A carboxyl-terminal tail peptide of neutrophil chemotactic receptor disrupts its physical complex with G protein

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Abstract: **The binding of G protein to the *N*-formyl peptide receptor of human neutrophils was investigated with site-specific synthetic peptides. Peptide CT^{336}_{322} (^{322}RALTEDSTQTSDTAT^{336}) from the carboxyl-terminal tail region of the receptor competed with the receptor for binding to bovine G_i protein. The peptide competition was assayed by dissociation of a GTP-sensitive, rapidly sedimenting (7S) form of receptor-G protein complex as analyzed by velocity sedimentation on linear sucrose density gradients. An IC_{50} of 590 μM was determined for CT^{336}_{322} peptide. A control peptide, with the reverse sequence, rCT^{322}_{336} (^{336}TATDSTQTSDETLAR^{322}), did not perturb the sedimentation of the reconstituted receptor-G protein complex up to the highest tested concentration, 3 mM. Other peptides tested, corresponding to central portions of the predicted intracellular loop regions CII^{140}_{127} (^{127}VLHPVWT QNHRTVS^{140}) and $CIII^{239}_{227}$ (^{227}KIHKQGLIKSSRP^{239}) of the receptor, failed to dissociate the reconstituted receptor-G protein complex. Control peptides from the extracellular region EII^{184}_{170} (^{170}KTGTVACTFNFSPWT^{184}) and an unrelated sequence matching a portion of neutrophil cytochrome b, CYT^{306}_{296} (^{296}KVVITKVVTHPFKTIE^{306}), were also ineffective. Our results suggest that the cytoplasmic tail of the formyl chemotactic peptide receptor is involved in its coupling to the signal-transducing G protein.** *J. Leukoc. Biol.* **54: 572-577; 1993.**

Key Words: *neutrophils • chemotactic receptor • G protein*

INTRODUCTION

Neutrophils contribute to immune function by their capacity to carry out chemotaxis, lysosomal enzyme secretion, and superoxide production [1]. Bacteria and mitochondria initiate protein synthesis with *N*-formylmethionine, whereas eukaryote protein synthesis begins with methionine. *N*-formyl peptides can act as chemoattractants by binding to specific neutrophil surface receptors, which then trigger the activation of superoxide production and other cellular functions [2-4]. A substantial body of evidence suggests that the *N*-formyl peptide chemoattractant receptor (FPR) mediates signal transduction through interaction with a guanyl nucleotide binding protein or G protein [5-8]. The FPR has been cloned and sequenced [3] and the derived amino acid sequence suggests that FPR belongs to a family of receptors called G protein-coupled heptahelical receptors (see refs. 9-11 for reviews).

The FPR can be identified by photoaffinity labeling with a derivative of *N*-formyl peptide and a heterobifunctional radioiodinated cross-linker, f-Met-Leu-Phe-N^{ε}-(2-(p-azido[^{125}I] salicyl-amido)ethyl-1,3'-dithiopropionyl)-Lys (fMLFK-[^{125}I]ASD, formerly referred to as FMLPL-[^{125}I]SASD) [12]. The affinity-labeled FPR from human neutrophils behaves as a monodisperse species of approximately 63 kd when analyzed by equilibrium sedimentation analysis in Triton X-100 [13]. Similarly, the FPR from differentiated HL60 cells elutes as a 66-kd species upon gel filtration [14]. These estimates are consistent with the apparent size of the affinity-labeled receptor on reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, where the liganded receptor migrates as a broad band between 50 and 70 kd [13, 15, 16]. Upon enzymatic deglycosylation, the receptor migrates as a sharp 35-kd band [17].

Photoaffinity-labeled, octyl glucoside-solubilized FPR exhibits two size forms with apparent sedimentation coefficients of approximately 4S and 7S. The 7S form can be converted to the 4S form by inclusion of GTPγS in the solubilizing buffer. This conversion occurred with a GTPγS EC_{50} of approximately 20 nM and appeared to correlate with a reduction in sedimentation rate of G α and βγ subunits [18].

Detergent-solubilized FPR retains the capability for interaction with endogenous G protein(s) upon reconstitution into phospholipid vesicles [19] or with exogenously added G protein(s) in a concentration-dependent manner in octyl glucoside [20]. The reconstituted 7S form is fully sensitive to guanyl nucleotides and is immunosedimentable by anti-G_{α} antibodies [20]. Silver staining and immunoblotting of sucrose gradient fractions containing the FPR-G protein complexes indicate that the G protein subunits cosediment with the 7S form of the receptor. These data suggest that the 7S form of the *N*-formyl chemotactic peptide receptor represents a physical complex with the G protein.

Such hydrodynamic studies have created a unique opportunity to conduct synthetic peptide competition studies of FPR binding to G protein to gain an understanding of the structural features of the chemotactic receptor that determine its interaction with its signal transduction partner. In this study, we probed the interaction of FPR and bovine G protein using (site-specific) synthetic peptides corresponding to predicted hydrophilic intracellular domains of FPR. Our results show that a 15-amino-acid peptide CT^{336}_{322} (^{322}RALT EDSTQTSDTAT^{336}) from the predicted cytoplasmic tail region of the receptor is able to disrupt the physical complex of G protein and the receptor. This result suggests that the carboxyl-terminal tail region of the FPR is involved in the physical coupling of FPR to G protein.

Abbreviations: fMLFK-[^{125}I]ASD, f-Met-Leu-Phe-N^{ε}-(2(*p*-azido[^{125}I]salicyl-amido)ethyl-1,3-dithiopropionyl)-Lys; FPR, *N*-formyl peptide chemoattractant receptor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Buffers, chemicals, and methods of cell preparation were as previously described [18, 21]. Chemicals used for G protein isolation were as described by Bokoch et al. [22].

Peptide synthesis

Peptides CT^{356}_{322} (^{322}RALTEDSTQTSDTAT^{336}), rCT^{322}_{336} (^{336}TAT DSTQTSDETLAR^{322}), CII^{140}_{127} (^{127}VLHPVWTQNHRTVS^{140}), (^{170}KTGTVACTFNFSPWT^{184}), and $CIII^{239}_{227}$ (^{227}KIHKQGL IKSSRP^{239}), corresponding to the predicted cytoplasmic surface regions of the FPR (**Fig. 1**), were synthesized by the fluorenyl-methoxy-carbonyl (FMOC) method with a Milligen 9050 automated peptide synthesizer. Deprotection and cleavage were carried out using reagent K (trifluoroacetic acid 97%, phenol 0.5%, H_2O 1%, ethanedithiol 1%, thioanisole 0.5%). Peptide purity was monitored by high-performance liquid chromatography using a Vydac reverse-phase C_{18} column and by electrospray mass spectrometry, which revealed a single molecular ion peak corresponding to the molecular weight of each of the peptides. Peptides corresponding to the extracellular receptor loop, EII^{184}_{170}, and neutrophil cytochrome b peptide, CYT^{306}_{296} (^{296}KVVITKVV THPFKTIE^{306}), were made as described previously [23]. The cytochrome b peptide (CYT^{306}) was a kind gift of Dr. Mark T. Quinn. Peptide stock solutions were made at 50–100 mM in the extraction buffer containing sodium azide (0.02%) and the pH was adjusted to 7.4.

Preparation of G protein

G_i was purified from bovine brain as previously described [24] and was separated 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 95% or higher as assessed by GTPγS binding and SDS-PAGE followed by silver staining. The G protein was the kind gift of Dr. Gary M. Bokoch.

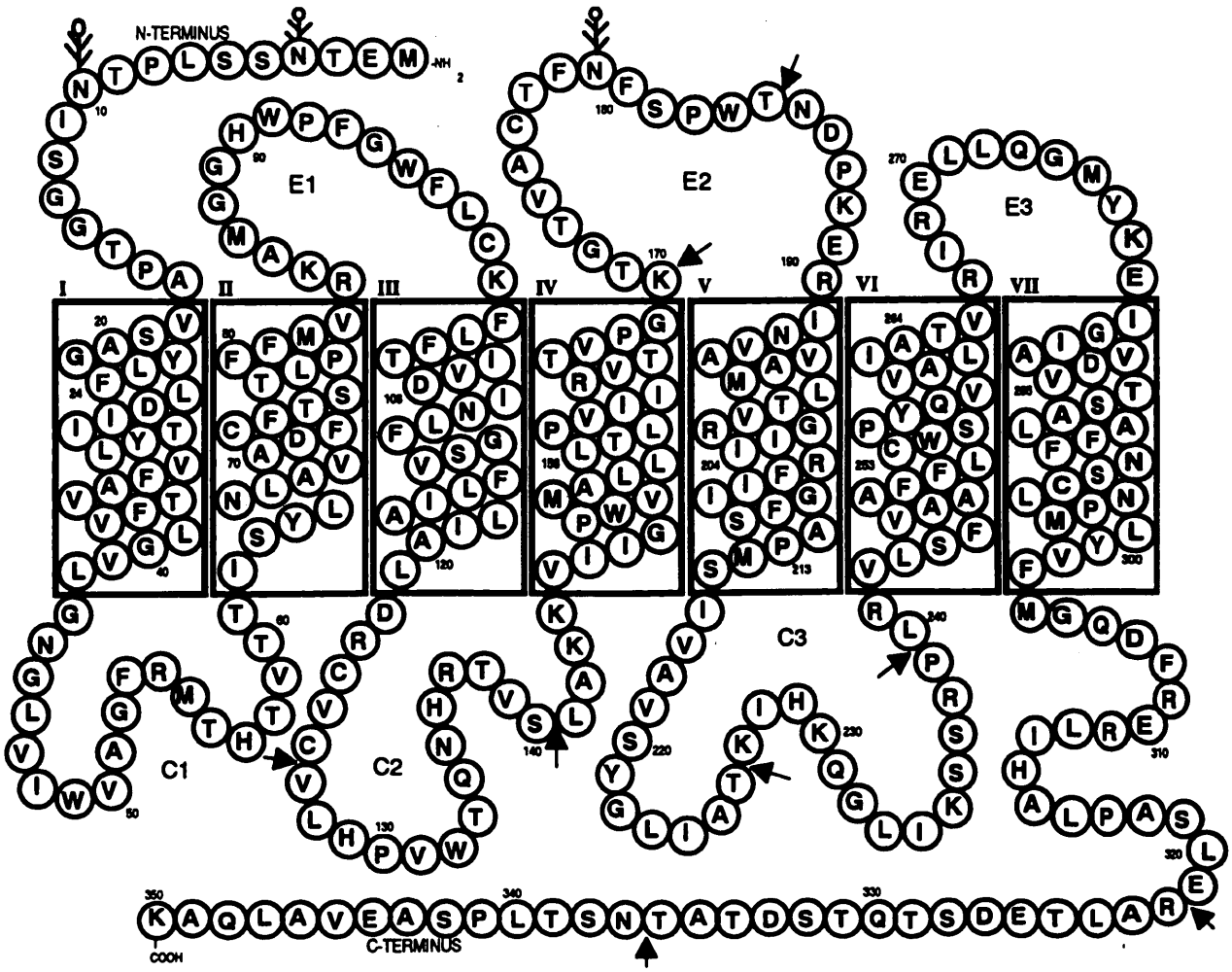

Fig. 1. Schematic model of the FPR polypeptide chain in the neutrophil plasma membrane. I through VII represent the putative lipid bilayer-spanning domains, which are connected by loops E1, E2, and E3 on the extracellular side and loops C1, C2, and C3 on the cytoplasmic side of the membrane. The asparagine residues at amino acid positions 4, 10, and 179 are predicted to be three potential glycosylation sites on the receptor. Similar seven-transmembrane-domain models have been proposed and validated to different degrees for a wide range of other integral membrane proteins, including bacteriorhodopsin [42, 43], rhodopsin [44], and adrenergic receptors [45]. The peptides between the arrowheads, 127–140, 170–184, 227–239, and 322–336, were tested in the reconstitution inhibition experiments.

Preparation of the 4S form of FPR

Plasma membranes, obtained from stimulated human neutrophils by nitrogen cavitation as described by Parkos et al. [21], were stored at −70°C until use. The membranes were washed with 1 M NaCl (in "relax buffer"; see below) and were resuspended in Hanks' buffer (pH 8.5) during the labeling step (at about 1×10^8 cell equivalents/ml). The FPRs were photoaffinity labeled with fMLFK-[^{125}I]ASD as described [12, 25]. Briefly, the NaCl-washed membranes were incubated with 20–30 nM fMLFK-[^{125}I]ASD for 30 min on ice in the dark in foil-covered plastic tubes. Nonspecific labeling was assessed in the presence of a 100-fold excess of unlabeled fMLFK-ASD. Covalent incorporation of the radiolabel was achieved by irradiating at 370 nm for 10 min using a Rayonet Ultraviolet Light Reactor as described previously [12].

The labeled membranes were treated with 10–20 μM GTPγS to prevent interference from the endogenous G protein during the reconstitution of the 7S form. GTPγS treatment was done at 4°C and for a duration of 5–10 min. The treated membranes were washed with 1 M NaCl three times to remove free GTPγS and peripheral proteins. The washed membranes were pelleted (Beckman Ti60 rotor at 45,000 rpm for 45 min) and resuspended at 1×10^9 cell equivalents/ml (approximately 1 mg/ml protein) in 10 mM HEPES, pH 7.4, 100 mM KCl, 10 mM NaCl, 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml chymostatin (relax buffer) plus 1% 1-octyl-β-D-glucopyranoside (octyl glucoside). The membranes were then allowed to extract on ice for 1–2 h and insoluble material was removed by centrifugation at 4°C in a Beckman Ti60 rotor at 45,000 rpm or 30 min.

Peptide effects on reconstitution of FPR with G_i protein

Linear sucrose density gradients (700 μl, 5–20%) were prepared by pouring step gradients (5, 10, 15, and 20% sucrose) and allowing the gradients to diffuse into a linear gradient for 10–12 h at 4°C. All sucrose and peptide solutions were made in the extraction buffer (1% octyl glucoside in relax buffer). The detergent extract containing the FPR was divided into 20-μl aliquots and G_i was added to a final concentration 450 nM. The estimated concentration of FPR was about 10–15 pM. The mixtures were incubated for 1–2 h on ice and then mixed with different concentrations of the peptides. One-, two-, or eight-hour incubations (with G_i) were equally effective in formation of the maximal 7S FPR complex. For most experiments, the peptide-containing mixtures were further incubated for 2–5 h on ice before layering on sucrose density gradients. However 1-, 2-, or 8-h incubations with peptide were found to be equally effective in disrupting the complexes. Moreover, identical results were obtained when the receptor peptides were preincubated with G protein before the addition of FPR. This suggests that these experiments were conducted at effectively equilibrium conditions. Appropriate concentrations of the relevant peptides were also included in the gradients to preclude re-formation of complex during sedimentation.

The gradients were then centrifuged in a SW55 Beckman swinging-bucket rotor for 8 h at 45,000 rpm at 4°C and receptor peaks were localized after fractionation of gradients into 55-μl fractions. Sedimentation experiments were calibrated with protein standards by centrifuging a mixture of 10 μg each of cytochrome c (2.1S), bovine serum albumin (4.4S), porcine immunoglobulin (7.7S), and bovine catalase (11.2S) in parallel with experimental gradients. Individual fractions were subjected to SDS-PAGE. G protein content was determined by SDS-PAGE and immunoblot analysis, confirming a relatively broad distribution [20] including the FPR-containing fractions. The receptor content was determined by the receptor radioactivity using storage phosphor technology and phosphorimage analysis [26] and also by traditional autoradiographic analysis as described previously [20]. The phosphorimage analysis and the quantitation of the receptor bands were done with a Molecular Dynamics (Sunnyvale, CA) 400E Phosphor Imager and software.

RESULTS AND DISCUSSION

Site-specific synthetic receptor peptides corresponding to the predicted interfacial contact sites between receptor and G protein have been observed to interfere with both physical and functional coupling of these macromolecules [27–31]. We report here the use of site-specific synthetic peptides to probe the physical coupling of the *N*-formyl peptide chemoattractant receptor (FPR) with its signal transduction partner G protein in detergent solution. We investigated the ability of three synthetic peptides corresponding to predicted cytoplasmic domains of the FPR to dissociate a reconstituted receptor–G protein complex. Our assay involves measuring peptide-induced changes in the rate of sedimentation of the receptor–G complex protein as analyzed by velocity sedimentation in linear sucrose density gradients [20].

The structural basis for the interaction between receptors and G proteins in other systems has been intensely studied by site-directed mutagenesis, competition studies using site-specific synthetic peptides, and conventional biochemical methods. Site-directed mutagenesis studies suggest that the second and third cytoplasmic loops of β-adrenergic receptor and rhodopsin are involved in receptor–G protein coupling (reviewed in ref 9). Synthetic peptide competition studies have implied participation of the second cytoplasmic loop, the NH_2-terminal and COOH-terminal regions of the third cytoplasmic loop, and regions of the cytoplasmic tail [9, 10, 29–32].

A schematic representation of the proposed transmembrane topology of FPR is shown in Figure 1. Comparison of sequences of the individual cytoplasmic domains between FPR and other G protein–coupled receptors indicates sequence identities ranging between 7 and 27% (except for the comparison of the first cytoplasmic loop between FPR and rhodopsin, which shows 46% identity). Such relatively low sequence identities suggest that these regions might confer specificity for receptor–G protein coupling. Selection of FPR peptides for our experiments was based on hydrophilicity [33] and predicted antigenicity [34]. We chose peptide segments CT^{336}_{322} (^{332}RALTEDSTQTSDTAT^{336}) of the carboxyl terminal tail, CII^{140}_{127} (^{127}VLHPVWTQNHRTVS^{140}) of the intracellular loop C2, and $CIII^{239}_{227}$ (^{227}KIHKQGLIKSSRP^{239}) of the intracellular loop C3, which are delimited by arrowheads in Figure 1. As control reagents, we also used reverse-sequence peptides, peptides with sequences from unrelated proteins CYT^{306}_{296} (^{296}KVVITKVVTHPFKTIE^{306} of neutrophil cytochrome b heavy chain), and peptide EII^{184}_{170} (^{170}KTGTVACTFNFSPWT^{184}) from the predicted extracellular loop E2 of the receptor.

To probe for interactive sites of the FPR–G protein pair, these peptides were added to the reconstitution mixture of FPR and bovine G_i as described in Materials and Methods. The peptides, at a concentration of 1 mM, were incubated with the receptor–G protein complex and the peptides were present throughout the gradient at the same concentration. However, inclusion of peptides during the receptor–G protein complexation or preincubation of the peptides with G protein produced identical results. **Figure 2** shows the

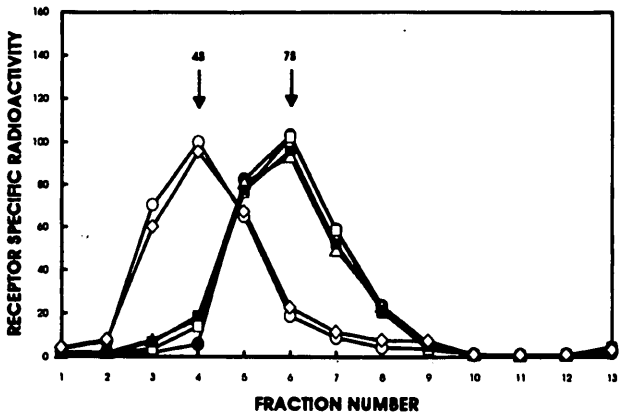

Fig. 2. Effect of various FPR peptides on the reconstituted receptor–G protein complexes. Octyl glucoside–extracted, photoaffinity-labeled FPR was incubated with 450 nM G_i as described in Materials and Methods. Separation of the complexes on the basis of size was achieved by subjecting the protein mixture to ultracentrifugation in detergent-containing sucrose gradient sedimentation for 8 h at 192,000g_{av} followed by fractionation into 13 equal fractions. The receptor content of each fraction, evaluated densitometrically, is plotted as a function of fraction number paralleling increasing sucrose concentration, as described previously [20]. The sedimentation distances in the gradient correspond to 4S and 7S globular proteins and are equivalent to fractions 4 and 6, respectively. Peptides at a concentration of 1 mM were used to assay disruption of the reconstituted 7S complex: $CIII^{239}_{227}$ (^{227}KIHKQGLIKSSRP^{239}) (△); CII^{140}_{127} (^{127}VLHPVWTQNHRTVS^{140}) (□); CT^{336}_{322} (^{322}RALTEDSTQTSDTAT^{336}) (◇); CYT^{306}_{296} (^{296}KVVITKVVTHPFK TIE^{306}) (■); no G_i and no peptide (○); 450 nM G_i and no peptide (●). The sedimentation profile of the receptor–G protein complex in the presence of the rCT^{322}_{336} (reverse sequence) peptide and the EII^{196}_{170} peptide was identical to that for the assay that did not contain any peptide (●). Data points represent the mean of two different experiments. Experiments with peptides other than CT^{336}_{322} (n = 3) were repeated a third time at a maximum peptide concentration of 3 mM and no disruption of 7S complex was observed.

effects of various peptides on the rate of sedimentation of the reconstituted FPR. Except for the 15-amino-acid CT^{336}_{322} peptide, none of the peptides were able to dissociate the reconstituted 7S complex as analyzed on detergent-containing sucrose gradients. It is noteworthy that a peptide of identical length but reverse sequence, rCT^{322}_{336} (^{336}TATDSTQTSDET-LAR^{322}), was also unable to perturb the reconstitution at the same or threefold (3 mM) higher concentration than that used for the native sequence (**Fig. 3**). These results indicate that the peptide-induced dissociation of the 7S receptor–G protein complex was not a result of nonspecific physicochemical effects. In fact, since the active peptide contains a seven-amino-acid palindrome constituting 42% identity with the reverse sequence, the inability of its reverse analog to dissociate the complex confirms the high level of specificity of this competition and localizes the probable active regions of the 15mer to its amino and/or carboxyl termini.

Densitometric analysis of the receptor bands on autoradiograms of gels were used to determine the receptor distribution in the gradient fractions [20]. Figure 3 indicates that the CT^{336}_{322} peptide disrupts the reconstituted 7S complex with an EC_{50} of about 590 μM. This result suggests that the interaction of this peptide stretch of the FPR and bovine G_i is of relatively low affinity but comparable to that observed for other receptor peptides [29, 30] and G protein peptides [27, 35] that dissociate receptor–G protein complexes. Another common feature of this concentration dependence is the relatively steep inhibition curve with a calculated Hill coefficient of 1.95 [36] corresponding to those calculated with the other systems mentioned above. The interaction between proteins is expected to be multivalent in nature, involving different sites on the interfacial regions. Thus the low affinity observed when a single peptide competes with the full protein-protein interface is not surprising.

Rhodopsin peptides compete with rhodopsin–G protein interactions in the fractional millimolar concentration range [27, 29]. The effects of rhodopsin peptides are synergistic when active peptides are used in combination, resulting in lower effective peptide concentrations [27, 29]. In contrast to the rhodopsin system, no synergism of FPR-G protein uncoupling was observed when peptides $CIII^{239}_{227}$ CII^{140}_{127}, and CT^{336}_{332} were added together (data not shown). This negative result could arise from the fact that peptides used to probe synergism may not have been taken from the correct portion of the cytoplasmic receptor surface. In fact, evidence from the rhodopsin system suggests that two highly conserved amino acids near the amino-terminal portion of the C-2 loop (glutamic acid and arginine) are involved in functional coupling of the receptor to its G protein [37], but these positions were not included in the peptides tested. FPR, like many other G protein–coupled receptors, contains aspartic acid and arginine at analogous positions (Fig. 1). If another peptide from the regions of the receptor could be found that also displays inhibition of receptor–G protein coupling, it would be interesting to investigate synergistic effects of such a peptide and CT^{336}_{322}.

The 15-amino-acid long CT^{336}_{322} peptide has 27–40% sequence identity and up to 73% sequence similarity to analogous segments on the cytoplasmic tail regions of various G protein–coupled receptors. In addition, the alignment of certain amino acids in the sequence appears to be common for several of the other receptors (**Table 1**). When the *entire* carboxyl tail region was compared, however, the amino acid sequence identity ranged between 27% (cAMP-R) and 40% (C5a receptor and rhodopsin). Such identities over the entire tail regions are almost equivalent to functionally unrelated proteins such as chymotrypsin and lysozyme, which show maximum identities of 27 and 20%, respectively. This suggests that the identities over the short stretches may be more

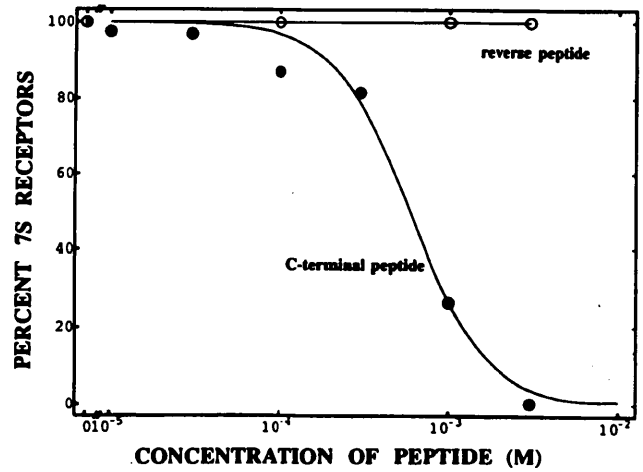

Fig. 3. Inhibition of FPR-G_i protein reconstitution as a function of the concentration of CT^{336}_{322} peptide. CT^{336}_{322} (●) and rCT^{322}_{336} (○) peptides were incubated with the reconstituted 7S complex, at concentrations indicated, for 2–4 h on ice and the complexes analyzed as described in Materials and Methods and in the legend of Figure 2. Complete inhibition would be equivalent to pure 4S receptor with no G_i added. Data points represent the mean of three different experiments and the standard deviations were 5.6, 8.2, 3.3, 1.0, and 8.5% at 10 μM, 30 μM, 100 μM, 300 μM, and 1 mM peptide, respectively. The solid line represents the computer-calculated fit of the data to the Hill equation as described in ref. 36.

TABLE 1. Comparison and Sequence Alignment of CT 322-336 Peptide of the Human Neutrophil *N*-Formyl Peptide Chemoattractant Receptor with Other Proteins[a]

Protein	Sequence	Percent identity	Percent similarity	Remarks
CT 322-336	322- R A L T E D S T Q T S D T A T -336	–	–	C-terminus
Rhodopsin	328- L G D D E A S T T V S K T E T -342	40	47	C-terminus
cAMP-R	314- G H P T G D D V Q C S S D M E -328	27	47	C-terminus
hum β2-R	375- K L L C E D L P G T E D F V G -389	33	73	C-terminus
hum sub P-R	380- L D L T S N C S S R S D S K T -394	33	60	C-terminus
hum a2-R	289- D A L D L E E S S S S D H A E -303	33	60	C-III loop
hum C5a-R	321- N V L T E E S V V R E S K S F T -336	40	67	C-terminus

[a]Sequences were compared by the *BestFit* routine of the Wisconsin GCG software package [46]. *BestFit* makes an optimal alignment of the best segment of similarity between two sequences. Percent similarity takes into account conservative substitutions in the sequences compared. *BestFit* uses the "local homology" algorithm of Smith and Waterman [47]. The location of the segment compared on various G protein-coupled receptors is given in the last column. The peptide segments are manually aligned to show the sequence homology. Highlighted regions represent either amino acid identity or conservative substitutions. The flanking numbers represent the location of the polypeptide segment in the protein. Abbreviations used: CTE 322-336, the CT^{336}_{322} peptide of the *N*-formyl chemoattractant peptide receptor; cAMP-R, cyclic AMP receptor from *Dictyostelium discoideum;* hum β2-R, human β_2-adrenergic receptor; hum sub P-R, human substance P receptor; hum α2-R, human α_2-adrenergic receptor; hum C5a-R, human C5a receptor. The shading of the amino acids was based on the similarity of the residues, which are divided into four groups: nonpolar (A, V, L, I, P, M, F, W), polar (G, S, T, C, Y, N, Q), positively charged (K, R, H), and negatively charged (D, E).

relevant to similarities of function of these receptor proteins.

The CT^{336}_{322} region on FPR is enriched in serine and threonine residues, similar to analogous cytoplasmic tail regions in rhodopsin and β-adrenergic receptors. The serine and threonine residues at positions 334-336 in rhodopsin [38] and numerous sites on the cytoplasmic tail of the β-adrenergic receptor (reviewed in ref. 9) were shown to be phosphorylated by specific receptor kinases. These phosphorylated regions, along with other phosphorylated serine and threonine residues nearby, are thought to mediate the binding of the regulatory molecule arrestin to rhodopsin and possibly β-arrestin to the β-receptor [39].

In contrast to earlier reports [40], serine at position 334 (CT 328), two threonine diads at positions 319-320 and 335-336 (CT 329), and an aspartic acid diad at position 330-331 on the carboxyl tail region of rhodopsin have been reported to be important for inhibition of reconstituted GTPase activity using purified rhodopsin and transducin [28]. Moreover, in a recent abstract, experiments were described in which a fusion protein containing the entire carboxyl tail of the FPR and maltose binding protein demonstrated inhibition of high-affinity ligand binding to FPR [41]. These results and the significant degree of local sequence identity with rhodopsin support our finding that the CT^{336}_{322} region on FPR appears to be important for the FPR-G_i coupling.

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Note added in proof: Recently, Schreiber et al. have confirmed our results, suggesting that at least two regions of the carboxyl terminal tail of the *N*-formyl peptide receptor interact with G protein. [Schreiber, R.E., Prossnitz, E.R., Ye, R.D., Cochrane, C.G., Bokoch, G.M. (1994) Domains of the human neutrophil N-formyl peptide receptor involved in G protein coupling: mapping with receptor-derived peptides. *J. Biol. Chem.* In press.] They also found that a peptide from the second intracellular loop is involved in this interaction. Our most recent evidence suggests that peptide mimetics corresponding to regions flanking the inactive ones in the second and third intracellular loops, described in the current study, are indeed active and capable of disrupting the 7S FPR/G protein complex [Bommakanti, R.K., Jesaitis, A.J., unpublished observations].

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