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Multiple Regions of $G_{\alpha 16}$ Contribute to the Specificity of Activation by the C5a Receptor

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SUMMARY

The C5a chemoattractant factor receptor, when expressed in COS-7 cells, can stimulate phosphoinositide-specific phospholipase C activity through the activation of the G_{16} isoform of the heterotrimeric G protein, but not through the G_{11} isoform. To identify the regions of the $G_{\alpha 16}$ subunit protein that are responsible for its activation by the C5a receptor, a series of chimeras between $G_{\alpha 16}$ and $G_{\alpha 11}$ were constructed and tested for their ability to be activated by the C5a receptor. Co-transfection experiments with chimeras in which the carboxyl-terminal regions of $G_{\alpha 11}$ were replaced with the corresponding regions of $G_{\alpha 16}$ demonstrated that changes in the carboxyl terminus, e.g., replacement of 134 amino acids, were not sufficient to confer receptor specificity. An additional segment encompassing residues 220–240 of $G_{\alpha 16}$ was required to confer C5a-induced activation. Testing of a reciprocal series of chimeras composed of $G_{\alpha 16}$ sequences at the amino terminus and $G_{\alpha 11}$ sequences at the carboxyl terminus revealed that certain sequences extending from the amino terminus to amino acid 209 of $G_{\alpha 16}$ were sufficient to endow the chimera with much of the specificity for C5a-induced activation. These results suggest that receptor specificity may involve specific conformations of the G protein stabilized by concerted interactions of multiple amino acid sequences distributed throughout the G_α protein.

Heterotrimeric G proteins couple the interaction of ligands with receptors to the subsequent regulation of a variety of intracellular effectors (reviewed in Refs. 1–4). The currently accepted model for the activation of G proteins suggests that ligand-activated receptor accelerates GDP/GTP exchange reactions on the α subunit of the heterotrimeric G protein. Seventeen different isoforms of the G_α subunit protein have been identified in mammalian organisms (5, 6). Different G proteins appear to interact preferentially with different receptors. Furthermore, the receptor may recognize the G protein as a heterotrimer, and the $\beta\gamma$ subunits can contribute to receptor specificity (7, 8). Nonetheless, the carboxyl-terminal 42 amino acids and the amino-terminal 34 amino acids of the G_α subunit have been implicated in determining the specificity of interaction with receptors (reviewed in Ref. 9). One of the most clearly defined regions of interaction is in the extreme carboxyl-terminal amino acid sequence of the G_α subunit. Several lines of evidence support this assignment. The *unc* mutation of $G_{\alpha s}$ corresponds to a change in an amino acid residue adjacent to the carboxyl terminus. The mutant was isolated from the S49 lymphoma cell line and cannot be activated by β-adrenergic receptor stimulation (10). Functional analysis of chimeric $G_{\alpha i}$/$G_{\alpha s}$ proteins showed that the carboxyl-terminal domain of $G_{\alpha s}$ specifies coupling of β-adrenergic receptors to the stimulation of adenylyl cyclase (11). In addition, the ADP-ribosylation-induced uncoupling of pertussis toxin-sensitive G_α subunits from their receptors also suggests that the carboxyl terminus of the G_α subunits, which is the region where the modification occurs, is involved in interactions with receptors (12). More recently, it was reported that substitution of three amino acids at the extreme carboxyl terminus of the G_α subunit is sufficient to switch receptor specificity from that of the $G_{\alpha q}$ class to that of $G_{\alpha i}$ (13). Studies on the interaction between rhodopsin and transducin have demonstrated that the amino terminus and the "G5 region" of the α subunit, which is located adjacent to the carboxyl terminus, are important in determining receptor contact. The prevention of the interaction of transducin with rhodopsin by synthetic carboxyl-terminal peptides and monoclonal antibodies specific for the amino and carboxyl termini of α-transducin suggests that the amino terminus of the G_α subunit, as well as its carboxyl terminus, may constitute structural elements required for interaction with specific receptors (14, 15). In a recent study it was shown that the activated form of rhodopsin, i.e., metarhodopsin II, induces a conformational change in the α-transducin carboxyl-terminal decapeptide, further supporting the argument for the involvement of the extreme carboxyl terminus of the G_α subunit in interaction with the receptor (16).

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ABBREVIATIONS: PI-PLC, phosphoinositide-specific phospholipase C; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; CMV, cytomegalovirus; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate.

Co-transfection of COS-7 cells has been used to assess the functional roles of specific receptors, G_{α}, $G_{\beta\gamma}$, and effectors (17–19). Cells are transiently transfected with specific cDNA clones encoding receptors, G proteins, or effectors, and ligand-specific activation can be determined by measuring changes in the levels of second messengers. Using these reconstitution methods, it was reported that the chemoattractant factor C5a receptor interacts specifically with $G_{\alpha 16}$, which is found primarily in cells of the hematopoietic lineage (20), but not with $G_{\alpha 11}$ (21). On the other hand, both $G_{\alpha 11}$ and $G_{\alpha 16}$ are capable of activating PI-PLC isoforms present in COS-7 cells (17, 22). Receptor selectivity in the absence of differential effector specificity allowed us to construct a series of chimeras between $G_{\alpha 16}$ and $G_{\alpha 11}$ and to examine their abilities to couple to the C5a receptor.

Experimental Procedures

Transient transfection and labeling of COS-7 cells. COS-7 cells were cultured in DMEM containing 10% fetal calf serum. One day before transfection, 1×10^5 cells/well were seeded in 12-well plates. One microgram of recombinant expression plasmids was mixed with 5 μl of Lipofectin (GIBCO-BRL) in 0.5 ml of Opti-MEM (GIBCO-BRL) and added to the cells in each well. Fetal calf serum (20%) in 0.5 ml of DMEM was added to each well 5 hr later. In co-transfection experiments, the total amount of plasmid DNA was kept constant (1 μg) even when a variety of different plasmids were used. One day after transfection, the medium was removed and the cells were washed with PBS and then labeled by incubation for 20–24 hr with 10 μCi/ml *myo*-[2-^{3}H]inositol (NEN) in 0.5 ml of inositol-free DMEM with 10% dialyzed fetal calf serum.

Analysis of ligand-induced inositol phosphate levels in intact COS-7 cells. Labeled COS-7 cells were washed once with PBS and then incubated in 200 μl of inositol-free DMEM containing 10 mM LiCl, in the absence or presence of stimulants [recombinant human C5a (Sigma) or 30 μM $AlCl_3$ plus 10 mM NaF], for 25 min at 37°. The 25-min time point was chosen because in kinetic experiments the C5a-induced release of [^{3}H]inositol phosphates was found to be linear with time for >25 min. Reactions were stopped by placing cells on ice and adding 200 μl of 10% perchloric acid. The medium was transferred to a microfuge tube and neutralized with 2 M KOH and 1 mM EDTA in the presence of pH indicator (pH-Hydrion). Total [^{3}H]inositol phosphates were then separated by anion exchange chromatography and the radioactivity of [^{3}H]inositol phosphates was counted with a liquid scintillation counter as described (23).

SDS-polyacrylamide gel electrophoresis and Western blotting. Transfected cells were washed, stimulated, and quenched as described above. The remaining cell pellets were collected in 50 μl of PBS and equal volumes of 2× SDS sample buffer were added, followed by boiling and SDS-gel electrophoresis. In some cases, the transfected COS-7 cells were homogenized and fractionated and their membranes were washed with 1 M KCl as described previously (17). The washed membranes, suspended in SDS sample buffer, were then boiled and subjected to electrophoresis. The gels were then electrotransferred onto nitrocellulose membranes. The chimeric proteins were detected with antibodies raised against the carboxyl-terminal sequence of $G_{\alpha 11}$ or $G_{\alpha 16}$, as described previously (17, 24).

Construction of recombinant G_{α} subunit chimeras. The G_{α} subunit chimeras between the $G_{\alpha 11}$ and $G_{\alpha 16}$ subunits were constructed using both pre-existing and created restriction sites located at equivalent positions in the G_{α} subunit cDNAs. χ1, χ4, χ6, χ7, and χ8 were constructed using the conserved *Bam*HI and *Afl*III restriction endonuclease sites located at equivalent positions in the $G_{\alpha 11}$ and $G_{\alpha 16}$ subunits. The construction of other chimeras was achieved as follows. For each of the chimeras, a pair of polymerase chain reaction fragments encoding various portions of $G_{\alpha 11}$ or $G_{\alpha 16}$ were designed by introducing silent mutations to create common restriction endonuclease sites, such as *Dra*I (χ2), *Bcl*I (χ3), *Aat*II (χ5 and χ9), *Eco*RI (χ10), *Bst*EII (χ11), and *Sal*I (χ12), at the junction positions. Each pair of polymerase chain reaction fragments were digested with corresponding restriction enzymes together with *Cla*I or *Not*I and were ligated to pCMV through *Cla*I and *Not*I restriction sites. All of the G_{α} subunit chimera constructs were verified by restriction enzyme analysis and DNA sequencing.

Results

Transient transfection of cDNAs into COS-7 cells was used to test the coupling of the $G_{\alpha 16}$ subunit to the C5a receptor. Application of the C5a ligand to COS-7 cells does not stimulate release of inositol phosphates, due to the absence of endogenous C5a receptors (25). However, COS-7 cells co-transfected with C5a receptor and $G_{\alpha 16}$ cDNAs showed a time-dependent, ligand-induced release of inositol phosphates in response to high levels of C5a (Fig. 1A). Fig. 1B shows that addition of C5a induced a concentration-dependent increase in inositol phosphate production in COS-7 cells co-transfected with the C5a receptor and $G_{\alpha 16}$ cDNAs. This interaction was specific, because co-transfection of the receptor with $G_{\alpha 11}$ or CMV did not produce ligand-dependent increases in inositol phosphate production. Half-maximal activity of the C5a-induced response was found at a concentration of approximately 1 nM, which is close to the level found to stimulate neutrophils (26).

A reciprocal series of chimeric $G_{\alpha 16}$ and $G_{\alpha 11}$ cDNA constructs were prepared to identify functional domains of $G_{\alpha 16}$ necessary for coupling to the C5a receptor. The ability of the chimeric $G_{\alpha 16}$ and $G_{\alpha 11}$ constructs to mediate C5a-induced release of inositol phosphates in co-transfected COS-7 cells was tested. Initially, we tested a series of chimeric constructs made of $G_{\alpha 11}$ at the amino terminus and $G_{\alpha 16}$ at the carboxyl terminus. The structures and the C5a-induced activities of $G_{\alpha 11}/G_{\alpha 16}$ chimeras are shown in Fig. 2. Because the expression levels of the

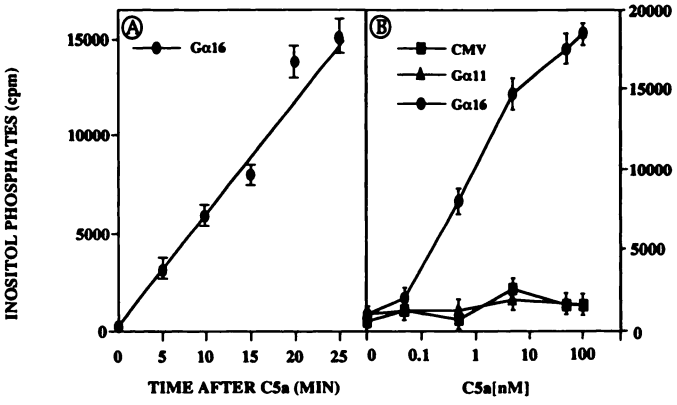

Fig. 1. Time course and dose-response curves for C5a-induced accumulation of [^{3}H]inositol phosphates. COS-7 cells were transiently co-transfected with cDNA encoding the human C5a receptor and cDNAs encoding CMV (■), $G_{\alpha 11}$ (▲), or $G_{\alpha 16}$ (●). A, Transfected cells were stimulated with C5a (100 nM) and the C5a-induced release of [^{3}H]inositol phosphates was measured at increasing time points. Background counts (2500 cpm for 0 min and 5000 cpm for 25 min) were subtracted. B, Transfected cells were stimulated with various concentrations of human recombinant C5a (range, 0–100 nM) and the C5a-dependent release of [^{3}H]inositol phosphates was measured. Background counts of 5000 cpm at 0 nM C5a were subtracted. In other experiments, saturation of the data was seen at levels between 75 and 200 nM C5a. Transfection, labeling, stimulation, and analysis of [^{3}H]inositol phosphates were as described in Experimental Procedures. Data shown are mean values of duplicate observations in a single representative experiment, and the variation is <10% of mean values.

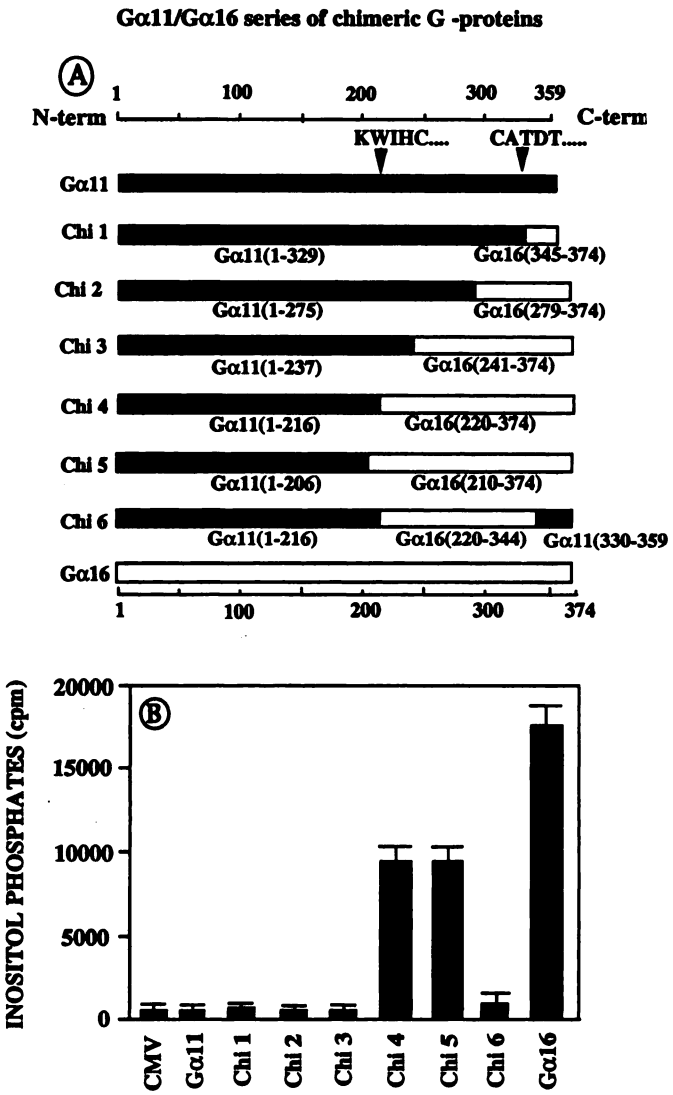

Fig. 2. Structures and C5a-induced release of [^{3}H]inositol phosphates for the $G_{\alpha 11}/G_{\alpha 16}$ series of chimeras. A, Linear structures of $G_{\alpha 11}/G_{\alpha 16}$ chimeras. The construction of the G_α chimeras is described in Experimental Procedures. B, C5a-induced release of inositol phosphates in COS-7 cells transiently co-transfected with cDNA encoding the human C5a receptor and cDNAs encoding CMV, $G_{\alpha 11}$, $G_{\alpha 16}$, or $\chi 1$–$\chi 6$. Labeled COS-7 cells were washed and incubated in 200 μl of inositol-free DMEM containing 10 mM LiCl, in the absence or presence of 100 nM human recombinant C5a. Transfection, labeling, and analysis of [^{3}H]inositol phosphates were as described in Experimental Procedures. Data shown are mean values of duplicate determinations in a single representative experiment, and the variation is <10% of mean values.

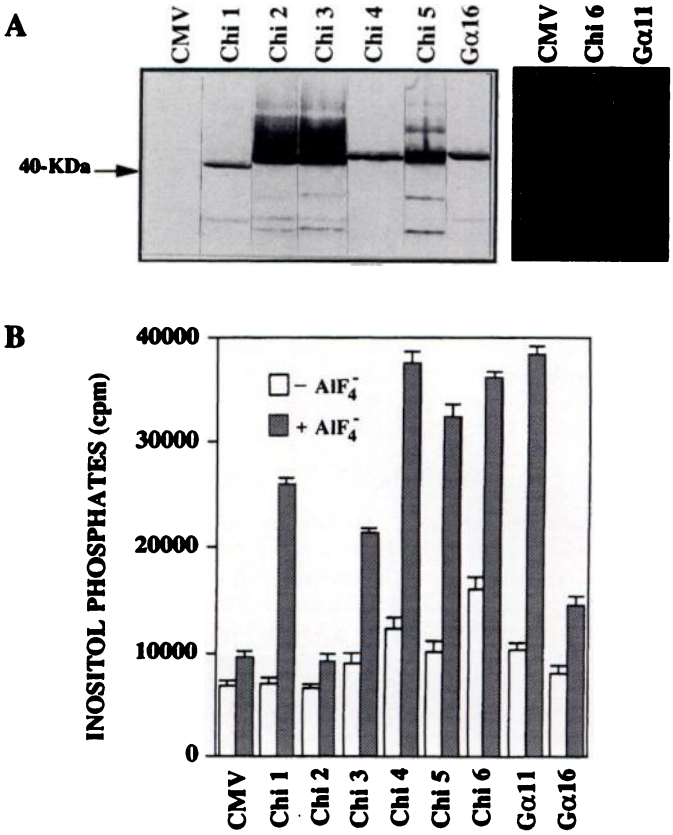

Fig. 3. Expression and AlF_4^--dependent activation of transfected $G_{\alpha 11}$, $G_{\alpha 16}$, and the $G_{\alpha 11}/G_{\alpha 16}$ series of chimeras. A, Western blot analysis of transfected COS-7 cells. Cells transfected with cDNAs encoding CMV, $G_{\alpha 16}$, or $\chi 1$–$\chi 5$ were lysed in SDS sample buffer. Membranes of COS-7 cells transfected with CMV, $G_{\alpha 11}$, or $\chi 6$ cDNAs were prepared and washed as described in Experimental Procedures. All of the samples were then boiled, and equal amounts were subjected to electrophoresis and Western blotting. $G_{\alpha 16}$ and $\chi 1$–$\chi 5$ proteins were detected with antibodies raised against the carboxyl terminus of $G_{\alpha 16}$. Chimeras $\chi 2$ and $\chi 3$ were produced at relatively high levels. $G_{\alpha 11}$ and $\chi 6$ proteins were detected with antibodies raised against the carboxyl terminus of $G_{\alpha 11}$. Low levels of cross-reaction are seen with endogenous protein in the CMV control. The apparent doublets seen in some lanes are the result of changes in mobility of the chimeric proteins. B, AlF_4^--dependent accumulation of [^{3}H]inositol phosphates in transfected COS-7 cells. Transfected cells were washed and stimulated with water (□) or 30 μM $AlCl_3$ plus 10 mM NaF (▨), in the presence of 10 mM LiCl. Transfection, labeling, stimulation, and analysis of [^{3}H]inositol phosphates were as described in Experimental Procedures. Data shown are mean values of duplicate determinations in a single representative experiment, and the variation is <10% of mean values.

transfected G_α proteins can affect their coupling efficiency, we measured the expression of the transfected G_α subunit chimeras using polyclonal antibodies raised against peptide sequences that were specific for $G_{\alpha 11}$ and $G_{\alpha 16}$ subunits. Fig. 3A shows that overproduced proteins reacted strongly with the specific antibodies. Control cells transfected with CMV showed much less or no reaction with the antibody (COS-7 cells express a basal level of $G_{\alpha 11}$ and do not express the $G_{\alpha 16}$ protein) (20, 22). $G_{\alpha 11}$ and $G_{\alpha 16}$ cDNAs encode 41-kDa and 43.5-kDa proteins, respectively, and the hybrid proteins migrated between 41 kDa and 43.5 kDa, depending on the portion of the molecule that underwent homologous replacement. Fig. 3A demonstrates that the $G_{\alpha 11}/G_{\alpha 16}$ chimeric proteins $\chi 1$–$\chi 6$ were expressed at levels similar to or higher than those of their parental proteins. An additional control experiment was performed to evaluate the functional integrity of the overproduced chimeric proteins. This was done by stimulating COS-7 cells transfected with and expressing the α subunit chimeras with AlF_4^- (30 μM $AlCl_3$ plus 10 mM NaF), which is thought to activate the GDP-bound form of the α subunit of various heterotrimeric G proteins by stabilizing them in the GTP-bound form. These activated α subunits can in turn stimulate endogenous PI-PLC activity. The endogenous PI-PLC activity may be due to the activation of PLC-β_1 and -β_3, which can be detected in COS-7 cells (data not shown) and, as reported previously, can couple to all of the α subunits of the G_q class (17, 22, 24, 27). Fig. 3B shows the accumulation of inositol phosphates released, after the addition of AlF_4^-, in

cells transfected with constructs expressing $G_{\alpha 11}$, $G_{\alpha 16}$, and the chimeras. As reported previously (28), $G_{\alpha 16}$ was poorly activated by AlF_4^-, compared with $G_{\alpha 11}$. There was a <50% increase in activity even with wild-type $G_{\alpha 16}$, whereas there was a 4-fold increase found with cells transfected with $G_{\alpha 11}$. Thus, activation by AlF_4^- was not always useful for assessing the activity of all of the chimeras. Nevertheless, the results suggest that chimeras χ1, χ3, χ4, χ5, and χ6 are as active as wild-type G_α protein in terms of α subunit-effector interactions, whereas χ2 has little or no activity.

As seen in Fig. 2, chimera χ1, which encodes the first 329 residues of $G_{\alpha 11}$ and the last 30 residues of $G_{\alpha 16}$, did not elicit ligand-induced inositol phosphate production, although it was expressed and could be activated by AlF_4^- (Fig. 3). The chimera χ2 has the last 84 amino acids of $G_{\alpha 11}$ replaced with the carboxyl-terminal 96 residues of $G_{\alpha 16}$. The expression level of χ2 was high (Fig. 3A); however, this chimera did not show AlF_4^- stimulation or C5a-induced inositol phosphate release (Figs. 2 and 3B). The χ3 chimera, in which the last 122 residues of $G_{\alpha 11}$ were substituted with the corresponding 134 amino acids of $G_{\alpha 16}$, had the ability to increase the release of inositol phosphates when stimulated with AlF_4^- (Fig. 3B). However, this chimera did not show C5a-induced inositol phosphate release (Fig. 2). This indicates that the carboxyl-terminal 134 amino acids of the $G_{\alpha 16}$ polypeptide are not sufficient to restore the ability of the chimera to be activated by the C5a receptor. The chimera χ4, in which the distal 143-amino acid segment of the $G_{\alpha 11}$ polypeptide was replaced with the corresponding 155 residues of $G_{\alpha 16}$, gained about 65% of the ability of $G_{\alpha 16}$ to liberate inositol phosphates in response to C5a stimulation. This observation implies that a short stretch of the $G_{\alpha 16}$ polypeptide extending from residue 220 to residue 240 is necessary for restoration of the ability to couple to the C5a receptor. The chimera χ5, which contains an additional 10 amino acids of $G_{\alpha 16}$ (residues 210–374), showed the same activity as found with χ4 (Fig. 2). Substitution of the carboxyl-terminal 30 residues of χ4 with the homologous stretch of $G_{\alpha 11}$ generated a polypeptide (χ6) that lost its functional coupling to the C5a receptor, although it was expressed and could be activated by AlF_4^- (Fig. 3). These findings imply that a contribution from the carboxyl-terminal 30 amino acid residues of $G_{\alpha 16}$ is necessary but not sufficient to restore interaction with the C5a receptor. Thus, we suggest that the short segment from residue 220 to residue 240 and the carboxyl-terminal 30-amino acid residue region of $G_{\alpha 16}$ each provide some of the specificity required for the coupling with the C5a receptor.

The reciprocal series of chimeras including $G_{\alpha 16}/G_{\alpha 11}$ was constructed and tested to further characterize the regions involved in the functional interaction of the $G_{\alpha 16}$ protein with the C5a receptor. Fig. 4 shows the structures and the receptor-coupling abilities of χ7–χ12. Again, we initially measured the levels of expression of the chimeric proteins and, as seen in Fig. 5A, χ7–χ12 were all expressed at levels similar to those of their parental G_α subunits. We also determined their potential to be activated with AlF_4^-. Fig. 5B shows that χ10, χ11, and χ12 had little or no activity, whereas chimeras χ7, χ8, and χ9 were at least as active as wild-type $G_{\alpha 16}$ protein and were able to activate endogenous PI-PLC. χ7, χ8, and χ9 are chimeras in which the carboxyl-terminal 30, 155, and 165 residues, respectively, of $G_{\alpha 16}$ are substituted with the equivalent portions of $G_{\alpha 11}$. The χ7 chimera, in which the carboxyl-terminal 30 residues of $G_{\alpha 16}$ were replaced with the homologous segment of $G_{\alpha 11}$, lost almost 35% of the receptor-coupling capacity of $G_{\alpha 16}$, although this chimera maintained a degree of AlF_4^--induced PI-PLC-stimulating activity similar to that of the $G_{\alpha 16}$ subunit (Fig. 5B). The further replacement of $G_{\alpha 16}$ subunit sequences with longer portions of $G_{\alpha 11}$, as in χ8 and χ9, led to further loss of the ability of the generated chimeras to couple to the C5a receptor. The χ9 construct showed 40% coupling to the C5a receptor; it lacks the carboxyl-terminal residues contributed by $G_{\alpha 16}$ and retains only the amino-terminal 209 residues of $G_{\alpha 16}$. These findings indicate that there are regions in both the amino-terminal part and the carboxyl-terminal part of $G_{\alpha 16}$ that contribute to specific activation by the C5a receptor. Although the maximal levels of activity found with χ7 and χ8 were markedly decreased, the level of the C5a ligand required

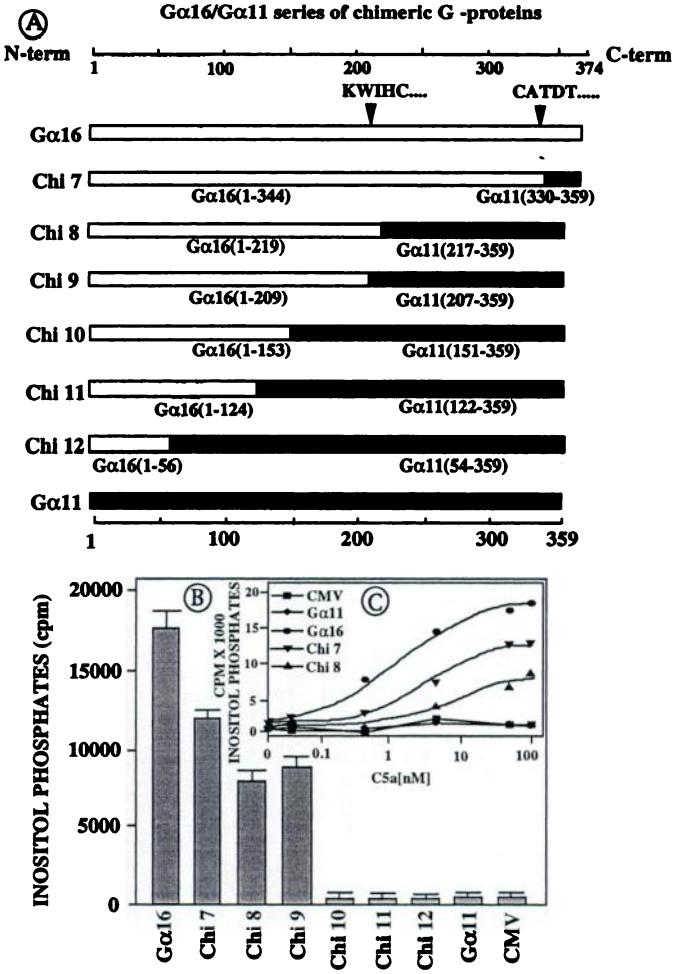

Fig. 4. Structures and C5a-induced release of [^{3}H]inositol phosphates for the $G_{\alpha 16}/G_{\alpha 11}$ series of chimeras. A, Linear structures of $G_{\alpha 16}/G_{\alpha 11}$ chimeras. The construction of the G_α chimeras is described in Experimental Procedures. B, C5a-induced release of inositol phosphates in COS-7 cells transiently co-transfected with cDNA encoding the human C5a receptor and cDNAs encoding CMV, $G_{\alpha 11}$, $G_{\alpha 16}$, or χ7–χ12. Labeled COS-7 cells were washed and incubated in 200 μl of inositol-free DMEM containing 10 mM LiCl, in the absence or presence of 100 nM human recombinant C5a. C, Dose-response curves for C5a-induced accumulation of [^{3}H]inositol phosphates in labeled COS-7 cells co-transfected with cDNA encoding the human C5a receptor and cDNAs encoding CMV, $G_{\alpha 11}$, $G_{\alpha 16}$, χ7, or χ8. Transfection, labeling, and analysis of [^{3}H]inositol phosphates were as described in Experimental Procedures. Data shown are mean values of duplicate observations in a single representative experiment, and the variation is <10% of mean values.

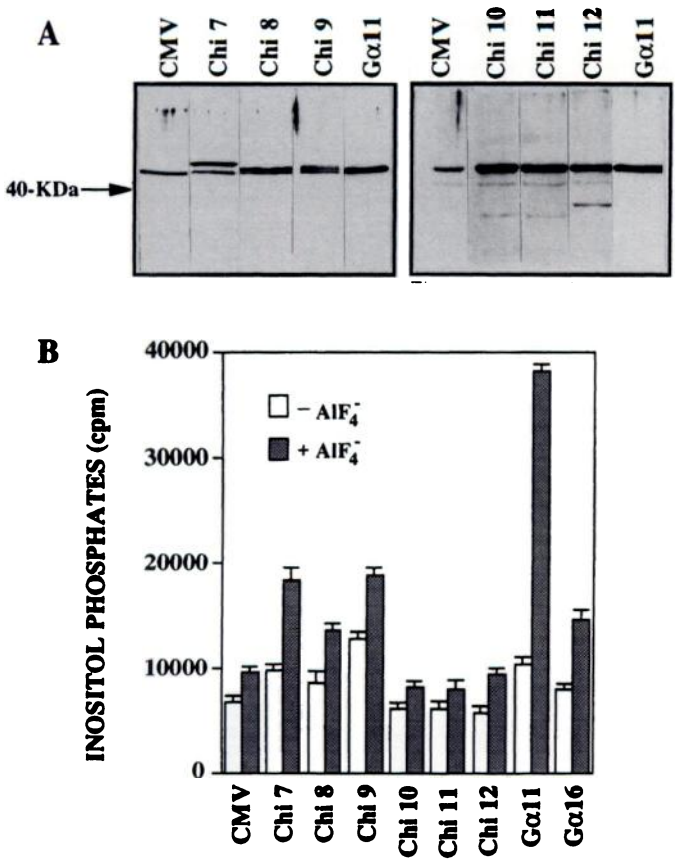

Fig. 5. Expression and AlF_4^--dependent activation of transfected $G_{\alpha 11}$, $G_{\alpha 16}$, and the $G_{\alpha 16}/G_{\alpha 11}$ series of chimeras. A, Western blot analysis of transfected COS-7 cells. Cells transfected with cDNAs encoding CMV, $G_{\alpha 11}$, or $\chi 10$–$\chi 12$ were lysed in SDS sample buffer. Membranes of COS-7 cells transfected with CMV, $G_{\alpha 11}$, or $\chi 7$–$\chi 9$ cDNAs were prepared and washed as described in Experimental Procedures. All of the samples were then boiled, and equal amounts were subjected to electrophoresis and Western blotting. $G_{\alpha 11}$ and $\chi 7$–$\chi 12$ proteins were detected with antibodies raised against the carboxyl terminus of $G_{\alpha 11}$. B, AlF_4^--dependent accumulation of [^{3}H]inositol phosphates in transfected COS-7 cells. Transfected cells were washed and stimulated with water (□) or 30 μM $AlCl_3$ plus 10 mM NaF (⊠), in the presence of 10 mM LiCl. Transfection, labeling, stimulation, and analysis of [^{3}H]inositol phosphates were as described in Experimental Procedures. Data shown are mean values of duplicate determinations in a single representative experiment, and the variation is <10% of mean values.

to induce half-maximal activity was only 3–4-fold higher with these two constructs than that found with $G_{\alpha 16}$ (Fig. 4C). This supports the notion that it is a change in the ability of the ligand-bound receptor to activate the G protein that is responsible for the lower activity of the chimeras. When larger areas of the polypeptide, extending from residues 153, 124, or 56 to the distal end of $G_{\alpha 16}$, were replaced with equivalent stretches of $G_{\alpha 11}$, the resulting chimeric proteins ($\chi 10$, $\chi 11$, and $\chi 12$, respectively) (Fig. 4), although expressed (Fig. 5A), lost their ability to respond to C5a and to show an AlF_4^--induced inositol phosphate release response (Fig. 5B).

The G protein is recognized by receptors as a heterotrimer; thus, it is possible that the chimeras did not show ligand-induced activity because they had lower affinity or different specificity for $\beta\gamma$ subunits. To test this notion we co-transfected a number of the chimeras and the wild-type $G_{\alpha 16}$ and $G_{\alpha 11}$ constructs with various combinations of $\beta\gamma$ subunits. Although small quantitative changes were found with some of the chimeras, the presence of excess levels of different $\beta\gamma$ subunits did not qualitatively change the results observed.

Discussion

More than 100 G protein-coupled receptor subtypes have been cloned and sequenced (29, 30). Three intracellular loops of the activated receptor have been proposed to interact with heterotrimeric G proteins (31, 32). It has been postulated that the carboxyl-terminal 42 amino acids and the amino-terminal 34 amino acids of the G_α subunit are important in determining the specificity of interaction with receptors (9, 14, 33–35). Our results show that multiple regions of $G_{\alpha 16}$ are necessary for the efficient activation of G protein by the C5a receptor. Some of these regions may interact directly with the receptor protein, whereas others may propagate or stabilize conformational changes induced by receptor interaction. It is clear, at least in the case of chimeras $\chi 1$ and $\chi 3$–$\chi 9$, that their ability to interact with effector is not impaired (Fig. 3), whereas their activation by receptor varies dramatically. Their ability to activate PI-PLC in response to AlF_4^-, which is used as a substitute for the γ-phosphate, indictes that these chimeras can undergo conformational change from a GDP-bound form to a GTP-bound form. Clearly, the carboxyl-terminal region plays a role in the G_α activation but does not account for all of the specificity. Incorporation of 134 carboxyl-terminal residues of $G_{\alpha 16}$ ($\chi 3$) did not restore coupling ability, and the region in the middle of the molecule (residues 220–240) and some elements in the amino-terminal portion also contribute to specificity. However, we do not assume that the requirement for these regions necessarily implies physical contact with receptor, even though in the crystal structure of transducin (36) the region homologous to residues 220–240 (the $\alpha 2$–$\beta 4$ region) is adjacent to the carboxyl terminus of the G_α subunit. Comparison of the crystal structures of transducin in the GDP- and GTPγS-bound forms demonstrates that there are three domains that move during nucleotide exchange (37). The region homologous to amino acids 220–240 is located between the switch II and switch III domains, and it is possible that this region is required for the ligand-induced conformational change that occurs during nucleotide exchange. It may be more useful to think of G_α function in terms of a series of concerted protein-protein interactions that control specificity, rather than in terms of discrete contact sites.

In chimeras where we replaced carboxyl-terminal regions of $G_{\alpha 11}$ with corresponding $G_{\alpha 16}$ segments, two different segments were required to recover specificity for the C5a receptor. The region of amino acids 345–374 may interact directly with the receptor, whereas the other segment (amino acids 220–240), which corresponds to the region between the switch II and switch III domains, may be involved in the global conformational change in G_α that leads to nucleotide exchange and stabilizes a receptor-G protein intermediate. The amino acid sequence differences between G_{11} and G_{16} are concentrated within each of these segments. Thus, when aligned sequences are compared in the residue 220–240 segment, there is a block of eight amino acids extending from residue 226 to residue 235 (226-Val-Ile-Ala-Leu-Ile-Tyr-Leu-Ala-Ser-Leu-235) in G_{16} that is replaced by a heterologous block extending from amino acid residue 223 to residue 232 (223-Val-Thr-Ser-Ile-Met-Phe-Leu-Val-Ala-Leu-232) in G_{11}. In the residue 345–374 segment, almost all of the amino acid sequence differences between G_{16}

and G_{11} are within the carboxyl-terminal 18 amino acids. These differences could drive local differences in conformation or could represent specific contact points that contribute to receptor specificity. However, individual local changes are not sufficient to account for receptor interaction. The global nature of the specificity is further seen in the chimeras where carboxyl-terminal portions of $G_{\alpha 16}$ were substituted by $G_{\alpha 11}$ segments. In these molecules the presence of an amino-terminal region of $G_{\alpha 16}$ (amino acids 1–209) is enough to ensure some C5a receptor specificity. Because a few of the chimeras ($\chi 10$–$\chi 12$) had very little or no activity in the AlF_4^- assay, it is not possible for us to define this region in greater detail. However, it might include a G protein receptor contact site or some other site that can stabilize the global conformational change induced by the ligand-bound receptor.

The precise steps in receptor activation of G proteins are not well understood. Activation requires both recognition of the heterotrimer and its ability to initiate nucleotide exchange and $\beta\gamma$ release. Until we can measure the intermediates in this process it will be very difficult to ascribe functional specificity to single amino acid changes. A more detailed analysis of structure-function relationships may be possible when the three-dimensional atomic structures of the different G_α proteins, in both of their nucleotide-bound forms, become available.

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