with 0.1 μM [^{3}H]GDP and 10 μM ATPγS for 90 min at 30°. Thereafter, one aliquot of membranes was pelleted by centrifugation for 10 min at 30,000 × *g* and the supernatant was removed. The pellet was resuspended in 50 μl of triethanolamine-HCl, pH 7.4. Supernatant, resuspended membranes, and the original membrane suspension (total) were subjected to acid denaturation and analyzed for [^{3}H]GTPγS by thin layer chromatography, as described in Experimental Procedures. Means ± standard deviations of triplicates, in pmol of [^{3}H]GTPγS formed per mg of membrane protein, are given.

	[^{3}H]GTPγS formed	
	pmol/mg of protein	*% of total*
Total	28.8 ± 3.4	100.0
Pellet	22.2 ± 3.6	77.1
Supernatant	3.4 ± 0.6	11.8

addition of GDP and ATPγS at the highest concentrations present in the combination experiment (0.1 μM and 10 μM, respectively) to exogenous GTPγS did not alter the potency of the GTP analog to inhibit ^{125}I-C5a receptor binding (see Fig. 1). Furthermore, as shown in Table 2, about 80% of the GTPγS formed from GDP (0.1 μM) and ATPγS (10 μM) was bound to the membranes, presumably to membrane G proteins. Taken together, these data indicate that the observed inhibition is due to formed and subsequently bound GTPγS.

Discussion

The data presented herein indicate that HL-60 membranes contain a nucleoside diphosphokinase capable of catalyzing the formation of GTPγS from GDP and ATPγS and that the GTPγS thus formed can potently and efficiently inhibit agonist binding to C5a receptors, similarly as reported before for regulation of formyl peptide receptor binding by nucleoside diphosphokinase-formed GTPγS (12). Most importantly, by measuring formation of GTPγS under identical assay conditions and with a wide range of ATPγS concentrations, we provide evidence that the nucleoside diphosphokinase-formed GTPγS is about 10 times more potent in inhibiting C5a receptor binding than is exogenously added GTPγS. The observed higher potency of formed GTPγS suggests that there is a close association of nucleoside diphosphokinase and the C5a receptor-G protein complex in HL-60 membranes. Throughout the literature, several lines of evidence are available that imply an association of nucleoside diphosphokinase with various G proteins. Kimura and Shimada (13) reported that the stimulatory G protein of adenylyl cyclase can be precipitated in a form complexed with nucleoside diphosphokinase. In HeLa S3 cells and Ehrlich ascites tumor cells, nucleoside diphosphokinase was found to be associated with low molecular mass G proteins (14, 15). Furthermore, a preferential phosphorylation of GDP over ADP was reported when nucleoside diphosphokinase in its intermediately phosphorylated form was combined with small or heterotrimeric G proteins (16). Finally, we provided evidence that activation of G proteins in human platelet membranes by nucleoside diphosphokinase-dependent formation of GTP or GTPγS can be regulated by agonist-liganded receptors (17, 18). All of these data support the notion that nucleoside diphosphokinase can be closely associated with G proteins and, as shown herein, thereby channel formed GTP or GTPγS into G proteins.

An hypothesis that may explain the observed high potency of nucleoside diphosphokinase-formed GTPγS is that the GDP bound in the α subunit of the inactive G protein is a substrate for nucleoside diphosphokinase. Such a reaction has been proposed for heterotrimeric G proteins; however, the authors retracted this conclusion because a dissociation of GDP from the α subunit, followed by its phosphorylation to GTP by nucleoside diphosphokinase and rapid rebinding of formed GTP to G proteins, could not be excluded (19, 20). Similarly, Randazzo *et al.* (21) recently reported that GDP bound to the low molecular mass G protein ADP-ribosylation factor is converted *in situ* into GTP by nucleoside diphosphokinase. Based on recently performed experiments, however, the same authors conclude that this interpretation is based on artifacts, particularly on underestimation of GDP release from G proteins.[1] The requirement of GDP addition for potentiation of the effect of ATPγS on C5a receptor binding appears to argue also against this hypothesis. However, GDP is only loosely bound to G_i proteins in HL-60 membranes and is rapidly released and degraded when membranes are incubated in the presence of Mg^{2+} at 30° (22). Thus, although formation of GTPγS from G protein-bound GDP by nucleoside diphosphokinase seems to be very unlikely, it cannot be excluded by our data.

The amount of formed GTPγS causing maximal inhibition of C5a receptor binding was about 30 pmol/mg of protein. This number is very similar to the amount of GTPγS bound to HL-60 membrane G proteins at a maximally effective concentration of exogenously added GTPγS (100 nM) (23). These data suggested that the vast majority of nucleoside diphosphokinase-formed GTPγS is bound to G proteins. This assumption was confirmed by direct measurement, showing that about 80% of the formed GTPγS was found to be membrane bound. We, therefore, propose that GTPγS is formed by nucleoside diphosphokinase in HL-60 membranes in close association with the G proteins interacting with C5a receptors. More work has to be done in order to elaborate the mechanisms by which

[1] P. A. Randazzo J. K. Northup, and R. A. Kahn, personal communication.

nucleoside diphosphokinase can apparently channel the formed guanine nucleoside triphosphate into G proteins.

Acknowledgments

We are indebted to Erwin Hagelskamp for expert technical assistance.

References

1. Morel, F., J. Doussiere, and P. V. Vignais. The superoxide-generating oxidase of phagocytic cells: physiological, molecular and pathological aspects. *Eur. J. Biochem.* **201**:523–546 (1991).
2. Gierschik, P., and K. H. Jakobs. Receptor-mediated ADP-ribosylation of a phospholipase C-stimulating G protein. *FEBS Lett.* **224**:219–223 (1987).
3. Gierschik, P., D. Sidiropoulos, and K. H. Jakobs. Two distinct G_i-proteins mediate formyl peptide receptor signal transduction in human leukemia (HL-60) cells. *J. Biol. Chem.* **264**:21470–21473 (1989).
4. Kikuchi, A., O. Kozawa, K. Kaibuchi, T. Katada, M. Ui, and Y. Takai. Direct evidence for involvement of a guanine nucleotide-binding protein in chemotactic peptide-stimulated formation of inositol bisphosphate and trisphosphate in differentiated human leukemic (HL-60) cells: reconstitution with G_i or G_o of the plasma membranes ADP-ribosylated by pertussis toxin. *J. Biol. Chem.* **261**:11558–11562 (1986).
5. Snyderman, R., M. C. Pike, S. Edge, and B. Lane. A chemoattractant receptor on macrophages exists in two affinity states regulated by guanine nucleotides. *J. Cell Biol.* **98**:444–448 (1984).
6. Gierschik, P., M. Steisslinger, D. Sidiropoulos, E. Herrmann, and K. H. Jakobs. Dual Mg^{2+} control of formyl-peptide-receptor-G-protein interaction in HL-60 cells: evidence that the low-agonist-affinity receptor interacts with and activates the G-protein. *Eur. J. Biochem.* **183**:97–105 (1989).
7. Siciliano, S. J., T. E. Rollins, and M. S. Springer. Interaction between the C5a receptor and G_i in both the membrane-bound and detergent-solubilized states. *J. Biol. Chem.* **265**:19568–19574 (1990).
8. Boulay, F., M. Tardif, L. Brouchon, and P. Vignais. Synthesis and use of a novel *N*-formyl peptide derivative to isolate a human *N*-formyl peptide receptor cDNA. *Biochem. Biophys. Res. Commun.* **168**:1103–1109 (1990).
9. Boulay, F., M. Tardif, L. Brouchon, and P. Vignais. The human *N*-formyl peptide receptor: characterization of two cDNA isolates and evidence for a new subfamily of G-protein-coupled receptors. *Biochemistry* **29**:11123–11133 (1990).
10. Gerard, N. P., and C. Gerard. The chemotactic receptor for human C5a anaphylatoxin. *Nature (Lond.)* **349**:614–617 (1991).
11. Seifert, R., W. Rosenthal, G. Schultz, T. Wieland, P. Gierschik, and K. H. Jacobs. The role of nucleoside-diphosphate kinase reactions in G protein activation of NADPH oxidase by guanine and adenine nucleotides. *Eur. J. Biochem.* **175**:51–55 (1988).
12. Wieland, T., J. Bremerich, and K. H. Jakobs. Contribution of nucleoside diphosphokinase to guanine nucleotide regulation of agonist binding to formyl peptide receptors. *Eur. J. Pharmacol.* **208**:17–23 (1991).
13. Kimura, N., and N. Shimada. Evidence for complex formation between GTP binding protein (G_s) and membrane-associated nucleoside diphosphate kinase. *Biochem. Biophys. Res. Commun.* **168**:99–106 (1990).
14. Ohtsuki, K., T. Ikeuchi, and M. Yokoyama. Characterization of nucleoside-diphosphate kinase-associated guanine nucleotide-binding proteins from HeLa S3 cells. *Biochim. Biophys. Acta* **882**:322–330 (1986).
15. Uesaka, H., M. Yokoyama, and K. Ohtsuki. Physiological correlation between nucleoside-diphosphate kinase and the enzyme-associated guanine nucleotide binding proteins. *Biochem. Biophys. Res. Commun.* **143**:552–559 (1987).
16. Ohtsuki, K., and M. Yokoyama. Direct activation of guanine nucleotide binding proteins through a high-energy phosphate-transfer by nucleoside diphosphate-kinase. *Biochem. Biophys. Res. Commun.* **148**:300–307 (1987).
17. Wieland, T., and K. H. Jakobs. Receptor-regulated formation of GTP[γS] with subsequent persistent G_s-protein activation in membranes of human platelets. *FEBS Lett.* **245**:189–193 (1989).
18. Jakobs, K. H., and T. Wieland. Evidence for receptor-regulated phosphotransfer reactions involved in activation of the adenylate cyclase inhibitory G protein in human platelet membranes. *Eur. J. Biochem.* **183**:115–121 (1989).
19. Kikkawa, S., K. Takahashi, K.-I. Takahashi, N. Shimada, M. Ui, N. Kimura, and T. Katada. Conversion of GDP into GTP by nucleoside diphosphate kinase on the GTP-binding proteins. *J. Biol. Chem.* **265**:21536–21540 (1990).
20. Kikkawa, S., K. Takahashi, K.-I. Takahashi, N. Shimada, M. Ui, N. Kimura, and T. Katada. Conversion of GDP into GTP by nucleoside diphosphate kinase on the GTP-binding proteins: additions and corrections. *J. Biol. Chem.* **266**:12795 (1991).
21. Randazzo, P. A., J. K. Northup, and R. A. Kahn. Activation of a small GTP-binding protein by nucleoside diphosphate kinase. *Science (Washington D. C.)* **254**:850–853 (1991).
22. Wieland, T., J. Kreiss, P. Gierschik, and K. H. Jakobs. Role of GDP in formyl-peptide-receptor-induced activation of guanine-nucleotide-binding proteins in membranes of HL 60 cells. *Eur. J. Biochem.* **205**:1201–1206 (1992).
23. Gierschik, P., R. Moghtader, C. Straub, K. Dieterich, and K. H. Jakobs. Signal amplification in HL-60 granulocytes: evidence that the chemotactic peptide receptor catalytically activates guanine nucleotide-binding regulatory proteins in native plasma membranes. *Eur. J. Biochem.* **197**:725–732 (1991).

Send reprint requests to: Dr. Thomas Wieland, Institut für Pharmakologie der Universität GH Essen, Hufelandstrasse 55, D-4300 Essen 1, FRG.