A Novel Site on the Ga-protein That Recognizes Heptahelical Receptors*

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Specific domains of the G-protein α subunit have been shown to control coupling to heptahelical receptors. The extreme N and C termini and a region between α4 and α5 helices of the G-protein α subunit are known to determine selective interaction with the receptors. The metabotropic glutamate receptor 2 activated both mouse Ga15 and its human homologue Gaα16, whereas metabotropic glutamate receptor 8 activated Gaα15 only. The extreme C-terminal 20 amino acid residues are identical between the Gaα15 and Gaα16 and are therefore unlikely to be involved in coupling selectivity. Our data reveal two regions on Gaα16 that inhibit its coupling to metabotropic glutamate receptor 8. On a three-dimensional model, both regions are found in a close proximity to the extreme C terminus of Gaα16. One module comprises α4 helix, α4–β6 loop (L9 Loop), β6 sheet, and α5 helix. The other, not described previously, is located within the loop that links the N-terminal α helix to the β1 strand of the Ras-like domain of the α subunit. Coupling of Gaα16 protein to the metabotropic glutamate receptor 8 is partially modulated by each module alone, whereas both modules are needed to eliminate the coupling fully.

The specific signaling pathway of a given G-protein-coupled receptor (GPCR1) depends on the subset of G-proteins it can activate. Transduction of signals by activated GPCR requires the interaction of the receptors with heterotrimeric G-proteins. Recent progress in defining the structure of G-proteins and site-directed mutagenesis studies of receptors and G-proteins bring increasingly more specific and precise descriptions of the contact sites between these proteins (1, 2). From the G-protein α, β, and γ subunits, probably both Ga and βγ dimers contact the receptors (3). The Ga subunit is likely to play a decisive role in discriminating between different receptor subtypes (4–6) and also between different functional states of receptors (7, 8).

Several regions along the sequence of the Ga-protein are involved in the selectivity of its activation by GPCR (1, 9, 10). The best characterized is the extreme C terminus, where residues at position −3 and −4 (the residue −1 being the last one) are decisive for coupling of Ga proteins with specific receptors (1, 11–15). Residue −4 in Gaα, Gaα, and Gaα4 is the cysteine residue that is ADP-ribosylated by pertussis toxin, a covalent modification that prevents the interaction of the G-protein with the receptors. In the Gi family, the residue −4 is a tyrosine residue that has to be phosphorylated for an efficient coupling to the PLC-activating receptors (16). Conformational changes in the C-terminal structures upon coupling to the GPCR probably cause activation of the Ga–protein. Another region determining coupling selectivity is the extreme N terminus. In the case of Gα11-proteins, this N terminus has been shown to restrict coupling of these G-proteins to a subset of GPCRs, namely to those known to activate PLC (17). Finally, the region between α4 and α5 helices that includes the L9 loop and β6 sheet (18) is also involved in coupling selectivity probably by directly interacting with the receptors of the rhodopsin-like family (family 1 GPCRs) (10, 19–22). The overall surface charge of a region that comprises the structures close to the C terminus is probably defining either selectivity or coupling properties in general (23).

Among the various G-proteins identified so far, the Ga15 subunit has unique properties. This G-protein that is found exclusively in the murine hematopoietic cell lineage shares the closest sequence similarity with the Gaα2 protein and activates PLCβ (24, 25). In cells normally expressing this G-protein as well as in heterologous expression systems it was found that it can couple to many GPCRs, including those that naturally do not stimulate PLC. This was observed with the members of family 1 GPCRs (26–28) and the mGlu receptor family (family 3 GPCRs) (29, 30). Ga12 is therefore a G-protein that is not able to discriminate between a variety of receptors (26). Functional characterization of the human homologue Ga16 revealed that this protein also couples to many GPCRs (27) but is not as promiscuous as Ga15. It is of interest to understand what determines the lack of selectivity of Ga15 and which sequences restrict promiscuity of the Ga16 subunit.

There are several examples of receptors that cannot activate Ga16 (31, 32). Among the family 3 receptors we reported that...


**EXPERIMENTAL PROCEDURES**

**Materials**—Chemicals including glutamate were obtained from Sigma (L'Isle d'Abeau, France) unless otherwise indicated. Serum, culture media, and other solutions used for cell culture were from Life Technologies, Inc. (Cergy Pontoise, France). The plasmids expressing mGlu receptors were as described previously (14) or modified (see below). The hemagglutinin epitope-tagged Gαq was kindly provided by Dr. Bruce Conklin (The Gladstone Institute, San Francisco, CA). The plasmids expressing the cDNA encoding the Gα15/Gα16 chimera were from Meylan, France, or the newly described polyclonal anti-Gα15 antibodies. The His 6-tagged receptors were detected using specific antibodies recognizing the MRGS-His epitope (Qiagen, Paris, Montpellier, France) or the newly described polyclonal anti-Gα15 antibodies. The His 6-tagged receptors were detected using specific antibodies recognizing the MRGS-His epitope (Qiagen, Paris, Montpellier, France) or the newly described polyclonal anti-Gα15 antibodies.

**Immunoblotting Analysis**—Cells were transfected and treated as described above. Cells cultured in one well were harvested for immunoblotting. Upper panel, immunoblot using the polyclonal anti-Gα15 antibodies. In the lower part, a portion of the same blot developed with the monoclonal anti-HA antibody. The bands corresponding to the G-proteins migrated with expected velocities. On the right, a schematic representation of the chimeric proteins between Gα7, HA, Gα15, and Gα16. The bars indicate prestained molecular mass standards migrating at molecular mass (from the top): 200, 118, 97, 66, 45, 31, and 21 kDa. The arrowhead indicates position of the G-protein.

The chimeric Gα15/Gα16 were constructed using a unique site of the cDNA encoding the Gα15-HA-tagged protein. The HA site was inserted between the C-terminal part of Gα15 and Gα16. The antiserum was tested in several dilutions (data not shown). The 1:2000 dilution gave optimal results. ECL chemiluminescence system was used to stain the secondary antibodies (Amersham Pharmacia Biotech, Paris, France).

The chimeric Gα15 and Gα16 were constructed using a unique XbaI site of the cDNA encoding the Gα15-HA-tagged protein. The XbaI sites in the C-terminal part of Gα15 and Gα16 were introduced by silent mutagenesis into the consensus sequence Met-Asp-Leu. This resulted in the expression of chimeric proteins that were detectable by both the HA antibodies and our anti-Gα15/Gα16 C-terminal antibody.

**Immunoblotting Analysis**—Cells were transfected and treated as described above. Cells cultured in one well were harvested for immunoblotting, and the rest of the cells were used for IP assay. Protein samples (10 μg per lane) were separated using SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membrane. Prior to the immunoblotting, total protein on the membranes was visualized with Ponceau S to confirm that the same amounts of protein were loaded on the gel. The G-proteins were detected using primary monoclonal antibodies against hemagglutinin-epitope (generous gift of Dr. B. Mouillac, Montpellier, France) or the newly described polyclonal anti-Gα15/Gα16 C-terminal antibodies. The His6-tagged receptors were detected using specific antibodies recognizing the MRGS-His epitope (Qiagen, Paris, France).

**Construction of Plasmids Expressing Higher Levels of Gα15**—We have noticed considerable lower expression levels of the human Gα15.
protein than those of Gα15 in transfected HEK 293 cells. To get higher expression levels of Gα15, we removed most of the 5′-untranslated region and introduced a Rokaz sequence corresponding to that of Gα16 by polymerase chain reaction using the unique EcoRI-PstI sites. These modifications were sufficient to raise the Gα15 protein expression obtained with this new Gα15PLUS construct to levels comparable with those reached by Gα16. This construct was used throughout the study and is referred to as Gα15 in this text.

**Construction of Chimeric G-proteins**—Silent mutagenesis approach using Pfu-based QuickChange™ technique (Stratagene) was used to introduce a BamHI, a PstI, and a BamHI restriction site at the positions indicated in Fig. 2 as sites 1, 2, and 4, respectively. Together with the existing XbaI site (position 3 in Figs. 2–4) and the HindIII site in the 3′-untranslated region of mGlu2, these sites were used to construct the chimeric proteins described in Figs. 3 and 4 using conventional subcloning techniques. The same approach was used for making the point mutations.

**Construction of Epitope-tagged Receptors**—The mGlu2 and mGlu8 receptors were tagged with MRGS-His6 epitope at the C termini as in our previous studies (14). Briefly, the pRK5 plasmid containing the sequence encoding the epitope MRGS-His6 flanked by a unique restriction site so that the stop codon was replaced by alanine followed by serine and the MRGS-His6 epitope (where MRGS indicates: methionine, arginine, glycine, serine).

**Molecular Modeling**—The three-dimensional model of the Gα15 subunit was constructed by homology using the coordinates of different molecular simulation (Molecular Simulation Inc.) and the CVFF force field. The extreme C-terminal residues (10 amino acid residues) common to both proteins (Figs. 1 and 2). The antiserum was raised in rabbits immunized with a peptide corresponding to the extreme C-terminal region (10 residues) of the resolved structure (37) (Protein Data Bank accession numbers 1TAG, 1GOT, 1GDD, and 1GP2, respectively). Some structural elements were deleted due to their inappropriate folding in the expected heterotrimeric form bound to the receptor. These deletions include the N-terminal residues of the resolved structure and the extreme C terminus (3 residues) of the α1 in its GDP plus Mg2+ form. Some constraints were also imposed during the modeling process of Gα15: an α helical secondary structure was imposed to residues 7–39, 305–325, 330–334, and 349–370. The sequence alignment and three-dimensional models were generated using the program Modeler (38) in the Insight-II environment (Molecular Simulation Inc., San Diego, CA) on a Silicon Graphic R10000 O2 work station. A statistical evaluation of the three-dimensional model was performed using the Verify 3D algorithm (39) and the Verify 3D Structure Evaluation Server. The model giving the best one-dimensional/three-dimensional scores was selected and subjected to energy minimization using the program Discover 2.9.7 (Molecular Simulation Inc.) and the CVFF force field. The extreme C terminus of the Gα15 subunit was modeled manually according to the resolved structure of the 10 C-terminal residues of transducin bound with rhodopsin (40). The β1γ2 subunits were added after superposition of the Gα15 subunit in its heterotrimeric complex.

**RESULTS**

**Characterization of Anti-Gα15,16 Antibodies**—The mGlu2 and mGlu8 receptors couple to the Gγ type of G-proteins and do not activate PLC pathway when expressed alone in HEK 293 cells. Because Gα15 and Gα16 activate PLCs, their efficient coupling to these mGlu receptors can easily be assayed by measuring the capability of glutamate to activate the PLC pathway in cells expressing both the receptor and one of these G-proteins. As mentioned in the introduction, we reported previously that glutamate activated PLC in HEK 293 cells coexpressing mGlu2 receptors and either Gα15 or Gα16. In contrast, glutamate activated IP formation in cells expressing mGlu8 receptors and Gα15 but not in those coexpressing this receptor with Gα16. To conclude that Gα15 can be activated by mGlu2 but not mGlu8 receptors, it was necessary to verify that both G-proteins were expressed at similar levels.

We generated a new polyclonal antibody, called anti-Gα15,16AB that recognizes equally well both Gα15 and Gα16 (Fig. 1). The antiserum was raised in rabbits immunized with a peptide corresponding to the extreme C-terminal region (10 amino acid residues) common to both proteins (Figs. 1 and 2). On Western blots, anti-Gα15,16AB recognized a single major band that migrated at a velocity appropriate for these G-proteins (Fig. 1). This band was detected only in lanes where membrane proteins from HEK 293 cells expressing proteins possessing C termini of Gα15 or Gα16 were separated. No ad-
ditional major band was detected, and no bands were detected in lanes where extracts from cells expressing other Ga-protein subunits were loaded (data not shown). To verify that anti-Ga15/16AB was recognizing both G-proteins at the same extent, we expressed chimeric proteins corresponding to the HA-tagged Gaq protein with its last 51 C-terminal residues replaced by their corresponding 63 residues of either Ga15 or Ga16 (chimeras GaqX5 and GaqX6, see Fig. 1). Those were expressed in HEK 293 cells at the same levels, as shown with the HA specific monoclonal antibody. When blots were reprobed with the new antibody anti-Ga15/16AB, the bands corresponding to chimeras bearing Ga15 and Ga16 C termini were stained with similar intensity (Fig. 1).

Optimization of Ga16 Expression—Employing the anti-Ga15/16AB antibody on immunoblots, we noticed that the level of expression of Ga16 was lower than that of Ga15 in cells transfected with the original vectors (data not shown). We therefore replaced the 5'-untranslated region of Ga16 cDNA, including the Kozak sequence with corresponding sequence from of Ga15 coding cDNA (see “Experimental Procedures”). This modification was found to raise the expression of Ga16 in transiently transfected HEK 293 cells to levels comparable with those of Ga15 (Figs. 1 and 3b).

In Contrast to mGlu2 Receptors, mGlu8 Receptors Do Not Efficiently Activate Ga16—Using the expression plasmids described above, we examined the coupling of mGlu2 and mGlu8 receptors to both Ga15 and the modified Ga16. As shown in Fig. 3a, glutamate stimulated IP formation in cells coexpressing mGlu2 receptors with either Ga15 or Ga16, an effect that is dose-dependent (Fig. 6). Using the new Ga16 vector, the maximal glutamate effect was found to be slightly lower than that obtained with Ga15 in cells expressing mGlu2 receptors, whereas it was 50% smaller when the original plasmid was used (29, 30). This is in agreement with the increased protein expression levels of Ga16 that resulted from the modification of the coding plasmid. In cells expressing mGlu8 receptors, a large increase in basal and glutamate-induced IP formation was detected when coexpressed with Ga15, but no change was observed with Ga16. All the glutamate effects were dose-dependent and the calculated EC50 values (for mGlu2 receptor-Ga15 pair the EC50 for glutamate was 8.2 ± 0.8 μM; mGlu2 receptor with Ga16 results in EC50 8.0 ± 0.3 μM glutamate, and mGlu8 receptor coexpressed with Ga16 determined EC50 for glutamate was 9.3 ± 1.6 μM) were close to those determined with these receptors using other assays (41). It was important to establish that the expression levels of both the receptors and the G-proteins are similar at their different combinations. At the receptor level this was confirmed by using His6-tagged receptors (data not shown). These data further confirm the discriminative coupling of Ga16 to mGlu2 and mGlu8 receptors.

Two Regions on Ga15 and Ga16 Are Recognized by mGlu Receptors—To identify the putative regions on the G-protein
that cause the differential coupling of \( \alpha_{15} \) and \( \alpha_{16} \) to mGlu8 receptor, we constructed a series of \( \alpha_{15} \)/\( \alpha_{16} \) chimeras (see panel a in Figs. 3–5). When transiently transfected into HEK 293 cells, all chimeras were expressed at levels similar to those of the original proteins as revealed by the antiserum anti-\( \alpha_{15} \)/\( \alpha_{16} \)AB (panel b in Figs. 3–5). Moreover, all constructs did couple well to mGlu2 receptors, as they all allowed an effective coupling of this receptor to IP formation upon glutamate application, showing that they were all functional. Coupling of each single constructs with the receptors is shown in Figs. 3–5 (panel c).

Chimera \( \alpha_{15}/\alpha_{16} \)C1, which corresponds to \( \alpha_{15} \) with the C-terminal 44 residues from \( \alpha_{16} \) (Fig. 3a), showed a smaller response to glutamate as compared with wild-type \( \alpha_{15} \) (Fig. 3c). This indicates that the C-terminal portion of \( \alpha_{15} \) contains a site that decreases G-protein coupling efficacy to mGlu8 receptors (Fig. 3c). Progressive exchange of \( \alpha_{15} \) C terminus with corresponding sequences of \( \alpha_{16} \), as in chimera \( \alpha_{15}/\alpha_{16} \)C2, resulted in a more pronounced decrease in coupling efficiency to mGluR8 (Fig. 3c). The converse chimera \( \alpha_{15}/\alpha_{16} \)C3, which corresponds to \( \alpha_{15} \) with the C-terminal 44 residues of \( \alpha_{16} \), was activated by mGlu8 receptors, although the glutamate response was smaller than that obtained with \( \alpha_{15} \) (Fig. 3b).

The module in \( \alpha_{16} \) responsible for discriminating between the two receptors is most likely a region that includes \( \alpha \) 4 helix, L9 loop, the \( \beta \) 6 sheet, and the \( \alpha \) 5 (residues 331–354). Residues that are different between \( \alpha_{15} \) and \( \alpha_{16} \) in these regions are:

- 10 in the \( \alpha \) 4 helix and N-terminal part of L9 loop,
- 3 in the C-terminal part of L9,
- 3 in \( \beta \) 6,
- 3 in \( \alpha \) 5 (Fig. 2).

Attempts to further characterize the critical residues in this C-terminal region failed. For example, the comparison of the IP formation obtained with mGlu8 receptors expressed with the C1 and C2 \( \alpha_{15}/\alpha_{16} \) chimera suggests that the region between positions 3 and 4 (Fig. 3a) decreases coupling efficacy of \( \alpha_{16} \). However, swapping this region of \( \alpha_{16} \) for that of \( \alpha_{15} \) in the chimera C4 did not improve the coupling compared with chimera C3, but rather diminished it. Also in chimera \( \alpha_{15}/\alpha_{16} \)C5, the exchange of the 3 residues of \( \alpha \) 5 in \( \alpha_{15} \) by those of \( \alpha_{16} \) lead to a decrease of functional responses obtained with both mGlu2 and mGlu8 receptors. The most likely explanation is that the region including \( \alpha \) 4, L9 loop, \( \beta \) 6, and \( \alpha \) 5 discriminates the GPCRs as a net of many forces, a situation where a given charge of a side chain of an amino acid residue is less important than the outcome of additions of neighboring forces. One can imagine this situation also as a mosaic, where the whole picture is the decisive one. This is in agreement with results obtained previously by other researchers (23). Since the result of swapping this part of the G-proteins had only partial effect on the mGlu8 receptor-G-protein coupling, there had to be another discriminatory region.
Swapping the N-terminal 32 residues of Ga16 by those of Ga15 resulted in chimera Ga15/16N1, which showed a feeble coupling to mGlu8 receptors (Fig. 4). When the first 101 residues of Ga16 were exchanged by the corresponding region in Ga15, chimera Ga15/16N2, a significant increase in the capacity of the chimeric G-protein to couple to mGlu8 receptors was observed. Interestingly, Ga15/16N2 did not perform any better than the Ga15/16C4 chimera (compare Figs. 3 and 4), suggesting that the region between position 101 and 313 did not play an important role in controlling coupling selectivity to mGlu2 and mGlu8 receptors. Taken together, these results indicate that two distinct regions in Ga16, encompassing residues 32–101 and 331–354, respectively, are responsible for the observed decrease in coupling efficacy to mGlu8 receptors (see Fig. 2). In agreement with this hypothesis, chimera Ga15/16N1C4 that is characterized by residues 331–354 of Ga15 and residues 32–101 of Ga16 was activated by mGlu8 receptors but not to the extent showed by wild type Ga15. Chimera Ga15/16N2C4, which is constituted by the backbone of Ga16 with both critical regions corresponding to residues 32–101 and 331–354 replaced by the respective Ga15 sequences, allowed a more efficient coupling of mGlu8 receptors to PLC. Considering the critical role played by residues 32–101 in conferring G-protein coupling specificity, we decided to make point mutations within this region in both Ga15 and Ga16.

The αN-β1 Loop of the Gα-Protein Selectively Modifies Coupling to mGlu Receptors—Within region 32–101, several amino acid residues differ between Ga15 and Ga16 (Fig. 2). Two of these nonconserved residues are located in the loop that links the N-terminal α helix and the first β strand of the Ras-like domain (the αN-β1 loop), the other residues are located in the aA helix of the helix-rich domain. Among this set of residues, those located in the αN-β1 loop are the most likely to affect G-protein coupling selectivity, since they are located in close proximity to the known G-protein-mGlu receptors interacting site, the extreme C terminus, and are exposed on the surface of the protein. Interestingly, the β1 sheet is highly conserved within the Gα-protein family, whereas the contiguous αN-β1 loop is extremely variable. As mentioned above, two residues in the αN-β1 loop are different between Ga15 and Ga16: glutamic acid 39 and 41 in Ga15 are replaced by an aspartic acid and a glycine in Ga16, respectively (Fig. 2). Our results indicate that both residues at positions 39 and 41 are involved in determining coupling selectivity to mGlu receptors. To reach maximal mGlu8 receptor coupling activity, both positions in Ga16 have to be replaced by the corresponding residues of Ga15 (chimera Ga15/16N2C4; Figs. 5 and 6); this is in agreement with what has been observed with chimera N2 (Fig. 5). Moreover, the reverse mutant Ga15D-G showed a significant decrease of coupling performance with mGlu8 receptor as compared with wild-type Ga15. All mutants exhibited comparable good coupling to mGlu2 receptor, even though with slightly lower efficacy than...
**DISCUSSION**

In this study we showed that two distinct regions along the sequences of Ga15 and Ga16 control their coupling to heptahelial receptors. One such region is between residues 311 and 354 that includes the C-terminal part of the a4 helix, L9 loop, the b6 strand, and the a5 helix. The second region comprises residues 39 and 41, which lie within the loop that links the N-terminal a helix with the first b strand of the Ras-like domain of the G-protein a subunit. Both regions are in close proximity to the extreme C terminus in the spatial arrangement of the proteins. These data further strengthen the role of the area located in close proximity to the C terminus of Ga—protein in controlling coupling to heptahelial receptors and extent the putative surface on Ga—protein that modulates the interaction with this class of receptors.

To better characterize the regions in G-proteins involved in GPCR recognition, we took advantage of the differential coupling of Ga15 and Ga16 to mGlu2 and mGlu8 receptors. The Ga15. Accordingly, basal IP formation induced by mGlu2 receptor was lower with these mutants than with Ga15 (Figs. 5 and 6). It is important to mention again that the expression levels in independent experiments performed in triplicates.

FIG. 6. Glutamate-induced IP formation in cells coexpressing mGlu2 or mGlu8 receptors with either Ga15 or Ga16 or the Ga16EE chimera. Cells coexpressing mGlu2 receptors with Ga15 (open circles) and the modified construct of Ga16 (closed circles) were stimulated with increasing concentrations of glutamate. b, same as a with mGlu8 receptor and the newly constructed vectors encoding Ga15, Ga16, and Ga16EE. Data represent the radioactivity in the IP fraction divided by the total radioactivity in the membranes. Values are means ± S.E. of three independent experiments performed in triplicates.
mGlu receptors and G\(_{\alpha 15/16}\) are unlikely to naturally interact. The mouse G\(_{\alpha 15}\) and human G\(_{\alpha 16}\) are known to be exclusively expressed in the hematopoietic cell lineage, whereas mGlu receptors are mostly expressed in neurons and/or in astrocytes in the central nervous system. However, several consideration prompted us to perform this study. The interaction between GPCR and the heterotrimeric G-protein is known to involve several regions on the G\(_{\alpha}\)-protein, the best characterized being the extreme C terminus. Since G\(_{\alpha 15}\) and G\(_{\alpha 16}\) share identical C termini, this study allowed us to identify additional regions with coupling-restrictive abilities. All G\(_{\alpha}\) subunits share high sequence similarity and therefore have highly conserved structures; thus, identification of additional regions involved in coupling selectivity of G\(_{\alpha 15/16}\) would help us to better map residues in all the \(\alpha\) subunits that control the coupling selectivity and/or efficacy to their partner receptors. The mGlu receptors are members of a subfamily of GPCRs (family 3) that is distinct from the rhodopsin-like family 1 GPCRs. Thus, identification of members of a subfamily of GPCRs (family 3) that is distinct from the rhodopsin-like family 1 GPCRs, would provide a valuable tool for the establishment of functional tests for receptors for which no information on the G-protein coupling selectivity is available (28). Identification of the specific epitopes on G\(_{\alpha 15/16}\) that influence their promiscuous coupling could constitute an helpful information for the construction of modified G-proteins with even lower selectivity. We were expecting that the residues responsible for the differential coupling of G\(_{\alpha 15}\) and G\(_{\alpha 16}\) toward mGlu8 receptors would be located in the L9 (\(\alpha 4-\beta 6\)) loop. This region was described as a key site in G-protein \(\alpha\) subunits discriminating between members of family 1 GPCRs (9, 10, 19–21). This loop, which is longer than the corresponding sequences of other G-protein \(\alpha\) subunits, is the most variable region between G\(_{\alpha 15}\) and G\(_{\alpha 16}\) proteins. Our results revealed that, as observed with family 1 GPCRs, \(\alpha 4\) helix, L9 loop, \(\beta 6\) sheet, and \(\alpha 5\) helix together play an important role in controlling their coupling of G-protein to family 3 receptors. This strengthens the hypothesis that these two distant heptahelical receptor types are recognized by similar molecular determinants on the G-protein \(\alpha\) subunits. Our results also indicate that differences in amino acid residues in the region comprised by \(\alpha 4\) and \(\alpha 5\) helices are not sufficient to explain the differential coupling sensitivity of G\(_{\alpha 15}\) and G\(_{\alpha 16}\) to mGlu8 receptors. Indeed, we identified a second region in G\(_{\alpha 16}\) that, once replaced by the equivalent G\(_{\alpha 15}\) sequence, lead to a chimera showing distinctive and measurable coupling to mGlu8 receptors. This novel region is characterized by a 2-amino acid residue exchange between G\(_{\alpha 15}\) and G\(_{\alpha 16}\), where Glu\(_{39}\) and Glu\(_{41}\) in G\(_{\alpha 16}\) are replaced by Asp and Gly in G\(_{\alpha 15}\), respectively. These two residues are located in the loop linking the N-terminal \(\alpha\) helix and the first \(\beta\) sheet of the Ras-like domain. Our three-dimensional model indicates that side chains of these residues are likely to be facing the surface of the protein in close proximity to the C terminus. As such they are likely to constitute a major determinant in defining the differential coupling observed for G\(_{\alpha 15}\) and G\(_{\alpha 16}\) to mGlu8 receptor. This region has never been previously reported as being involved in the control of receptor G-protein coupling, and it is therefore a novel possible contact site between the G-proteins and family 3 GPCRs. An alternative that could not be ruled out in the present study is that these residues may affect flexibility of the N-terminal helix. Since the N terminus of G\(_{\alpha}\)-protein contacts the \(\beta\) subunit, this might influence \(\beta-\gamma\) coupling and so coupling to receptors (43).

Taken together, our data indicate that at least two sets of residues in G\(_{\alpha 15}\) and G\(_{\alpha 16}\) contribute to coupling restriction of these G-proteins to the mGlu8 receptors. The residues comprised in these two regions are located on both sides of the extreme C-terminal domain known to interact with the intracellular loops of the heptahelical receptors. This cooperativity between different portions of the G-protein \(\alpha\) subunit, that leads to differential coupling to heptahelical receptors, is reminiscent of the cooperativity between different intracellular portions of the receptors in controlling their coupling to G-proteins. This strengthens the concept that coupling efficacy and/or selectivity between heptahelical receptors and the G-proteins results from a multiplicity of contact zones. It remains to be clarified which portion of a G-protein is recognized by a given intracellular region on the receptors.

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Jaroslav Blahos, Thierry Fischer, Isabelle Brabet, Daniela Stauffer, Giorgio Rovelli, Joël Bockaert and Jean-Philippe Pin

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