Identification of Common and Distinct Residues Involved in the Interaction of α₁₂ and αₛ with Adenylyl Cyclase*

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The G protein α subunits, αₑ and αₛ, have stimulatory and inhibitory effects, respectively, on a common effector protein, adenylyl cyclase. These effects require a GTP-dependent conformational change that involves three α subunit regions (Switches I-III). αₑ residues in three adjacent loops, including Switch II, specify activation of adenylyl cyclase. The adenylyl cyclase-specifying region of α₂ₛ is located within a 78-residue segment that includes two of these loops but none of the conformational switch regions. We have used an alanine-scanning mutagenesis approach within Switches I-III and the 78-residue segment of α₁₂ to identify residues required for inhibition of adenylyl cyclase. We found a cluster of conserved residues in Switch II in which substitutions cause major losses in the abilities of both α₁₂ and αₑ to modulate adenylyl cyclase activity but do not affect αₛ subunit expression or the GTP-induced conformational change. We also found two regions within the 78-residue segment of α₁₂ in which substitutions reduce the ability of α₁₂ to inhibit adenylyl cyclase, one of which corresponds to an effector-activating region of αₑ. Thus, both αₑ and αₛ interact with adenylyl cyclase using: 1) conserved Switch II residues that communicate the conformational state of the α subunit and 2) divergent residues that specify particular effectors and the nature of their modulation.

Upon activation by cell surface receptors, heterotrimeric G proteins transmit signals to effector proteins that regulate a wide variety of cellular processes (1–4). Receptors activate G proteins to transmit signals to effector proteins that regulate a variety of cellular processes (1–4). G proteins interact with effector proteins that regulate a variety of cellular processes (1–4).

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1 The abbreviations used are: GTP-S, guanosine 5′-O-(thiotriphosphate); hGH, human growth hormone; FDE, cGMP phosphodiesterase; PLC, phosphoinositide phospholipase C.
α₂ interact with adenyl cyclase using two types of residues: 1) conserved residues within Switch II that signal that the α subunit is in the GTP-bound active conformation and 2) divergent residues that specify activation or inhibition of this effector enzyme.

To identify the α₂ residues involved in specifying inhibition of adenyl cyclase, we substituted alanines for solvent-exposed residues within the 78-residue segment. We found two regions of sequence in which mutations impaired the ability of α₂ to inhibit adenyl cyclase, the amino terminus of α₃ and the α₄β₆ loop. The α₄β₆ loop is also important for the effector interactions of α₁ (12) and α₁ (16, 17). These substitutions did not cause as much of a decrease in adenyl cyclase inhibition as the Switch II mutations did, suggesting that Switch II residues are the primary contributors to the interaction between α₁ and adenyl cyclase.

EXPERIMENTAL PROCEDURES

Generation of Plasmids—α₂ mutants were constructed from the mouse α₂ cDNA (18), and α₁ mutants were constructed from the rat α₁ cDNA (19). Two modifications were made to each of the α subunits to facilitate their detection at their expression levels. The arginine at position 179 in α₂ and 201 in α₁ was mutated to cysteine to inhibit GTPase activity and produce constitutive activation (20, 21). An epitope, referred to as the EE epitope (22) was generated by mutating the rat α₁ cDNA (19) by mutating arginine 179 to cysteine and residues DYQPE (167–172) to EYMPTE (single letter amino acid code, mutated residues are underlined). The resultant constructs were designated α₁-RCCEE and α₁-RCCE respectively. α₁-RCCEE was generated from the rat α₁ cDNA (19) by mutating arginine 179 to cysteine and residues DYQPE (167–172) to EYMPTE.

The α₁-RCCEE cDNA (gift of Ann Pace and Henry Bourne, University of California, San Francisco) was subcloned into pcDNA I/Amp (Invitrogen) as an EcoRI fragment. The α₁-EE cDNA (gift of Paul Wilson and Henry Bourne, University of California, San Francisco) was subcloned into pcDNA I/Amp as a HindIII fragment. To produce the α₁ RC cDNA, the α₁ RCHA cDNA (12), which contains the HA epitope from influenza virus (23), was digested with XbaI and EcoRI to yield a fragment containing the R201C mutation but not the HA epitope. XbaI-EcoRI restriction of α₁EPcDNA I/Amp removed a fragment containing the EE epitope, which was replaced by the XbaI-EcoRI fragment from the α₁-RCCE cDNA to produce α₀RCpDNA I/Amp. To generate α₁RCEEpcDNA I/Amp, α₁RCpDNA I/Amp was digested with AlwII I to yield a fragment containing the R201C mutation, which was ligated into α₁EPcDNA I/Amp in place of the analogous fragment to produce a α₁ cDNA containing both the R201C mutation and the EE epitope.

All mutagenesis procedures were verified by oligonucleotide-directed in vitro mutagenesis (24) using the Bio-Rad Mutagen kit except for those in the GTPases. Constructs 2 and 3, which were 2 residues SDYIPTQ (166–172) to EEYMPTE and i2 residues SDYIPTQ (166–172) to EEYMPTE (single letter amino acid code, mutated residues are underlined). The resultant constructs were designated α₁-RCCEE and α₁-RCCE respectively. α₁-RCCEE was generated from the rat α₁ cDNA (19) by mutating arginine 179 to cysteine and residues DYQPE (167–172) to EYMPTE.

RESULTS

Characterization of Mutant α₁ Constructs Using cAMP Assay—To characterize mutant α₁ subunits after transient expression in HEK-293 cells, two features were included, as in a previous study (14), to enable measurement of their functions without interference from the activities of the α₁ proteins endogenous to these cells. First, a conserved arginine (R179C) was replaced by cysteine. This mutation constitutively activates α₁ by inhibiting its GTPase activity (20) and made it possible to measure inhibition of adenyl cyclase without requiring receptor-mediated activation of the mutant α₁ subunits. Second, the α₁ constructs include an epitope from an internal region of polyoma virus medium T antigen, referred to as the EE epitope (22), which does not interfere with the α₁-adenyl cyclase interaction (27).

We measured the ability of recombinant α₂ subunits to inhibit adenyl cyclase in HEK-293 cells by co-expressing them with the constitutively activated α₁ mutant, α₁ RC, in which arginine 201 is mutated to cysteine (21). As in a previous study (14), transient expression with 0.1 µg of vector containing α₁ RC resulted in an approximately 18-fold increase in cAMP production compared with cells transfected with vector alone. Co-transfection with 0.3 µg of vector containing α₁ RCEE resulted in ~60% inhibition of the cAMP response to α₁ RC, while co-transfection with the same amount of vector containing α₁ RCEE inhibited the response to α₁ RC by only ~15% (Fig. 1). We used α₁ RCEE as a negative control because α₁ has been shown to have little or no ability to inhibit adenyl cyclase (10, 11).
Alanine Substitutions within Conformational Switch Regions—Since the GTP-bound form of \( \alpha_{2} \) inhibits adenylyl cyclase much more effectively than the GDP-bound form does (11), it was surprising that the effector-specifying region of \( \alpha_{2} \), as defined by the 78-residue segment, residues 245–322 (14), did not include any of the three regions, Switches I-III (6, 8), that undergo GDP-dependent conformational changes. However, the sequences of these regions are highly conserved in \( \alpha_{2} \) and \( \alpha_{q} \). 7 of the 11 Switch I residues, 18 of the 21 Switch II residues, and 6 of the 12 Switch III residues are identical in the sequences of \( \alpha_{2} \) and \( \alpha_{q} \). Therefore, the importance of these regions as effector binding sites could have been missed using homologous sequence substitutions.

To directly test the importance of Switches I-III as effector contact sites, we mutated solvent-exposed residues within each of these regions to alanine residues. Substitutions using alanine residues eliminate the side chain beyond the \( \beta \) carbon but generally do not alter the main chain conformation and do not impose significant electrostatic or steric effects (28). We identified clusters of solvent-exposed residues by inspection of the x-ray crystal structures of the GTP-bound forms of \( \alpha_{1} \) (7) and \( \alpha_{2} \) (5) and calculations of fractional accessibility values (29) from the coordinates. As shown in Fig. 1, we mutated three clusters of residues in Switch I (6 residues), five clusters of residues in Switch II (8 residues), and four clusters of residues in Switch III (7 residues).

We found that alanine substitutions of three residues in Switch II, Arg-209, Lys-210, and Ile-213, blocked \( \alpha_{2} \)RCEE from inhibiting adenylyl cyclase (Fig. 1). These residues are located in the middle of the \( \alpha2 \) helix and are highly conserved among \( \alpha \) subunits (see Fig. 7). We previously found that substituting \( \alpha_{2} \) homologs for three \( \alpha \) subunits in \( \alpha_{2} \)RCEE, and membranes were prepared, treated with trypsin, and immunoblotted as described under “Experimental Procedures.” The first lane in each set is the control (no trypsin). The second and third lanes show the result of trypsin digestion in the presence or absence, respectively, of GTPγS.

Criteria for Specificity of Mutations—Mutations that prevent \( \alpha_{2} \)RCEE from inhibiting adenylyl cyclase could do so for reasons other than disruption of residues that interact with this effector. Therefore, we subjected constructs with these mutations to the following criteria for specificity. The first criterion was that the mutants should be expressed at wild-type levels in HEK-293 cell membranes. This criterion was tested by performing immunoblots on membranes prepared from cells expressing the mutants. The second criterion was that the mutants should be able to bind GTP and undergo the GTP-dependent conformational change that is detected as the acquisition of resistance to trypsin cleavage (30–32). This second criterion is quite stringent because it requires not only proper GTP binding but also the ability to respond to this binding with an activating conformational change. Under the conditions of this assay, in the presence of GTPγS, trypsin removes a short segment from the amino terminus but leaves most of the protein intact (Fig. 2). However, in the absence of GTPγS, trypsin degrades \( \alpha_{2} \)RCEE to small fragments not seen on SDS-polyacrylamide gels.

We found that the Switch II mutant constructs, (R209A)\( \alpha_{2} \)RCEE and (K210A,I213A)\( \alpha_{2} \)RCEE, were expressed as well as \( \alpha_{2} \)RCEE and achieved the GTP-dependent activated conformation, as measured by the trypsin assay (Fig. 2). Therefore, by our
criteria, residues Arg-209, Lys-210, and Ile-213 are specifically required for interaction with adenylyl cyclase. In contrast, although the Switch I mutant construct, (I185A,E187A) α2RCEE, exhibited resistance to trypsin in the presence of GTPγS, it was expressed very poorly (Fig. 2). The role of residues Ile-185 and Glu-187 in effector interaction is, therefore, uncertain.

In the course of these studies, we mutated the αi2 residue, Arg-209, that corresponds to the GTPγS-protected trypsin site determined by amino-terminal sequencing of tryptic peptides from αi2 and αo (31). Elimination of this cleavage site would be expected to result in an α subunit that was resistant to trypsin cleavage in both the presence and absence of GTPγS. However, (R209A)αi2RCEE was resistant to trypsin cleavage in the presence but not the absence of GTPγS (Fig. 2). Similar results were obtained upon mutation of each of the other potential trypsin sites in Switch II, Arg-206, Lys-210 (Fig. 2), and Lys-211, as well as mutation of all four residues simultaneously. These results suggest that, although Switch II may contain cleavage sites that change conformation upon GTP binding, there are also other sites outside of this region that are preferentially cleaved by trypsin in the absence compared with the presence of GTPγS. Nevertheless, the ability of the trypsin assay to detect GTP-dependent conformational changes in Switch II is demonstrated by the fact that the Switch II αs mutant, G226Aαs, which is unable to undergo the activating conformational change required for dissociation from βγ,

2 C. H. Berlot, unpublished observations.
FIG. 6. Mapping of effector-interacting residues of $\alpha_{i2}$ and $\alpha_i$ onto the x-ray crystal structure of the GTP$_\gamma$S-bound form of $\alpha_{i1}$. A, space-filling model showing $\alpha_{i2}$ residues required for inhibition of adenylyl cyclase. Residues that were mutated are shown in red, magenta, orange, and dark blue, as follows. Residues in Switch II specifically required for inhibition of adenylyl cyclase are red. Residues in Switch I in which mutations reduce both inhibition of adenylyl cyclase and expression level are magenta. Residues within the 78-residue segment in which mutations cause a partial loss of adenylyl cyclase inhibition are orange. Residues in which mutations do not affect inhibition of adenylyl cyclase are dark blue. Residues that were not mutated in this study are shown in green, light blue, and white, as follows. Residues outside of the 78-residue segment and Switches I-III are green. Also green are the residues within the 78-residue $\alpha_{i2}$ segment that are conserved between $\alpha_{i2}$ and $\alpha_i$. The $\alpha_{i2}$ residues within this segment that differ from $\alpha_i$ residues but are not identical among the three $\alpha_i$ isoforms are light blue. Residues in Switches I-III that were not mutated and residues within the 78-residue segment that differ from $\alpha_i$ residues and are conserved among the three $\alpha_i$ isoforms, but were not mutated, are white. Main chain backbone atoms are gray. The GTP is yellow. The numbers on the model refer to $\alpha_{i2}$ residues. The model on the right is rotated approximately 90° about the vertical axis relative to the model on the left. B, ribbon diagram showing comparison of
A Conserved Region of Switch II Is Specifically Required for the Effector Interactions of Both \(\alpha_s\) and \(\alpha_q\)—To determine whether the highly conserved middle region of Switch II (see Fig. 7) is required for the activation of adenylyl cyclase by \(\alpha_s\), we tested the effects of substituting alanines for the \(\alpha_s\) residues (Arg-232 and Ile-235) that correspond to Lys-210 and Ile-213 in \(\alpha_q\). We introduced these substitutions into \(\alpha_s\)RCEE, which contains the EE epitope, previously shown to have no effect on the interaction between \(\alpha_s\) and adenylyl cyclase (34). The substitutions almost entirely prevented \(\alpha_s\)RCEE from activating adenylyl cyclase without affecting the GTP-dependent conformational change measured by the trypsin assay (Fig. 3). Thus, the same region of Switch II is required for the interaction of both \(\alpha_s\) and \(\alpha_q\) with adenylyl cyclase.

Alanine Substitutions within the 78-Residue Segment—Since \(\alpha_q\), but not \(\alpha_s\), inhibits adenylyl cyclase (10, 13) and an \(\alpha_q/\alpha_q/\alpha_q\) chimera containing only 78 \(\alpha_q\) residues (245–322) inhibits adenylyl cyclase as well as \(\alpha_q\) does (14), the \(\alpha_q\) residues that specify inhibition of adenylyl cyclase must be located within this 78-residue segment. To identify these effector-specifying residues, we tested the effects of mutating nine clusters of solvent-exposed residues (22 residues total) to alanine residues (Fig. 4). Within the 78-residue segment of \(\alpha_q\), 65 residues are identical among the three \(\alpha_s\) isoforms, which have equal abilities to inhibit adenylyl cyclase (11). Of these 65 residues, 28 are different in \(\alpha_q\) and therefore might account for the ability of \(\alpha_q\) to inhibit adenylyl cyclase. 20 of the substitutions were in residues that are identical among the three \(\alpha_q\) subunits, and 18 were in residues that differ between \(\alpha_q\) and \(\alpha_q\). The thoroughness of our mutational analysis is illustrated in Fig. 6A.

As shown in Fig. 4, substitutions of three sets of residues: His-245 (Construct 1), Lys-313, Asp-316, and Thr-317 (Construct 8), and Arg-314, Lys-315, and Glu-319 (Construct 9), significantly reduced inhibition of adenylyl cyclase. However, in contrast to the Switch II mutations, which entirely blocked the ability of \(\alpha_q\)RCEE to inhibit adenylyl cyclase, the mutations in Constructs 1, 8, and 9 had only partial effects. The other six clusters of mutations (15 residues) did not significantly impair the ability of \(\alpha_q\)RCEE to inhibit adenylyl cyclase.

All of the constructs that inhibited adenylyl cyclase to a similar or decreased extent compared with \(\alpha_q\)RCEE were expressed in HEK-293 cell membranes and were able to undergo the GTP-dependent conformational change that results in increased resistance to trypsin digestion (Fig. 5). However, since scanning densitometry of immunoblots showed that Constructs 1, 8, and 9 were expressed at lower levels than \(\alpha_q\)RCEE was, their decreased abilities to inhibit adenylyl cyclase may be due to effects of the mutations on protein folding and/or stability. Nevertheless, since we have substituted alanines for the majority of solvent-exposed residues within the effector-specifying 78-residue segment (see Fig. 6A) and the other substitutions did not significantly reduce adenylyl cyclase inhibition, the residues in Constructs 1, 8, and 9 are, by default, the most likely candidates for specifying inhibition of adenylyl cyclase.

Comparison of the Effector-Interacting Surfaces of \(\alpha_q\) and \(\alpha_s\)—We used the x-ray crystal structure of the GTP-\(S\)-bound form of \(\alpha_q\) (7) to map the results of our mutagenesis studies. 88% of the residues in \(\alpha_q\) can be aligned with identical residues in \(\alpha_{i1}\), while 67% of the \(\alpha_{i1}\) residues can be aligned with identical residues in \(\alpha_{i2}\). Since the structures of the active (GTP-\(S\)-bound) forms of \(\alpha_{i1}\) (7) and \(\alpha_{i2}\) (5) are virtually identical, the structure of \(\alpha_{i1}\) is an excellent model for that of \(\alpha_{i2}\). Our mutagenesis analysis of Switches I-III in \(\alpha_{i2}\) and the 78-residue effector-specifying \(\alpha_q\) segment, residues 245–322, focused on solvent-exposed residues. In addition, most of the alanine substitutions in the 78-residue segment were of residues that are: 1) different from the homologous \(\alpha_q\) residues and 2) conserved among the \(\alpha_q\) isoforms. The thoroughness of this study is demonstrated by the fact that the residues in Switches I-III that were not mutated and the residues in the 78-residue segment that meet criteria 1 and 2 but were not mutated represent a very small fraction of the available surface area (shown in white in Fig. 6A).

The alanine substitutions that caused the largest decrease in the ability of \(\alpha_q\)RCEE to inhibit adenylyl cyclase were in the middle of the \(\alpha_2\) helix in Switch II (red in Fig. 6A). The effector-interacting surfaces of \(\alpha_q\) and \(\alpha_s\) overlap exactly in this region (magenta in Fig. 6B) where the sequences of the two \(\alpha_q\) subunits are highly conserved (Fig. 7). However, the \(\alpha_2\) helix at the carboxyl-terminal end of Switch II is important for the interaction of \(\alpha_q\) (12) but not \(\alpha_{i2}\) (Fig. 1) with adenylyl cyclase (blue in Fig. 6B).

The alanine substitutions within the 78-residue effector-specifying segment that caused a moderate reduction in the ability of \(\alpha_q\)RCEE to inhibit adenylyl cyclase (orange in Fig. 6A) were in the amino terminus of \(\alpha_3\) (Construct 1) and in the \(\alpha_2\) helix loop (Constructs 8 and 9) (Fig. 6B). The amino terminus of \(\alpha_3\) (red in Fig. 6B) is important for the effector interactions of \(\alpha_{i2}\) (4), but not \(\alpha_q\) (12), while mutations in the \(\alpha_2\) helix loop (blue in Fig. 6B) disrupt interaction between \(\alpha_q\) and adenylyl cyclase (12) but do not have a significant effect on the \(\alpha_q\)-adenylyl cyclase interaction (Fig. 4). Residues in the \(\alpha_2\) helix loop found to be important for specifying the effector interactions of both \(\alpha_{i2}\) and \(\alpha_q\) are magenta in Fig. 6B.
DISCUSSION

The studies reported here investigated two key aspects of α subunit-effector interactions, GTP-dependence and specificity. We found that in the case of α₂, these two components of effector interaction are mediated by distinct regions of surface residues. GTP-dependent effector interaction is mediated by Switch II residues that are conserved among α subunits (Fig. 1) while specificity (inhibition of adenylyl cyclase) is mediated by nonconserved residues (the amino terminus of α3 and the α4/β6 loop) outside of the conformational switch regions (Fig. 4). In contrast, in the case of α₁, Switch II plays a role in regulating both the GTP dependence of effector interaction as well as effector specificity. The conserved Switch II region is required for GTP-dependent activation of adenylyl cyclase (Fig. 3) while nonconserved Switch II residues, as well as residues outside of the conformational switch regions (the α3/β5 and α4/β6 loops), are involved in regulating effector specificity (12). In the case of α₁, the conformational switch regions and regions that don’t switch conformation (α3 and the α3/β5 loop) interact with distinct regions of the effector molecule, PDE (35).

Taken together, our results and those of others indicate that two α subunit regions, Switch II and the α4/β6 loop, may be important for effector interactions in general (Fig. 7). The conserved middle region of Switch II has been shown to be important for the interaction between α₁ and PDE. Mutation of a conserved tryptophan in α₁ reduces binding to PDE (36) while mutation of a conserved glutamate causes constitutive activation of PDE by the GDP-bound form of α₁ (37). The α4/β6 loop is involved in specifying the effector interactions of at least three α subunits (Fig. 7). We previously found that replacement of α₂ residues in this region by their α₁ homologs prevents α₁ from activating adenylyl cyclase without preventing the mutant protein from attaining the GTP-dependent active conformation (12). Rarick et al. (16) found that a 22-amino acid peptide (α₁ residues 293–314) activates PDE. Within this region, Spickofsky et al. (17) identified five residues in which substitutions of homologs from other α subunits block PDE activation by peptides. Three of these residues are in the α4 helix and two are in the α4/β6 loop. Mutations in the α4/β6 loop of α₁ and α₂, but not in α₃ cause decreases in effector modulation. In the case of α₃, α₄ and the α₄/β₆ loop have been implicated in PLC activation in studies using peptides (38). However, chimera studies showed this region could be replaced with α₁ sequence without affecting PLC activation (39).

Since α₁ and α₂ have opposite effects on adenylyl cyclase activity, the conserved region of Switch II required for the effector interactions of both α subunits is most likely involved in regulating GTP-dependent effector binding. Of the three residues found to be important for inhibition of adenylyl cyclase by α₁, Arg-209 and Ile-213 are identical in the sequences of α₁ and α₂ (see Fig. 7). The third residue is conserved but not identical between the two α subunits (Lys-210 in α₂, Arg-232 in α₁). However, α₁/α₂ chimera studies showed that substitution of lysine for arginine at position 232 in α₁ has no effect on activation of adenylyl cyclase (12). Furthermore, the α₁ residue corresponding to Lys-210 is an arginine residue and α₁/α₂ chimera studies showed that substitution of arginine at this position does not affect inhibition of adenylyl cyclase (14). Therefore, these Switch II residues do not determine the nature of adenylyl cyclase modulation by α₁ and α₂.

Although all α subunits are conserved in this Switch II region, other α subunits do not modulate adenylyl cyclase, with the exception of a weak inhibition of type I adenylyl cyclase by α₁ (11). A possible explanation for this selectivity is that other α subunits contain residues that preclude a productive adenylyl cyclase interaction. If so, then replacing α₁ residues in the amino terminus of α₃ and in the α₄/β₆ loop with the homologous residues from α₁ or other α subunits might cause a larger reduction in ability to inhibit adenylyl cyclase than was observed for alanine substitutions.

Our studies show that the effector-specifying regions of α₁ and α₂ overlap but are not identical (see Fig. 6B). Studies using α₁ subunit chimeras localized the region of α₂ that specifies inhibition of adenylyl cyclase to a 78-residue segment (amino acids 245–322) that extends from α₃ to β₆ (14). Residues corresponding to two of the three α₁ regions that specify activation of adenylyl cyclase (12, 15), the α₃/β₅ and α₄/β₆ loops, are included in this segment. The only region of overlap that we have found among the effector-specifying regions of α₁ and α₂ is in the α₄/β₆ loop. Effector-specifying regions unique for α₁ are located in the α₃/β₅ loop and in the carboxyl-terminal part of Switch II (12). Similarly, mutation of a single residue in the amino terminus of α₃ reduces the ability of α₂ to inhibit adenylyl cyclase but is not required for the activation of adenylyl cyclase by α₁ (12).

Since both α₁ and α₂ interact with adenylyl cyclase, the effector-specifying residues of each α subunit presumably determine whether activation or inhibition will result from α₁ binding. However, the effector-specifying residues of α₁ appear to contribute more to the interaction with adenylyl cyclase than do those of α₂. Substitutions in the effector-specifying segment of α₂ do not cause as large a decrease in the ability to inhibit adenylyl cyclase as do substitutions in the conserved middle part of Switch II. However, mutations in two of the effector-specifying regions of α₁, the nonconserved carboxy-terminal part of Switch II and the α₃/β₅ loop, decrease effector activation to the same extent as do mutations in the conserved Switch II region. Consistent with our results, Tausig et al. (11) found that replacing α₁ residues with α₂ homologs in the α₃/β₅ loop results in an α subunit that weakly activates certain adenylyl cyclase isoforms. Thus, the effector-specifying regions of α₂ appear to be dominant over those of α₁.

Mutagenesis studies of hGH and its receptor, for which a structure of the hormone-receptor complex is available (40), have characterized the functional importance of residues in the binding interface. Individual replacements of residues in hGH (41) and its receptor (42) demonstrated that only a small subset of the residues at the center of the contact region contribute substantially to binding affinity. However, hGH residues in the periphery of the interface, which do not contribute much to the affinity of binding (41), are important for the specificity of binding (43).

In a similar manner, our studies of the interaction between α₂ and adenylyl cyclase implicate Switch II residues as being the major contributors to this binding interaction. Substitutions in the effector-specifying segment of α₂ have a more modest effect on the ability of α₁/RCCE to inhibit adenylyl cyclase. In the absence of any structures of α subunit-effector complexes, we predict that interactions between these proteins will include the conserved Switch II region as well as nonconserved specificity regions but that, as seen in the case of hGH and its receptor (41, 42), the contact surfaces may be larger than the “functional epitopes” defined by our mutagenesis studies.

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