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Reconstitution of a Physical Complex between the *N*-Formyl Chemotactic Peptide Receptor and G Protein

INHIBITION BY PERTUSSIS TOXIN-CATALYZED ADP RIBOSYLATION*

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Photoaffinity-labeled *N*-formyl chemotactic peptide receptors from human neutrophils solubilized in octyl glucoside exhibit two forms upon sucrose density gradient sedimentation, with apparent sedimentation coefficients of approximately 4 and 7 S. The 7 S form can be converted to the 4 S form by guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) with an EC_{50} of ~20 nM, suggesting that the 7 S form may represent a physical complex of the receptor with endogenous G protein (Jesaitis, A. J., Tolley, J. O., Bokoch, G. M., and Allen, R. A. (1989) *J. Cell Biol.* 109, 2783–2790). To probe the nature of the 7 S form, we reconstituted the 7 S form from the 4 S form by adding purified G protein. The 4 S form, obtained by solubilizing GTPγS-treated neutrophil plasma membranes, was incubated with purified (>95%) G_i protein from bovine brain (containing both $G_{i\alpha1}$ and $G_{i\alpha2}$) or with neutrophil G protein (G_n), and formation of the 7 S complex was analyzed on sucrose density gradients. The EC_{50} of 7 S complex formation induced by the two G proteins was 70 ± 25 and 170 ± 40 nM for G_n and G_i, respectively. No complexation was measurable when bovine transducin (G_t) was used up to 30 times the EC_{50} for G_n. The EC_{50} for G_i was the same for receptors, obtained from formyl peptide-stimulated or unstimulated cells. The addition of 10 μM GTPγS to the reconstituted 7 S complex caused a complete reversion of the receptor to the 4 S form, and anti-G_i peptide antisera immunosedimented the 7 S form. ADP-ribosylation of G_i prevented formation of the 7 S form even at 20 times the concentration of unribosylated G_i normally used to attain 50% conversion to the 7 S form. These observations suggest that the 7 S species is a physical complex containing *N*-formyl chemotactic peptide receptor and G protein.

The binding of *N*-formyl peptides to specific cell surface receptors (1–3) on human leukocytes results in the activation of a variety of cell functions, including chemotaxis, lysosomal enzyme secretion, and superoxide production (4). There is a substantial body of evidence suggesting that the receptor for these peptides mediates transduction through interaction with a guanyl nucleotide-binding protein or G protein (5–7). The *N*-formyl chemotactic peptide receptors in membranes (8) or permeabilized neutrophils (9) exist in a high affinity state for agonist that can be specifically converted to a low affinity state by guanyl nucleotides. It has also been shown that GTPγS[1] inhibits high affinity binding of fMet-Leu-[^{3}H]Phe to fMet-Leu-Phe receptors with an EC_{50} value of about 20 nM (10). We have recently (11) presented data that indicate that the octyl glucoside-solubilized *N*-formyl chemotactic peptide receptor from unstimulated neutrophils exhibits two forms with apparent sedimentation coefficients of approximately 4 and 7 S. The 7 S form could be converted to the 4 S form by GTPγS with an EC_{50} value of about 20 nM, suggesting that the 7 S form represented a receptor-G protein complex. The inhibition of high affinity agonist binding to G protein coupled-receptors by guanyl nucleotides is generally accepted to be indicative of uncoupling of receptor and G protein (5). The similar GTPγS concentration dependence of the 7 to 4 S conversion (11) and the inhibition of high affinity agonist binding by GTPγS (10) further substantiates that the 7 S form of the fMet-Leu-Phe receptor represented the G protein-coupled form.

Pertussis toxin-catalyzed ADP-ribosylation of a 40-kDa membrane substrate in neutrophils also disrupts the functional coupling of the *N*-formyl chemotactic peptide receptor to neutrophil activation (12). The cholera toxin-induced ADP-ribosylation of this substrate in membranes can be modulated by *N*-formyl peptide binding (13, 14), suggesting an interaction with the ligand-occupied receptor. Finally, chemotactic *N*-formyl peptides stimulate guanyl nucleotide binding and pertussis toxin-sensitive GTPase activity in the neutrophil membranes (15, 16). The molecular mechanisms of the uncoupling however, remain unclear.

Pertussis toxin substrates of 40 and 41 kDa in neutrophils and HL60 cells have been purified and appear to be identical with G_{i2} and G_{i3} (17–19). G_{i2} (also termed G_n) has been shown to be the major substrate in mature human neutrophils (20) and HL60 cells (18, 21). Both G_{i2} and G_{i3} have been demonstrated to be functionally coupled to *N*-formyl chemotactic peptide receptors in HL60 cells (21). Several studies have demonstrated that high affinity formyl peptide binding, formyl peptide-stimulated GTPase activity, and formyl peptide-mediated activation of phospholipase C (22, 23) can be re-

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[1] The abbreviations used are: GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, SASD, sulfosuccinimidyl-2-(*p*-azidosalicylamido)ethyl-1,3′-dithiopropionate; FMLPL-^{125}I-SASD, *N*-formyl-Met-Leu-Phe-Lys-N^{ϵ}-(2-(*p*-azido-^{125}I-salicylamido)ethyl-1,3′-dithiopropionate.

stored by rat or brain G_i/G_o proteins. These various criteria clearly indicated that restoration of function of receptor-G protein coupling can be demonstrated. To date, however, there has been no demonstration of the formation of a physical complex of the *N*-formyl chemotactic peptide receptor with GTP-binding protein(s).

Active functional receptor G protein complexes have clearly been demonstrated in rhodopsin, β-adrenergic, muscarinic, dopamine, and adenosine receptor systems among the many now investigated (24). In nearly all cases, evidence for such association has been the GTP sensitivity of agonist binding (or phototransformation in the case of rhodopsin) or agonist/light-induced stimulation of the GTPase activity. In a number of detergent-based systems (25–28) used for the study of such interactions, including the formyl peptide receptor system (11, 29), receptors or G proteins have been shown to be released from apparent complexation upon exposure to GTP or its nonhydrolyzable derivatives. Reconstitution of the functional activities described above have also been demonstrated in both membrane- and detergent-based systems. To our knowledge no studies have reported direct measurement of such complexes by hydrodynamic analysis of solubilized radiolabeled receptors.

In this report, we provide evidence for the direct formation of a 7 S complex between 4 S receptors and either pure bovine brain G_i or purified endogenous neutrophil G_n. We additionally show that stoichiometric ADP-ribosylation of G_i by pertussis toxin prevents the reconstitution of 7 S complexes with G protein. Moreover, the structurally related G protein of the visual system, G_t, does not reconstitute a 7 S complex, indicating specificity of the receptor for G_n and G_i. These observations suggest that the 7 S complexes are the result of physical association of receptor and G protein, whereas the 4 S form is uncoupled from G proteins.

MATERIALS AND METHODS

Buffers, chemicals, and methods of cell preparation were as previously described (11, 30). Chemicals used for G protein isolation were as described by Bokoch *et al.* (20, 31). Anti-G protein antisera were prepared as described in Ref. 20.

Identification of 4 or 7 S forms of N-formyl Chemotactic Peptide Receptor—Plasma membrane fractions were prepared from degranulated neutrophils as described previously by Parkos *et al.* (30). The *N*-formyl chemotactic peptide receptors were specifically labeled with FMLPL-^{125}I-SASD by the procedures of Allen *et al.* (32, 33). In order to prevent isolation of endogenous 7 S receptor forms during the membrane solubilization and reconstitution step, the labeled membranes were treated with 10 μM GTPγS and were subsequently washed with "relax buffer" (10 mM Hepes, pH 7.4, 100 mM KCl, 10 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml chymostatin, containing 1.0 M NaCl). Labeled membranes were pelleted and resuspended at 0.5–0.8 mg of protein/ml in the relax buffer containing 1.0% 1-octyl-β-D-glucopyranoside (octyl glucoside) and then incubated on ice for 2 h. Insoluble material was removed by sedimentation at 45,000 rpm in a Beckman 60Ti rotor for 30 min at 4 °C. Supernatant extracts (100 μl) were layered onto 5-ml 5–20% linear sucrose density gradients prepared in the extraction buffer. Gradients were centrifuged in an SW 55 Beckman swinging bucket rotor for 16 h at 45,000 rpm and fractionated into 13 × 400-μl fractions. Alternatively, to conserve material and reduce sedimentation time, experiments were also carried out using 25-μl samples on 0.7-ml gradients sedimenting for 8 h in the same rotors. In some cases, fractions were diluted 1:10 in extraction buffer, concentrated to the original volume, and resedimented by the first procedure. Sedimentation was calibrated with known protein standards by centrifuging a mixture containing 25 μg each of cytochrome *c* (2.1 S), bovine serum albumin (4.4 S), porcine immunoglobulin (7.7 S), and bovine catalase (11.2 S) in parallel with the experimental gradients. Photoaffinity-labeled receptor content was measured by autoradiography of receptors separated on SDS-PAGE or by the ^{125}I content of fractions, as described previously (11).

Reconstitution of Receptor with G Protein and Analysis—Octyl glucoside extracts were divided into individual incubation mixtures with the indicated levels of G protein under the conditions described in the text. Incubations were carried out for 2 h on ice. Samples were then layered onto 5-ml 5–20% linear sucrose gradients, and receptor sedimentation was analyzed as described above. The G protein concentration dependence of the reconstitution of the 7 S form of the receptor was fitted with the Hill equation using a nonlinear nonweighted curve-fitting computer program (34). Endogeneous G protein did not couple with the receptor, as evidenced by the identical sedimentation pattern at zero G protein in the presence of 10 μM GTPγS.

Preparation of G Proteins—G_i was purified from bovine brain as described (35) and resolved from G_o by chromatography on a 20-ml DEAE-Sephacel column equilibrated with 25 mM Tris-HCl, pH 8, 1 mM EDTA, 1 mM NaCl, 0.6% Lubrol, and eluted with a linear gradient (200 ml) of 0–250 mM NaCl in the same buffer. Purity was judged to be 95% or higher, as assessed by SDS-PAGE and silver staining. Concentrations given for the G proteins are based on the protein concentration determined after the final elution step. The G proteins used were functionally intact, as determined by [^{35}S]GTPγS binding (20). Neutrophil G_n (or G_{i2}) was isolated as described by Bokoch *et al.* (20, 31) up until the heptylamine-Sepharose chromatography, with the exception that purifications were performed in the absence of $AlCl_3$, $MgCl_2$, and NaF. The G_n was further purified to apparent homogeneity by DEAE-Sephacel chromatography as described above, concentrated by hydroxyapatite chromatography, and switched into Lubrol-containing buffers by G-25 gel filtration, as described in Ref. 36. Functional bovine transducin, able to stabilize the Meta-II form of rhodopsin, was prepared by the method of Stryer *et al.* (37) and was the kind gift of Dr. H. Hamm (Department of Physiology and Biophysics, University of Illinois at Chicago College of Medicine).

ADP-ribosylated G_i was prepared by incubation of 350 μg of G_i with 20 μg/ml pertussis toxin in 100 mM Tris-HCl, pH 8, 1 mM EDTA, 2.5 mM $MgCl_2$, 10 mM thymidine, 1 mM ATP, 0.1 mM GTP, 0.5 mM dimyristoyl phosphatidylcholine, 10 mM dithiothreitol, and 2 mM NAD for 30 min at 30 °C. The level of ADP-ribosylation of the G_i was assessed with a parallel incubation in the presence of [^{32}P]NAD (specific activity, 300 cpm/pmol) that was analyzed by trichloroacetic acid precipitation/filtration, as in Ref. 36, as well as by subsequent [^{32}P]NAD labeling of the G_i preribosylated with unlabeled NAD (see Fig. 4, *inset*). Determination of [^{35}S]GTPγS binding to G_i and Amido Black protein analyses were as described in Bokoch *et al.* (36).

RESULTS

In detergent (Triton X-100 and digitonin), the covalently liganded *N*-formyl chemotactic peptide receptor from human neutrophils behaves as a monodisperse species of ~63 kDa upon sedimentation equilibrium analysis (38). The receptor from differentiated HL60 cells eluted in deoxycholate as a ~66-kDa species upon gel filtration (29). These estimates are consistent with the apparent size of the affinity-labeled receptor upon reduced SDS-PAGE, where the liganded receptor migrates as a broad band of 50–70 kDa (38–40). It has been demonstrated that the octyl glucoside-solubilized *N*-formyl chemotactic peptide receptor retains the capability for interaction with endogenous neutrophil G protein(s) upon reconstitution into phospholipid vesicles (41).

We have previously shown that the photoaffinity-labeled, octyl glucoside-solubilized *N*-formyl peptide receptor from human neutrophils exhibits two forms upon sucrose density gradient sedimentation, with apparent sedimentation coefficients of approximately 4 and 7 S (11). The 7 S form could be converted to the 4 S form by GTPγS with an EC_{50} of ~20 nM, and this size change in the receptor appeared to correlate with a reduction in sedimentation rate of G_n α and β subunits. These data suggested that the 7 S form of the *N*-formyl chemotactic peptide receptor might represent a physical complex with endogenous G_n protein (11).

To show that the 7 S form is indeed a complex of 4 S receptor and G_n, we attempted its reconstitution with purified G_n (20, 31). The 4 S form of the photoaffinity-labeled receptor

was isolated from GTPγS-treated neutrophil membranes by octyl glucoside extraction and was incubated with different concentrations (10–600 nM) of purified G_n. Each incubation was subsequently analyzed on a velocity sucrose density gradient, and a representative experiment is shown in Fig. 1. It can be clearly seen that there is a major shift of receptors (identified autoradiographically (11) and quantitated densitometrically) from the 4 S form (*peak fraction 4*) to the 7 S form (*peak fraction 7*). A small percentage of receptor (7–8%) did not undergo the shift. The reconstituted 7 S receptor form was fully sensitive to guanyl nucleotides, as no such shift was detectable in the presence of GTPγS (see Fig. 4). In addition, the reconstituted 7 S form could be reversed to the 4 S form when incubated with GTPγS. Resedimentation of the peak or flanking fractions from the zero G protein condition resulted in distributions virtually indistinguishable (not shown) from the original profile, suggesting that the distributions were representative of unassociated receptor.

SDS-PAGE analysis of these gradients confirmed the presence of G_n in these 7 S fractions (not shown), as had been previously shown by Western blot analysis of the endogenous 7 S complexes (11). Moreover, when 1:8 anti-G protein antiserum (11, 12) was included in similar gradients and in the reconstitution mixture containing 300 nM G_i, about 30% of the receptor in the 7 S fraction was shifted into fractions 9 S and higher, whereas less than 1% was shifted when equal amounts of prebleed control serum were added. Together, these results suggest that the 7 S form of the *N*-formyl chemotactic peptide receptor may be a physical complex of receptor and G protein.

Autoradiographic analysis of SDS-PAGE gels of each fraction of these runs confirmed that a major portion of the radioactivity measured was derived from receptor. Fig. 2*A* shows the shift in sedimentation position of the 4 S receptor to the 7 S reconstituted receptor-G protein complex at a G_n concentration of 200 nM. The two autoradiograms indicated that the receptor was shifted in position (not molecular weight) in the sedimentation profile and relative to the "internal standard" of nonspecifically labeled 68-kDa SASD-binding protein (32, 33), the amount of which varied in the preparation depending on the age of the ligand and membrane batch used.

To evaluate the selectivity of the receptor for its endogenous

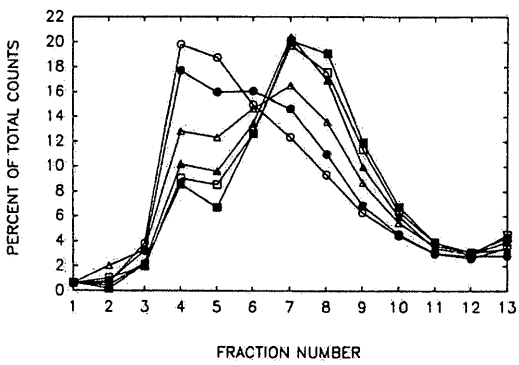

FIG. 1. **Reconstitution of 7 S *N*-formyl chemotactic peptide receptor complexes with purified human neutrophil G_n.** Octyl glucoside-extracted, photoaffinity-labeled *N*-formyl chemotactic peptide receptor was incubated with different concentrations of G_n, as described under "Materials and Methods." The mixtures were sedimented in an ultracentrifuge for 16 h at 192,000 × g_{av} and fractionated into 13 equal fractions. The receptor content of each fraction evaluated densitometrically is plotted as a function of the fraction number. The 4 and 7 S migration distances correspond to fractions 5 and 7, respectively. The concentrations of G_n were 0 (○), 10 (●), 100 (△), 200 (▲), 400 (□), and 600 nM (■).

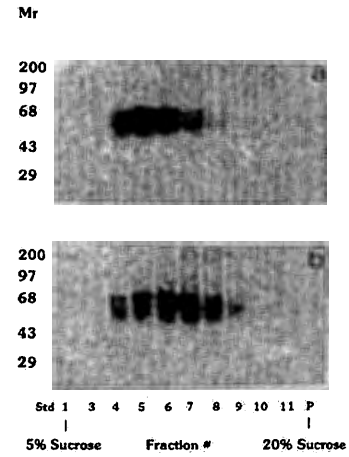

FIG. 2. **Autoradiograms of ^{125}I affinity-labeled and reconstituted formyl chemotactic peptide receptor separated on sucrose density gradients.** *A*, fractions from the density gradients containing the reconstitutions with 0 nM (*a*) and 200 nM G_n (*b*) shown in Fig. 1 were solubilized in SDS and run on 9% SDS-polyacrylamide gels. The gels were dried and developed for autoradiography after 7 days of exposure with Du Pont Cronex Quanta III intensifying screens as described originally by Allen *et al.* (32). The formyl peptide receptor is observed as a broad species between the 68- and 43-kDa markers in both *a* and *b*. Also detectable is the presence of a nonspecifically labeled 68-kDa SASD-binding protein, which serves as an internal sedimentation standard (3.5 S) and is variably present in membrane batch and ligand age.

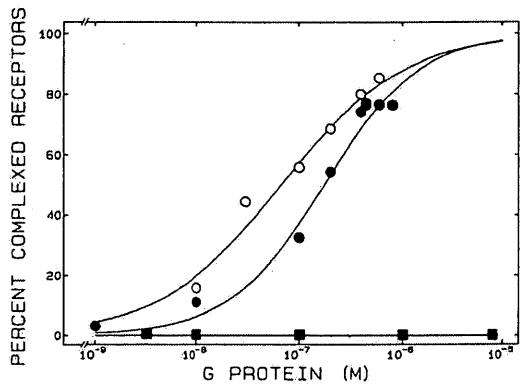

FIG. 3. **Quantitative comparison of the ability of G_n, G_i, or G_t to reconstitute a *N*-formyl peptide receptor G protein complex.** Reconstitution analyses as described in Fig. 1 were used to determine the avidity of *N*-formyl peptide receptor for G protein by measuring the increase in the 7 S form as a percentage of the saturation value observed with excess G protein. ○, G_n ($n = 3$); ●, G_i ($n = 6$); ■, G_t ($n = 2$).

G-protein transduction partner G_n *versus* G proteins from other sources, a similar analysis was performed using G_i purified from bovine brain (consisting of G_{i1} and G_{i2} (42)), and transducin (G_t) from retina. Averaging of the results from multiple sedimentation runs permitted us to estimate the amount of brain G_i or neutrophil G_n necessary to convert 50% of the 4 S receptor form to the 7 S form (Fig. 3). The EC_{50} for reconstitution of the 7 S form of the receptor was 170 ± 40 nM for G_i and 70 ± 25 nM for G_n, based on a computer calculated fit of the data to the Hill equation (34). Functionally active bovine transducin (G_t) was incapable of complexing with the formyl peptide chemotactic receptor in this system, as it showed no shift in receptor sedimentation rate even at 5 μM added transducin. It is noteworthy that the EC_{50} for the reconstitution of the 7 S form of the receptor-G_i protein complex was the same when using receptors obtained from membranes of unstimulated neutrophils and were compared

with receptors obtained from agonist-stimulated cells.

Evidence for the functional significance of the reconstitution of the physical interaction of receptors and G proteins is obtained by determining the ability of the receptor to reconstitute with ADP-ribosylated G protein. Pertussis toxin-catalyzed ADP-ribosylation of G_i proteins is known to uncouple the G_i protein from their receptor transduction partners (5). This uncoupling has been demonstrated by examining receptor-stimulated functions in intact cells, membranes, or reconstituted systems, including phospholipase C activation (23), adenylate cyclase inhibition (43), receptor-stimulated GTPase activity (16, 44), and high affinity agonist binding to the *N*-formyl chemotactic peptide receptor (16), and by other means (44, 45). However, physical uncoupling of receptor and G proteins by ADP-ribosylation has not, to our knowledge, been demonstrated. Indeed, one can postulate that functional uncoupling could take place without physical disruption of the receptor-G complex. We therefore examined the ability of ADP-ribosylated G_i to reconstitute the *N*-formyl chemotactic peptide receptor-G protein (7 S) complex.

Table I documents the preparation of ADP-ribosylated G_i. The protein was ADP-ribosylated to an extent of 0.96 pmol of ADP-ribose/pmol of GTPγS binding, as determined by direct analysis of [^{32}P]NAD incorporation. That the protein was totally ADP-ribosylated is also shown by the inability to significantly label with [^{32}P]NAD subsequent to initial ADP-ribosylation with unlabeled NAD (Fig. 4, *inset*). In order to confirm that the ADP-ribosylated G_i was still functional after the ADP-ribosylation reaction, we determined (see Table I) that GTPγS binding to the ribosylated G_i reached a level of 0.9 pmol of GTPγS/pmol of protein, which is the same value obtained with the nonribosylated (control) protein.

As shown in Fig. 4, when the stoichiometrically ADP-ribosylated G_i protein was used to attempt reconstitution of the *N*-formyl chemotactic peptide receptor-G protein (7 S) complex, we were not able to shift the 4 S receptor to the faster sedimenting form. Even though we raised the concentration of ADP-ribosylated G_i to 20 times the concentration that resulted in 50% complexation of receptor by control G_i, receptor sedimentation profiles showed no conversion of receptor to the 7 S form. These data suggest that the pertussis toxin-catalyzed ADP-ribosylation of G proteins prevents their physical complexation with receptors. This result also suggests that previous studies showing only partial uncoupling by the 14-amino acid peptide mastoparan as a receptor analog (40) is not fully representative of complete receptor-G protein interaction.

DISCUSSION

In this study, we demonstrated that the sedimentation rate of octyl glucoside-solubilized *N*-formyl chemotactic peptide receptor was increased when incubated with either the endogenous neutrophil pertussis toxin substrate, G_n, or purified G_i protein(s) from bovine brain but was unchanged when incubated with bovine transducin. These changes were prevented by guanine nucleotides and, in the case of bovine G_i, by pertussis toxin-catalyzed ADP-ribosylation. The most likely explanation for these observations is that the 7 S form of the receptor is a physical complex containing the heterotrimeric G protein and receptor. Although it is conceivable that such a change could result from a G protein-dependent oligomerization of the receptor with itself or other proteins, it is highly unlikely for several reasons. First, our results are consistent with the large body of biochemical evidence measuring functional parameters, such as agonist binding, GTPase activity, and phototransformation observed in many receptor systems and interpreted as evidence for complexation of receptors and G proteins (24). Second, a G protein-dependent oligomerization of receptors with other proteins would necessarily imply that the newly formed complex would have to interact with G proteins again in the GTP-bound form in order to explain the sensitivity of the 7 S form to guanyl nucleotides. Evidence for a second collisional interaction of receptor and G protein does not exist. Finally, our data indicating specific immunosedimentation of receptor by anti-G protein antisera, suggests that stable receptor G-protein complexes have been formed.

The 4 and 7 S forms of the *N*-formyl chemotactic peptide receptor appears relatively broad in Figs. 1 and 2 (half-band width, ~35% of gradient) in contrast to other membrane proteins (half-band width, 15–20%) (46, 47). Such broadening could arise from a number of sources, including heterogeneous glycosylation of receptor (48), variable amounts of bound lipid and/or detergent on this very hydrophobic protein (38), or even the presence of oligomeric forms of the receptor (49) or other proteins (50). Resedimentation of receptors in the peak and flanking fractions of the 4 S form (zero G protein runs) indicate that the distributions do not narrow or shift in position, suggesting that these latter two possibilities are unlikely. Technical considerations, such as anomalous zone broadening, wall effects, and diffusion (51), are sufficient to explain the broad receptor bands, since narrower distributions are observed (half-band width, 12% of gradient) when shorter gradients in narrower tubes were used to shorten sedimentation time and reduce wall effects (Fig. 4).

Our reconstitution evidence is also consistent with quantitative estimates of requirement for functional interactions within the cell. The *N*-formyl chemotactic peptide receptor was not purified in this work, and we estimate that it was present in the starting membranes at a level of 5–10 pmol/mg of membrane protein. Endogenous G_n protein was present at a level of ~1 nmol of $G_{n\alpha}$ subunit/mg of membrane protein (20, 52). Therefore, the amount of total $G_{n\alpha}$ subunit relative to receptor was approximately 100 to 1 in the native membrane. If one makes the assumption that only the $\alpha\beta\gamma$ complex of G_n is able to effectively interact with receptor, this ratio is reduced to 25 to 1, since $\beta\gamma$ subunits appear to be present at levels approximately one-fourth that of α subunits (20). We induced approximately 80% of the maximum conversion of the labeled receptor from the 4 to the 7 S form (Fig. 3) at 300 pmol/ml G_n. This level is similar to the level of the oligomeric G_n that is present in the native membrane. We are not using a huge excess of G protein relative to what exists in the cell

TABLE I

Characterization of G_i ADP-ribosylation by Pertussis Toxin

Sample	Protein	GTPγS bound	GTPγS/ protein	ADP-ribose incorporated	ADPr/ protein	ADPr/ GTPγS
	pmol/ml	*pmol/ml*	*pmol/pmol*	*pmol/ml*	*pmol/pmol*	*pmol/pmol*
Control G_i	0.495	0.470	0.95			
ADP-ribosylated G_i	2.66	2.34	0.88	2.24	0.84	0.96

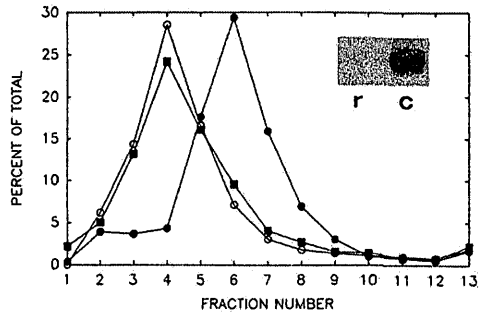

FIG. 4. **ADP-ribosylated G_i is incapable of forming physical complexes with the *N*-formyl peptide receptor.** Reconstitution of the receptor was attempted with 400 nM G_i + 10 μM GTPγS (○), 400 nM G_i (●), and 3000 nM ADP-ribosylated G_i (■), as performed in Fig. 1, except that this sedimentation was performed in smaller tubes for shorter times (8 h). The percent of the total sedimentable radioactivity in the gradient is plotted as a function of fraction number of the octyl glucoside-containing velocity sucrose gradient. The *inset* shows the labeling of either control (*c*) or pre-ADP-ribosylated G_i (*r*) (as described in Table I) with [^{32}P]NAD and pertussis toxin. The autoradiogram was overexposed to demonstrate that all of the G_i protein was stoichiometrically ADP-ribosylated by the initial (cold) ADP-ribosylation reaction (see "Materials and Methods").

to achieve the reconstitution of the receptor-G complex *in vitro*. Both *in vitro* and *in vivo* the G protein levels are considerably in excess (>100-fold) of the *N*-formyl peptide receptor itself.

The similar values for the EC_{50} for complex formation seen with the brain and neutrophil G proteins may reflect identical efficiency in the coupling of G_n and the G_{i2} portion present in the mixed brain G_i preparation. Our experiments, however, are not able to determine if the receptor interacts with both G_{i1} and G_{i2} in the brain preparation. At least three polypeptide stretches of the α subunit of G_{i2} and G_n (corresponding to residues 8–23, 311–328, and 340–350 of transducin) may be interacting with receptors based on an analogy from peptide competition studies of the interaction of rhodopsin and transducin (53, 54). The α subunits of bovine G_{i2} and human G_n are 100% identical in these stretches. The identity between the α subunits of G_n and G_{i1} in these regions is 87, 100, and 100%, respectively. The overall identity of G_{i1} and G_{i2} α subunits, moreover, is 90%, suggesting the differences are rather small but significant. Transducin, which does not interact with the formyl peptide receptor, has a sequence that is only 44, 78, and 91% identical with G_n in the above polypeptide regions. Although these regions are 88, 83, and 100% homologous, if conservative substitutions are considered, there appears to be insufficient similarity to allow transducin to interact with the human chemotactic receptor. These results therefore suggest that the 7 S complex formation is selective and can recognize structural differences in G proteins. Thus, the methods described in this report should be suitable for detailed studies of the specificity of interaction of the *N*-formyl peptide receptor with various other forms of G proteins.

The technique might also be applicable to investigations of functional modification of receptor. Results from our laboratory using labeled *N*-formyl peptide receptor prepared from unstimulated cells indicate that its interaction with G protein is as avid as the receptor prepared from stimulated cells described in this study (data not shown). If it can be shown that the receptor from desensitized cells and unstimulated or stimulated cells are basically equivalent in their affinity for G protein, then it would support the hypothesis that the lateral segregation of receptor from its transduction partner in the plane of the plasma membrane (11, 55) may be sufficient to explain receptor specific desensitization in neutrophils.

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