J. Microbiol. Biotechnol. (2004), **14**(5), 1031–1037

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Characterization of Segments of $G\alpha_{16}$ Subunit Required for Efficient Coupling with Chemoattractant C5a, IL-8, and fMLP Receptors

HA, JI-HEE[1], CHUL-HOON LEE[2], AND CHANG-HO LEE[3]*

[1]*Department of Cancer Research and Molecular Biology, Fels Institute, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, U.S.A.*
[2]*Department of Medical Genetics, College of Medicine, Hanyang University,* [3]*Department of Pharmacology and Institute of Biomedical Sciences, College of Medicine, Hanyang University, Seoul 133-791, Korea*

Received: June 24, 2004
Accepted: September 21, 2004

Abstract The interaction of chemoattractant receptors and $G\alpha_{16}$ was studied to provide the molecular basis to elucidate the interaction of chemoattractant receptors with $G\alpha_{16}$ subunit, thereby possibly contributing to finding novel targets for designing new type of G protein antagonists with anti-inflammatory effects. Experiments were performed to characterize the $G\alpha_{16}$ subunit domains responsible for efficient coupling to chemoattractant receptors. Thus, a series of chimeric $G\alpha_{11}/G\alpha_{16}$ and $G\alpha_{16}/G\alpha_{11}$ cDNA constructs were expressed, and the ability of chimeric proteins to mediate C5a, IL-8, and fMLP-induced release of inositol phosphate in transfected Cos-7 cells was tested. The results showed that short stretches of residues 154 to residue 167 and from residue 174 to residue 195 of $G\alpha_{16}$ contribute to efficient coupling to the C5a receptor. On the other hand, a stretch of amino acid residues 220–240 of $G\alpha_{16}$ that is necessary for interacting with C5a receptor did not play any role in the interaction with IL-8 receptor. However, a stretch from residue 155 to residue 195 of $G\alpha_{16}$ was found to be crucial for efficient coupling to IL-8 receptor in concert with C-terminal 30 amino acid residues of this α subunit. Coupling profiles of a variety of chimeras, composed of $G\alpha_{11}$ and $G\alpha_{16}$, to fMLP receptor indicate that the C-terminal 30 amino acids are most critical for the coupling of $G\alpha_{16}$ to fMLP receptor. Taken together, $G\alpha_{16}$ subunit recruits multiple and distinctive coupling regions, depending on the type of receptors, to interact.

Key words: $G\alpha_{16}$, chemoattractant receptor, IL-8, fMLP, G-protein antagonist

Multiple structural elements on the G protein molecules have been suggested to be involved in dictating G protein-receptor coupling selectivity [6]. One of the well-defined regions of interaction resides in the extreme C-terminal amino acid sequence of the Gα subunit [8, 9, 24, 30]. However, additional structural determinants on the G protein have been proposed for interacting with receptors, suggesting that various segment sequences, such as the C-terminus and sequences distributed throughout the Gα subunit, may dictate the specific receptor interaction [14, 18, 19, 25].

Our previous study indicated that the C-terminal 30 amino acids and certain sequences extending from the N-terminus to amino acid 209 of $G\alpha_{16}$ as well as a segment encompassing residues 220–240, particularly Ala 228, were responsible for the ability of chemoattractant C5a receptor to induce $G\alpha_{16}$-mediated inositol phosphate release, suggesting that the multiple regions of $G\alpha_{16}$ account for its ability to interact with C5a receptor [13, 21].

In the present study, experiments were undertaken to characterize the segment extending from the N-terminus to amino acid 209 of $G\alpha_{16}$ subunit in order to find short stretches of amino acid sequence that may play key roles in the efficient coupling of $G\alpha_{16}$ with C5a receptor. Furthermore, coupling determinants on the $G\alpha_{16}$ subunit were also examined for their ability to interact with the fMLP receptor and the IL-8 receptor to investigate if there are any common and/or specific structural elements that are responsible for the $G\alpha_{16}$-chemoattractant receptor interaction.

The results of this study are expected to provide a molecular basis for elucidation of the interaction of chemoattractant receptors with $G\alpha_{16}$ subunit, thereby possibly contributing to finding novel targets for designing a G protein antagonist with anti-inflammatory effect that can inhibit the specific interaction of chemoattractant receptors with $G\alpha_{16}$.

*Corresponding author
Phone: 82-2-2290-0655; Fax: 82-2-2292-6686;
E-mail: jennysue@hanyang.ac.kr

MATERIALS AND METHODS

Materials and cDNAs

Myo-[2-^{3}H]inositol (10 µCi/ml) was purchased from Amersham (Buckinghamshire, England). DMEM (high glucose), fetal bovine serum, Lipofectamin, and opti-MEM were from Gibco-BRL (Gaithersburg, MD, U.S.A.), chemiluminescence Western blot detection kit from Intron (Seoul, Korea), and Vent DNA polymerase from New England Bio Lab (Beverly, MA, U.S.A.). Other chemicals were from Sigma (St. Louis, MO, U.S.A.). Antibodies against Gα subunits, cDNAs for the chemoattractant receptors C5a, fMLP and IL-8, and $G\alpha_q$ class subunit G proteins were kindly provided by Mel. I. Simon (Caltech, Pasadena, CA, U.S.A.).

Cos-7 Cell Expression Vector

cDNAs encoding receptors of C5a, IL-8, and fMLP and cDNAs encoding α subunits of G -proteins $G\alpha_{11}$, $G\alpha_{16}$, and various chimeric G proteins were carried by the cytomegalovirus vector pCMV.

Construction of the Recombinant Gα Subunit Chimeras

Various chimeric G proteins (χ_1-χ_9) in pCMV were constructed as described [21]. Another series of chimeric G proteins χ_{10}, χ_{11}, χ_{12}, and χ_{13} in pCMV were constructed by overlap extension PCR [17]. Briefly, fragments from $G\alpha_{16}$ and $G\alpha_{11}$ that are to be recombined were generated in separate PCR. Primers for the preparation of $G\alpha_{16}$ part of chimeras were: forward primer, AT AGC ATC GAT AAA AGG CAG CCT CCC TGC GCA CCC which corresponds to the sequences encoding N-terminal 24 nucleotides of $G\alpha_{16}$ and *Cla*I site (underlined); various reverse primers, GAT GAT GTT CTC CAG GTC AAA CGG GTA CTC GTT GAT GCC AGT GGT (χ_{10}), CTC CTC GGT GAT GCG CTC CAG GTG GGA (χ_{11}), CAG GTG GGA CAG GTA GTA CAC GGC (χ_{12}), GTA CTT AGC CGA GTC AGA TAG CTG GAA TTC CCG CCG ACG CTC ATA GCA (χ_{13}). Primers for the preparation of $G\alpha_{11}$ part of chimeras were: forward primers, CCC ACC ACT GGC ATC AAC GAG TAC CCG TTT GAC CTG GAG AAC ATC ATC (χ_{10}), TCC CAC CTG GAG CGC ATC ACC GAG GAG GGC TAC CTG CCC ACC CAG CAG GAT GTG (χ_{11}), GCC GTG TAC TAC CTG TCC CAC CTG GAC CGC ATC GCC ACA GTA GGC TAC (χ_{12}), TGC TAT GAG CGT CGG CGG GAA TTC CAG CTA TCT GAC TCG GCT AAG TAC (χ_{13}); reverse primer, A TAC CTC GAG CGG TCA CAC CAG GTT GTA CTC CTT that corresponds to the sequence encoding C-terminal 1116- 1139 of $G\alpha_{11}$ and *Xho*I site (underlined). Each parts of the PCR products were purified, mixed, and subjected to PCR by Vent DNA polymerase in the presence of forward primer, AT AGC ATC GAT AAA AGG CAG CCT CCC TGC GCA CCC which corresponds to the sequences encoding N-terminal 24 nucleotides of $G\alpha_{16}$ and *Cla*I site (underlined) and reverse primer, A TAC CTC GAG CGG TCA CAC CAG GTT GTA CTC CTT that corresponds to the sequence encoding C-terminal 1116- 1139 of $G\alpha_{11}$ and *Xho*I site (underlined). The resultant PCR fragment was digested with restriction enzymes, *Cla*I and *Xho*I, and ligated to pCMV through *Cla*I and *Xho*I restriction sites. The cDNA constructs encoding all the chimeras were verified by restriction enzyme analysis and DNA sequencing.

Transient Transfection of Cos-7 Cells

Cos-7 cells were cotransfected with different plasmids, including cDNAs of various chemoattractant receptors, Gα subunits, and chimera Gα subunits. For this, adequate amount of each plasmid was mixed (total 1.0 µg) with 4 µl of lipofectamin in 0.5 ml of opti-MEM, and then was added to the cells. In control experiments, the total amount of cDNA was maintained constant by adding pCMV DNA. Twenty % fetal bovine serum (FBS) in 0.5 ml of DMEM was added after 5 h.

Labeling of Cells with Myo-[2-^{3}H]Inositol and Analysis of C5a, IL-8, and fMLP-Induced Inositol Phosphates Levels in Intact Cells

One day after transfection of Cos-7 cells, the cells were labeled by incubating with 10 µCi/ml of *myo*-[2-^{3}H]inositol in 0.4 ml of inositol-free DMEM containing 10% dialyzed FBS for 24 h. Finally, the labeled Cos-7 cells were washed once with inositol-free DMEM and then incubated in 200 µl of inositol-free DMEM medium containing 10 mM LiCl in the presence of stimulants, 100 nM recombinant human C5a, 50 nM IL-8, and 500 nM fMLP, or 30 µM $AlCl_3$ plus 10 mM NaF (AlF_4^-) for 15 min at 37°C. Then, changes in the level of inositol phosphates were measured.

SDS-Polyacrylamide Gel Electrophoresis and Western Blot

Cos-7 cells transfected with the cDNAs encoding pCMV, chemoattractant receptors, $G\alpha_q$ class subunits, and various chimeras were washed twice with PBS. Then, cell membranes (50 µg protein) were collected in 45 µl of RIPA buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 50 mM NaF, 1 mM PMSF, 4 µg/ml aprotinin, 2 µg/ml leupeptin, 200 µM vanadate, and 0.1% β-mercaptoethanol in PBS). Fifteen µl of 4× Laemmli's buffer was added, followed by boiling, and the samples were subjected to SDS-gel electrophoresis. The gels were then transferred onto PVDF membranes (Bio-Rad). The expression of $G\alpha_q$ class subunits and various chimeric G proteins were detected with antibodies raised against the C-terminal sequence of $G\alpha_{11}$, as described previously [3]. The level of expression was visualized with a Westzol chemiluminescence Western blot detection kit.

RESULTS AND DISCUSSION

Heterotrimeric G proteins, which are molecular switches in the receptor-mediated transmembrane signaling system, have been implicated in a variety of cellular functions. More than twenty heterotrimeric G protein α subunits have been identified in mammalian cells and classified into four subfamilies, $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$, depending on their amino acid sequence homology [15, 31]. Among them, $G\alpha_{16}$ subunit, a member of $G\alpha_q$ subfamily, is primarily expressed in hematopoietic cells, therefore, it is suggested to regulate hematopoiesis [3]. The $G\alpha_{16}$ subunit can functionally couple to various seven transmembrane receptors [27]. Especially, interaction of $G\alpha_{16}$ with the C5a, fMLP, and IL-8 receptors that are expressed in cells of hematopoietic lineage and mediate chemotactic responses in leukocytes, could play an important role in inflammatory and immune responses, when stimulated with any of the corresponding ligand, respectively [1, 2, 33].

It is, therefore, feasible to target the receptor-G protein interface with a low molecular weight compound. The molecules that can block these domains are defined as G protein antagonists, and this produces effects that are not achieved by receptor antagonists [10]. G protein antagonist might impede the interaction of a given receptor with only one type of G protein oligomer, thus inhibiting only one signaling pathway that is normally regulated by the receptor while other pathways are uninterrupted [7, 11]. Peptides derived from the G protein α subunits are likely to be antagonists, provided that they uncouple receptors and G proteins [12]. This can be achieved with peptides that encompass the residues contacted by the receptor.

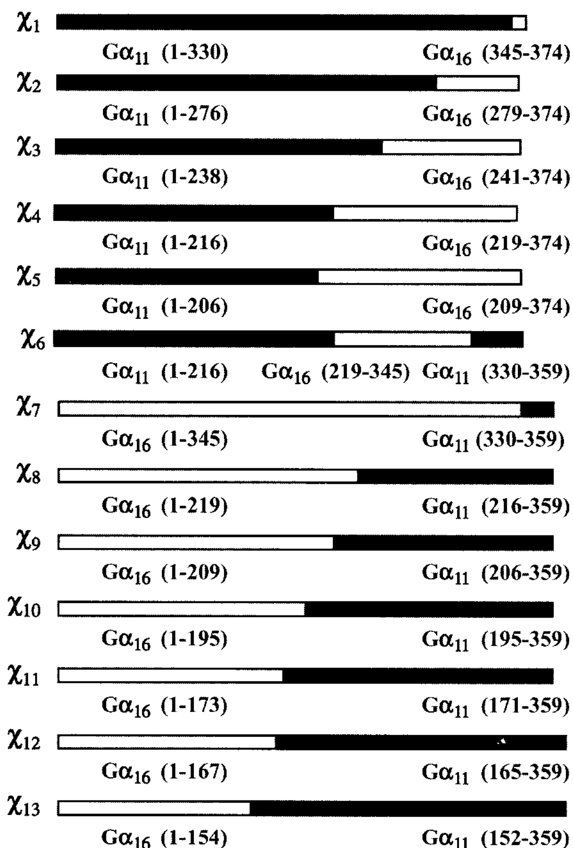

Fig. 1. Construction of the recombinant $G\alpha$ subunit chimeras.
cDNAs encoding a series of chimeric G proteins (χ_1-χ_{13}), composed of $G\alpha_{11}$ and $G\alpha_{16}$ subunits, were constructed as described in Materials and Methods. The cDNAs were carried by the cytomegalovirus vector pCMV to be expressed in Cos-7 cells.

In the present study, to provide unique segment(s) for designing a G protein antagonist that can block a variety of inflammatory and immune functions via activation of C5a, IL-8, and fMLP recptors, the structural elements on $G\alpha_{16}$ that are responsible for interacting with chemoattractant C5a, IL-8, and fMLP receptors were characterized by analyzing a gain or loss of function of a series of chimeric G proteins, composed of $G\alpha_{11}$ and $G\alpha_{16}$ subunit, in the interaction with each ligand-activated receptors. For this, Cos-7 cells were transiently transfected with specific cDNA clones encoding C5a, fMLP, and IL-8 receptors, and various chimeric G proteins, and ligand-specific activation was then determined by measuring changes in the levels of inositol phosphates that are the products of phosphatidylinositol hydrolysis.

It has been postulated that the region extending from the N-terminus to residue 206 of $G\alpha_{16}$ is sufficient to endow the chimera with much of the specificity for C5a-induced activation [21]. In order to further characterize the regions of $G\alpha_{16}$ protein responsible for the functional interaction with the C5a receptor, a new series of chimeric G protein cDNA constructs, such as χ_{10}, χ_{11}, χ_{12}, and χ_{13}, in which the C-terminal 179, 201, 207, and 220 residues of $G\alpha_{16}$ were substituted with equivalent portions of $G\alpha_{11}$, respectively, were prepared and the receptor coupling activity was then tested.

Control experiments were carried out to show the functional integrity of the overproduced chimeric proteins,

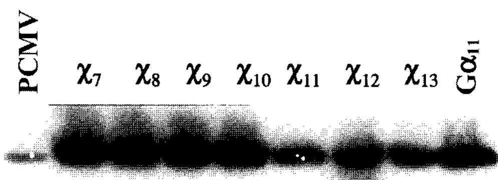

Fig. 2. Expression of various chimeric G proteins in Cos-7 cells.
Membranes of Cos-7 cells transfected with cDNAs, encoding pCMV, $G\alpha_{11}$, and various chimeras χ_7-χ_{13}, were prepared in 45 µl of RIPA buffer, followed by boiling, and equal amounts of samples were then subjected to SDS-gel electrophoresis and Western blotting, as described in Materials and Methods. The chimeric G proteins were detected with antibodies raised against the carboxyl-terminal sequence (QLNLKEYNLV) of $G\alpha_{11}$.

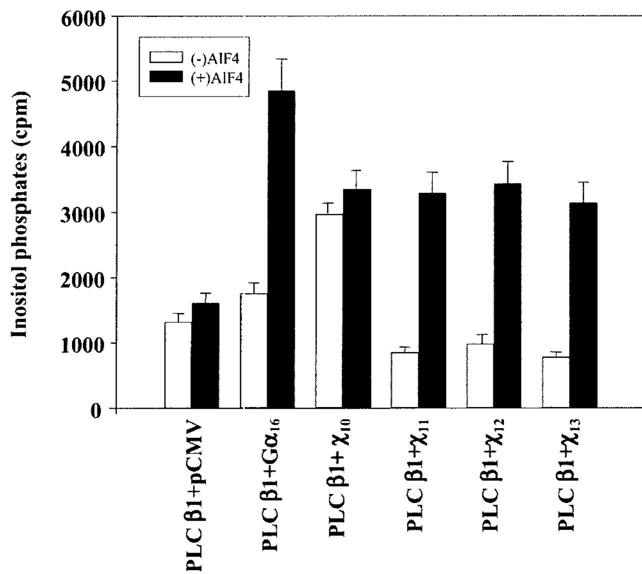

Fig. 3. Activities of χ_{10}, χ_{11}, χ_{12}, and χ_{13}.
AlF_4^--induced accumulation of [^{3}H]inositol phosphate in Cos-7 cells transfected with cDNAs, encoding phospholipase C-β1 together with pCMV, $G\alpha_{16}$, χ_{10}, χ_{11}, χ_{12}, and χ_{13}, were measured. Transfected Cos-7 cells were washed once with inositol-free DMEM and then stimulated with water (white bars) or AlF_4^- (30 μM $AlCl_3$ plus 10 mM NaF) (black bars) for 15 min at 37°C in 200 μl of inositol-free DMEM containing 10 mM LiCl.

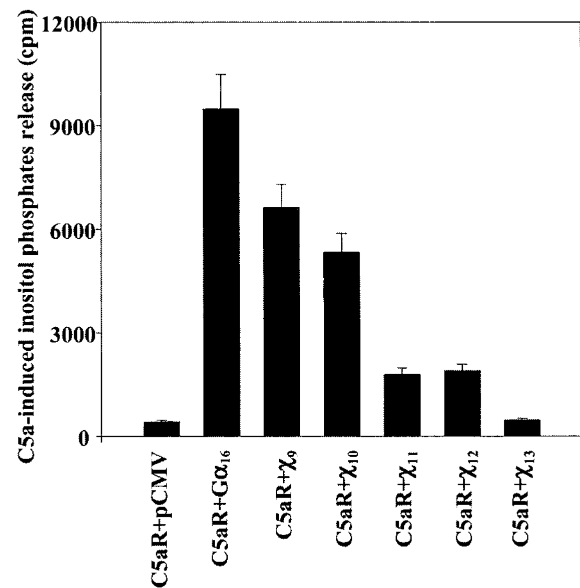

Fig. 4. Interaction of C5a receptor with a series of chimeric G proteins.
Cos-7 cells transfected with cDNAs, encoding pCMV, $G\alpha_{16}$, χ_9, χ_{10}, χ_{11}, χ_{12}, and χ_{13}, were washed once with inositol-free DMEM and then stimulated with 100 nM recombinant C5a for 15 min at 37°C in 200 μl of inositol-free DMEM containing 10 mM LiCl. Background counts in the absence of C5a were subtracted, and C5a-dependent release of [^{3}H]inositol phosphate was then calculated.

which was done by stimulating the Cos-7 cells that expressed chimeric G proteins and phospholipase C-β1 with AlF_4^-. Figure 3 shows the accumulation of inositol phosphates released after the addition of AlF_4^- in cells transfected with constructs which expressed χ_{10}, χ_{11}, χ_{12}, and χ_{13} as well as $G\alpha_{16}$ and pCMV. All chimeras, except χ_{10}, were properly activated by AlF_4^- and they were as active as $G\alpha_{16}$. Although the χ_{10} was not activated by AlF_4^- it was still able to couple to and activate phospholipase C-β1, thus releasing [^{3}H]inositol phosphate. Apparently, the χ_{10} behaves like an activated form of Gα with impaired GTPase activity, and its property remained established. As seen in Fig. 4, the χ_9, which encodes the first 209 residues of $G\alpha_{16}$ and C-terminal 165 residues of $G\alpha_{16}$ substituted with equivalent portion of $G\alpha_{11}$, showed about 70% of the receptor coupling capacity of $G\alpha_{16}$. Further replacement of $G\alpha_{16}$ subunit sequences with longer portions of $G\alpha_{11}$, such as in χ_{10}, χ_{11}, χ_{12}, and χ_{13}, led to further loss of the ability of the chimeras to interact with C5a receptor. The receptor coupling capacity of χ_{11} decreased dramatically, as compared with χ_{10}, and the χ_{13} chimeric G protein further lost its C5a receptor coupling capacity. Considering the level of expression of these chimeric proteins, short stretches of $G\alpha_{16}$ extending from residues 154 to residue 167 and residues from residue 174 to residue 195 appear to be necessary for endowing the ability to couple to the C5a receptor. The chimera χ_{12}, that lost an additional 6 amino acids of $G\alpha_{16}$, showed the same activity as seen with χ_{11}.

The chemoattractant C5a, IL-8, and fMLP receptors share significant sequence homology with each other, and their regulation of phospholipase C activity via $G\alpha_{16}$ protein has well been characterized. Therefore, a series of chimeras between $G\alpha_{11}$ and $G\alpha_{16}$ were also examined for their ability to interact with the IL-8 and fMLP receptors in order to find out if there are any common structural elements on $G\alpha_{16}$ that are involved in interacting with the chemoattractant receptors. χ_7, χ_8, and χ_9 depicted in Fig. 1 are chimeras in which the C-terminal 30, 155, and 165 residues of $G\alpha_{16}$, respectively, were substituted with equivalent portions of $G\alpha_{11}$, and these chimeras showed about 40–65% coupling to the C5a receptor [21]. However, as seen in Fig. 5, the chimeras χ_7, χ_8, and χ_9 still maintained almost 70–80% of the IL-8 receptor-coupling capacity of $G\alpha_{16}$, and the reduction in receptor coupling efficiency might perhaps be due to the removal of C-terminal segment of $G\alpha_{16}$. When additional 14 amino acids of $G\alpha_{16}$ subunit sequences were replaced with longer portions of $G\alpha_{11}$ as in χ_{10}, there was no further loss of the ability of the chimera to couple to the IL-8 receptor. When 220 amino acid residues of C-terminal of $G\alpha_{16}$ were replaced with homologous portion of $G\alpha_{11}$, as in χ_{13}, almost 80% of the ability of $G\alpha_{16}$ to couple to the IL-8 receptor were decreased (Fig. 5). This indicates that a stretch from the residue 155 to residue 195 is responsible for interacting with IL-8 receptor.

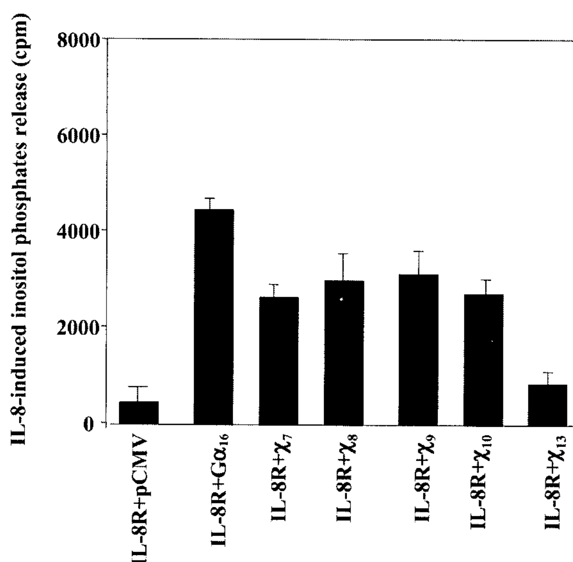

Fig. 5. Interaction of IL-8 receptor with a series of chimeric proteins.

Cos-7 cells transfected with cDNAs, encoding pCMV, χ_7, χ_8, χ_9, χ_{10}, χ_{13}, and $G\alpha_{16}$, were washed once with inositol-free DMEM and then stimulated with 100 nM recombinant IL-8 for 15 min at 37°C in 200 µl of inositol-free DMEM containing 10 mM LiCl. Background counts in the absence of IL-8 were subtracted, and IL-8-dependent release of [^{3}H]inositol phosphate was then calculated.

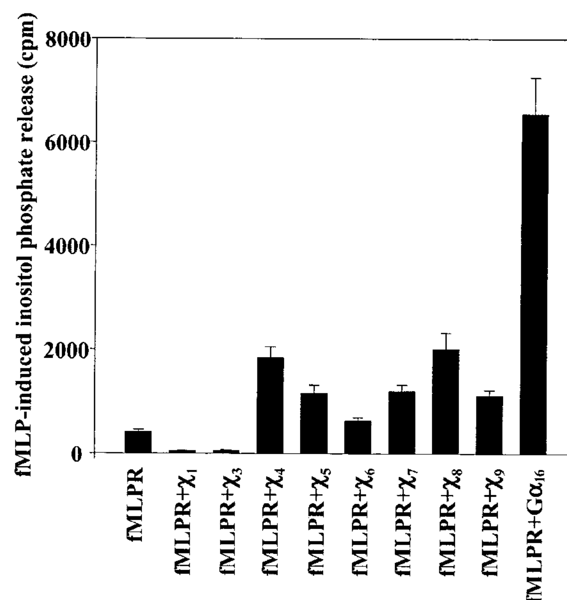

Fig. 6. Interaction of fMLP receptor with a series of chimeric proteins.

Cos-7 cells cotransfected with cDNAs, encoding fMLP receptor together with chimeras χ_1, χ_3, χ_4, χ_5, χ_6, χ_7, χ_8, χ_9, and $G\alpha_{16}$, were washed once with inositol-free DMEM and then stimulated with 500 nM recombinant fMLP for 15 min at 37°C in 200 µl of inositol-free DMEM containing 10 mM LiCl. Background counts in the absence of fMLP were subtracted, and fMLP-dependent release of [^{3}H]inositol phosphate was then calculated.

A coupling profile of a variety of chimeras, composed of $G\alpha_{11}$ and $G\alpha_{16}$, to fMLP receptor is shown in Fig. 6. Application of fMLP to the cells expressing fMLP receptor, but not together with $G\alpha_{16}$, did not induce inositol phosphate production. Chimeras χ_1 and χ_3 did not elicit fMLP-induced inositol phosphate production. However, χ_4 restored about 25- 30% of $G\alpha_{16}$ ability to couple to fMLP receptor. The chimera χ_5, which contains an additional 10 amino acids of $G\alpha_{16}$ (residues 209- 374) showed slightly less activity than χ_4. The chimera χ_6, which was prepared by substituting C-terminal 30 residues of χ_4 with a homologous stretch of $G\alpha_{11}$, completely lost its functional coupling to the fMLP receptor. These findings indicate that a segment of residues 220- 240 as well as C-terminal 30 residues are necessary, but not sufficient to restore interaction with the fMLP receptor. The reciprocal series of chimeras, including χ_7, χ_8, and χ_9, lost about 70% of coupling ability of $G\alpha_{16}$ to fMLP receptor. This implies that the C-terminal 30 amino acids are most critical for the coupling of $G\alpha_{16}$ to fMLP receptor.

It has been suggested that G protein interacts with receptor via multiple interaction sites. We have demonstrated that a series of chimeras composed of $G\alpha_{16}$ and $G\alpha_{11}$ lost or gained the ability to interact with chemoattractant receptors, suggesting that sequences encompassing the residues 154- 167 and residues 174- 195 were necessary for the efficient coupling to C5a, and the residues 155- 195 as well as C-terminal 30 amino acids of $G\alpha_{16}$ were necessary for the efficient coupling to IL-8 receptor and fMLP receptor, respectively. Besides their distinctive interaction with specific receptor proteins, these regions of $G\alpha_{16}$ propagate or stabilize conformational changes induced by receptor interaction. As shown in our results, the C-terminal region of $G\alpha_{16}$ that is known to be involved in direct interaction with receptor protein plays an essential role in determining interaction specificity with chemoattractant receptors. However, the extent of its contribution to the receptor coupling specificity varies, depending on the types of receptor with which $G\alpha_{16}$ subunit interacts. In addition to the C-terminal segment of $G\alpha_{16}$, a segment encompassing the residues 174- 195 and a segment of the residues 155- 195 of $G\alpha_{16}$, that includes the region of switch I domain, are necessary for interaction with the C5a receptor and IL-8 receptor, respectively. Moreover, a segment 155- 195 includes a part of the α-helical domain (αE and αF) that is known to function as GTPase activating protein [23] or effector coupling [22]. Collectively, these sequences are involved in the global conformational changes in Gα subunit that lead to nucleotide binding and hydrolysis.

As reported previously [21], however, the segment 220- 240 of $G\alpha_{16}$ that was required to recover specificity for the C5a receptor does not appear to be necessary for coupling of IL-8 receptor. Considering the fact that C5a receptor and IL-8 receptor share significant sequence

homology, this is an interesting observation. The region homologous to the residues 220-240 is located between the switch II and switch III domains. Perhaps, this region is required for the ligand-induced conformational change that occurs during nucleotide exchange. These data led us to suggest that $G\alpha_{16}$ subunit recruits multiple and distinctive regions, depending on the type of the receptor with which it interacts. When the three-dimensional structure of G protein-C5a, IL-8, and fMLP receptor complexes become available, the precise domains required for interaction of G protein with these receptors are expected to be elucidated.

Taken together, the results will provide the molecular basis to elucidate the interaction of chemoattractant receptors with $G\alpha_{16}$ subunit, thereby possibly contributing to finding novel targets for designing a G protein antagonist with anti-inflammatory effect that can inhibit the specific interaction of chemoattractant receptors with $G\alpha_{16}$.

Acknowledgment

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ2-PG3-21501-0001).

REFERENCES

1. Amatruda, 3rd T. T., S. Dragas-Graonic, R. Holmes, and H. D. Perez. 1995. Signal transduction by the formyl peptide receptor. Studies using chimeric receptors and site-directed mutagenesis define a novel domain for interaction with G-proteins. *J. Biol. Chem.* **270:** 28010-28013.
2. Amatruda, 3rd T. T., N. P. Gerard, C. Gerard, and M. I. Simon. 1993. Specific interactions of chemoattractant factor receptors with G-proteins. *J. Biol. Chem.* **268:** 10139-10144.
3. Amatruda, 3rd T. T., D. A. Steele, V. Z. Slepak, and M. I. Simon. 1991. $G\alpha_{16}$, a G protein α subunit specifically expressed in hematopoietic cells. *Proc. Natl. Acad. Sci. USA* **88:** 5587-5591.
4. Beckmann, M. P., W. E. Munger, C. Kozlosky, T. VandenBos, V. Price, S. Lyman, N. P. Gerard, C. Gerard, and D. P. Cerretti. 1991. Molecular characterization of the interleukin-8 receptor. *Biochem. Biophys. Res. Commun.* **179:** 784-789.
5. Buhl, A. M., B. J. Eisfelder, G. S. Worthen, G. L. Johnson, and M. Russell. 1993. Selective coupling of the human anaphylatoxin C5a receptor and G alpha 16 in human kidney 293 cells. *FEBS. Lett.* **323:** 132-134.
6. Cabrera-Vera, T. M., J. Vanhauwe, T. O. Thomas, M. Medkova, A. Preininger, M. R. Mazzoni, and H. E. Hamm. 2003. Insights into G protein structure, function, and regulation. *Endocr. Rev.* **24:** 765-781.
7. Chang, M., L. Zhang, J. P. Tam, and E. Sanders-Bush. 2000. Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-HT(2C) receptors in choroid plexus epithelial cells. *J. Biol. Chem.* **275:** 7021-7029.
8. Conklin, B. R., Z. Frafel, K. D. Lustig, Julius, and H. R. Bourne. 1993. Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. *Nature* **363:** 274-276.
9. Dratz, E. A., J. E. Furstenau, C. G. Lambert, D. L. Thireault, H. Rarick, T. Schepers, S. Pakhlevaniants, and H. E. Hamm. 1993. NMR structure of a receptor-bound G-protein peptide. *Nature* **363:** 276-281.
10. Freissmuth, M., M. Waldhoer, E. Bofill-Cardona, and C. Nanoff. 1999. G protein antagonist. *Trends Pharmacol. Sci.* **20:** 237-245.
11. Gilchrist, A., J. F. Vanhauwe, A. Li, T. O. Thomas, T. Voyno-Yasenetskaya, and H. E. Hamm. 2001. G alpha minigenes expressing C-terminal peptides serve as specific inhibitors of thrombin-mediated endothelial activation. *J. Biol. Chem.* **276:** 25672-25679.
12. Gilchrist, A., M. R. Mazzoni, B. Dineen, A. Dice, J. Linden, W. R. Proctor, C. R. Lupica, T. V. Dunwiddie, and H. E. Hamm. 1998. Antagonists of the receptor-G protein interface block Gi-coupled signal transduction. *J. Biol. Chem.* **273:** 14912-14919.
13. Ha, J. H., N. Dhanasekaran, H. C. Koh, and C. H. Lee. 2000. Single amino acid of $G\alpha_{16}$ (Ala^{228}) is responsible for the ability of chemoattractant C5a receptor to induce $G\alpha_{16}$-mediated inositol phosphate release. *Biochem. Biophys. Res. Commun.* **278:** 426-431.
14. Hamm, H. E., D. Deretic, A. Arendt, T. A. Hargrave, B. Koenig, and K. P. Hofmann. 1988. Site of G protein binding to rhodopsin mapped with synthetic peptides from the alpha subunit. *Science* **241:** 832-835.
15. Hepler, J. R and A. G. Gilman. 1992. G proteins. *Trends Biochem. Sci.* **17:** 383-387.
16. Holmes, W. E., J. K. Lee, W. J. Kuang, G. C. Rice, and W. I. Wood. 1991. Structure and functional expression of a human interleukin-8 receptor. *Science* **253:** 1278-1280.
17. Horton, R. M., H. D. Hunt, S. N. Ho, J. K. Pullen, and L. R. Pease. 1989. Engineering hybrid genes without the use of restriction enzymes: Gene splicing by overlap extension. *Gene* **77:** 61-68.
18. Kosteinis, E., M. Y. Degtyarev, B. R. Conklin, and J. Wess. 1997. The N-terminal extension of Galpha q is critical for constraining the selectivity of receptor coupling. *J. Biol. Chem.* **272:** 19107-19110.
19. Krieger-Brauer, H. I., P. K. Medda, U. Hebling, and H. Kather. 1999. An antibody directed against residues 100-119 within the alpha-helical domain of Galpha(s) defines a novel contact site for beta-adrenergic receptors. *J. Biol. Chem.* **274:** 28308-28313.
20. Le, Y., B. Li, W. Gong, W. Shen, J. Hu, N. M. Dunlop, Oppenheim, and J. M. Wang. 2000. Novel pathophysiological role of classical chemotactic peptide receptors and their communications with chemokine receptors. *Immunol. Rev.* **177:** 185-194.
21. Lee, C. H., A. Katz, and M. I. Simon. 1995. Multiple regions of G alpha 16 contribute to the specificity of activation by the C5a receptor. *Mol. Pharmacol.* **47:** 218-223.

22. Liu, W. and J. K. Northup. 1998. The helical domain of a G protein alpha subunit is a regulator of its effector. *Proc. Natl. Acad. Sci. USA* **95:** 12878-12883.
23. Markby, D. W., R. Onrust, and H. R. Bourne. 1993. Separate GTP binding and GTPase activating domains of a G alpha subunit. *Science* **262:**1895-901.
24. Masters, S. B., K. A. Sullivan, R. T. Miller, B. Beiderman, N. G. Lopez, J. Ramachandran, and H. R. Bourne. 1988. Carboxyl terminal domain of Gs alpha specifies coupling of receptors to stimulation of adenylyl cyclase. *Science* **241:** 448-451.
25. Mazzoni, M. R., J. A. Malinski, and H. E. Hamm. 1991. Structural analysis of rod GTP-binding protein, Gt. Limited proteolytic digestion pattern of Gt with four proteases defines monoclonal antibody epitope. *J. Biol. Chem.* **25:** 14072-14081.
26. Murphy, P. M. and H. L. Tiffany. 1991. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* **253:** 1280-1283.
27. Offermanns, S. and M. I. Simon. 1995. G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C. *J. Biol. Chem.* **270:** 15175-15180.
28. Prossnitz, E. R. and R. D. Ye. 1997. The N-formyl peptide receptor: A model for the study of chemoattractant receptor structure and function. *Pharmacol. Ther.* **74:** 73-102.
29. Rossi, D. and A. Zlotnik. 2000. The biology of chemokines and their receptors. *Annu. Rev. Immunol.* **18:** 217-242.
30. Sullivan, K. A., R. T. Miller, S. B. Masters, B. Beiderman, W. Heideman, and H. R. Bourne. 1987. Identification of receptor contact site involved in receptor-G protein coupling. *Nature* **330:** 758-760.
31. Wilkie, T. M., D. J. Gilbert, A. S. Olsen, X. N. Chen, T. T. Amatruda, J. R. Korenberg, B. J. Trask, P. de Jong, R. R. Reed, and M. I. Simon. 1992. Evolution of the mammalian G protein alpha subunit multigene family. *Nat. Genet.* **1:** 85-91.
32. Wu, D., A. Katz, and M. I. Simon. 1993. Activation of phospholipase C beta 2 by the alpha and beta gamma subunits of trimeric GTP-binding protein. *Proc. Natl. Acad. Sci. USA* **90:** 5297-5301.
33. Wu, D., G. J. LaRosa, and M. I. Simon. 1993. G protein-coupled signal transduction pathways for interleukin-8. *Science* **261:** 101-103.