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A mutation in the heterotrimeric stimulatory guanine nucleotide binding protein α-subunit with impaired receptor-mediated activation because of elevated GTPase activity

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ABSTRACT **It has been reported that substitution of Arg^{258}, a residue within the GTPase domain of the heterotrimeric guanine nucleotide binding protein (G protein) α-subunit (α_s), to alanine (α_s-R258A) results in decreased activation by receptor or aluminum fluoride (AlF_4^-) and increased basal GDP release. Arg^{258} interacts with Gln^{170} in the helical domain, and, presumably, loss of this interaction between the GTPase and helical domain leads to more rapid GDP release, resulting in decreased activation by AlF_4^- and increased thermolability. In this study, we mutate Gln^{170} to alanine (α_s-Q170A) and demonstrate that this mutant, like α_s-R258A, has decreased activation by AlF_4^-, increased thermolability (both reversed in the presence of excess guanine nucleotide), and an increased rate of GDP release. However, unlike α_s-R258A, α_s-Q170A does not have impaired receptor-mediated activation. Therefore, this interdomain interaction is critical to maintain normal guanine nucleotide binding (and hence normal activation by AlF_4^-) but is not important for receptor-mediated activation. In single turnover GTPase assays, the catalytic rate for GTP hydrolysis of α_s-R258A was 14-fold higher than normal whereas that of α_s-Q170A was unaffected. Examination of the α_s crystal structure suggests that Arg^{258}, through interactions with Glu^{50}, might constrain the position of Arg^{201}, a residue critical for catalyzing the GTPase reaction. This is an example of a mutation in a heterotrimeric G protein that results in an increased intrinsic GTPase activity and provides another mechanism by which G protein mutations can impair signal transduction.**

Heterotrimeric guanine nucleotide binding proteins (G proteins) mediate the activation of intracellular effectors by heptahelical receptors (reviewed in refs. 1–3). Each G protein has a distinct α-subunit that binds guanine nucleotide and modulates the activity of specific downstream effectors. For the stimulatory G protein α-subunit (α_s), these include the stimulation of adenylyl cyclase and modulation of ion channels (4, 5). In the basal (inactive) state, GDP-bound α-subunit is associated with a βγ-dimer. Activation by receptor results in a conformational change in the α-subunit, leading to the exchange of GTP for GDP and dissociation from βγ. The GTP-bound α-subunit interacts with and regulates specific effectors. Hydrolysis of bound GTP to GDP by an intrinsic GTPase activity within the α-subunit returns the G protein to the inactive state. Mutations that slow this intrinsic GTPase activity lead to constitutive activation, and such activating mutations are found in sporadic endocrine tumors (6) and in patients with the McCune-Albright syndrome (7). G proteins also can be activated by guanosine-5′-*O*-3-thiotriphosphate (GTPγS), a nonhydrolyzable analogue of GTP, or aluminum fluoride (AlF_4^-), which binds to GDP-bound α-subunits and mimics the γ-phosphate of GTP.

G protein α-subunits have two domains, a *ras*-like GTPase domain, which includes the sites for guanine nucleotide binding and effector interaction, and a helical domain (8–14). Because the guanine nucleotide resides in a cleft between the two domains, the helical domain may be important in slowing GDP release in the inactive state. Comparison of the structures of inactive (GDP-bound) and activated (GTPγS- or AlF_4^--bound) α-subunits reveals three regions within the GTPase domain (named switches 1, 2, and 3) that undergo conformation changes on activation. On activation, switches 2 and 3 move toward each other and form multiple interactions that stabilize the active state (15–17). Residues in switch 3 also make contact with residues in the helical domain, and these interactions have been implicated in the maintenance of guanine nucleotide binding (12, 18) and in receptor-mediated activation (18–20).

An α_s mutation in a patient with Albright hereditary osteodystrophy in which the switch 3 residue Arg^{258} is substituted with tryptophan§ has been identified, and it has been demonstrated that this substitution (as well as the Arg^{258} to alanine substitution, α_s-R258A) leads to increased GDP release (resulting in decreased activation by AlF_4^- and increased thermolability) and impaired receptor-mediated activation (18). Based on the α_s crystal structure (14, 18), Arg^{258} interacts with residue Gln^{170} within the helical domain. Loss of this interaction would be predicted to open the cleft between the GTPase and helical domain, resulting in more rapid GDP release, as observed for the Arg^{258} substitution mutants. It also has been suggested that interactions between Arg^{258} and the helical domain are important for receptor-mediated activation (18–20). In this study, we show that mutating Gln^{170} to alanine (α_s-Q170A) also leads to increased GDP release (along with decreased activation by AlF_4^-) but does not affect receptor-mediated activation. Therefore, interactions between Arg^{258} and Gln^{170} are important for maintaining guanine nucleotide binding but are not important for activation by receptor. We further show that α_s-R258A (but not α_s-Q170A) has a markedly elevated intrinsic GTPase rate, which will lead to more rapid inactivation. Arg^{258}, through mutual interactions with Glu^{50}, may constrain Arg^{201}, a residue critical for catalyzing GTP hydrolysis. Disruption of the interaction between Arg^{258} and Glu^{50} may relieve this constraint and allow Arg^{201} to more efficiently interact with the γ-phosphate of GTP in the transition state. This is an example of a mutation in a heterotri-

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Abbreviations: G protein, guanine nucleotide binding protein; α_s, stimulatory G protein α-subunit; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); WT, wild-type.

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§All numbering is based on the α_s-1 sequence reported by Kozasa *et al.* (21).

meric G protein that increases the intrinsic GTPase activity and provides another mechanism by which receptor signaling can be impaired by G protein mutations.

MATERIALS AND METHODS

Generation of α_s *in Vitro* Transcription/Translation Products. To generate α_s-Q170A, PCR was performed by using wild-type α_s (α_s-WT) cDNA as template (18), upstream primer 5′CCCTCCCGAATTCTATGAGCATGCCAAGGCTCTGTGGGAGGATGAAGGAGTGCGTGCCTG3′, and downstream mutagenic primer 5′CTGCTTGATCACGTCGATCTTGTCCAGGAAGTACTGGGCACAGTCAATCAGCGCGTACTCG3′. The PCR product was digested with *Hin*cII and *Sse*8387I and was ligated into pBluescript II SK(+) (Stratagene) containing α_s-WT cDNA (splice variant α_s-1; ref. 21) in which the same *Hin*cII-*Sse*8387I restriction fragment had been removed. *In vitro* transcription/translation was performed on α_s plasmids as described (18, 22). Synthesis of full-length α_s from each construct was confirmed by immune precipitation of *in vitro* translated products with RM antibody, directed against the α_s C-terminal decapeptide (23).

Adenylyl Cyclase and Trypsin Protection Assays. *In vitro* transcription/translation products (10 μl of translation medium) were reconstituted into 25-μg purified S49 cyc-plasma membranes and were tested for stimulation of adenylyl cyclase in the presence of various agents at 30°C as described (18, 22, 24). Trypsin protection assays were performed as described (18, 22). In brief, [^{35}S]methionine-labeled *in vitro*-translated α_s was incubated at 30 or 37°C for 1 h with either no activators, 100 μM GTPγS, 10 mM NaF/10 μM $AlCl_3$ (AlF_4^-), or AlF_4^- plus 2 mM GDP, and then was digested with 200 μg/ml tosyl-L-phenylyalanine chloromethyl ketone-trypsin for 5 min at 20°C (18, 22). Digestion products were separated on 10% SDS polyacrylamide gels.

GTPγS Binding Assays. Plasmid pQE60 containing the long form of bovine α_s cDNA with a hexa-histidine extension at the C terminus was mutated to α_s-Q170A by site-directed mutagenesis by using the Quickchange kit (Stratagene). α_s proteins were purified from *Escherichia coli* and rates of GTPγS binding were measured and calculated as described (18, 25, 26).

Single Turnover GTPase Assays. Purified α_s (100 pmol, 40 nM) was incubated in 50 mM Hepes (pH 8.0), 1 mM DTT, 5 mM EDTA, 0.1% Lubrol-PX, and 5 μM [^{32}P-γ]GTP (3,000 cpm/pmol) at 30°C for 30 min (α_s-WT) or 20°C for 20 min (α_s-R258A and -Q170A). Reactions were placed on ice for 5 min, and the GTPase reaction was initiated by the addition of $MgCl_2$ and GTP to final concentrations of 20 mM and 200 μM, respectively. At various time points, 50-μl aliquots (corresponding to 10 pmol of α_s) were removed and immediately added to 750 μl of ice-cold 5% (wt/vol) Norit-SA3 (Aldrich) in 50 mM NaH_2PO_4 (pH 2.0). After microcentrifugation, ^{32}P was measured in the supernatant by liquid scintillation. k_{cat} was determined by fitting the data into the equation $P = P_{max}(1 - e^{-kt})$ (where P represents phosphate released at time t and P_{max} represents maximal phosphate release) by using PRISM 2.01 (GraphPad, San Diego).

RESULTS

Substitution of α_s Gln^{170} Leads to Impaired Activation by AlF_4^- but Normal Receptor-Mediated Activation. A mutation in a patient with Albright hereditary osteodystrophy encoding a substitution of residue Arg^{258} within the switch 3 region of α_s has been identified, and it has been demonstrated that substitution of this residue impairs AlF_4^-- and receptor-mediated activation whereas activation by GTPγS is unaffected (18). The mutant has an increased rate of GDP release, and excess GDP restores AlF_4^--induced activation. Because GDP binding is a prerequisite for AlF_4^- binding and activation, the impaired activation by AlF_4^- is a function of the decreased ability of this mutant to maintain the GDP-bound state. Arg^{258} interacts with the conserved residue Gln^{170} within the helical domain (18), and disruption of this interaction would be predicted to open the cleft between the GTPase and helical domains, leading to increased GDP release.

To further characterize the role of this interdomain interaction in maintaining the GDP-bound basal state and in receptor-mediated activation, we examined the effect of substituting Gln^{170} on G protein function. A Gln^{170} to alanine substitution mutant (α_s-Q170A) was cloned into the transcription vector pBluescript, and the *in vitro* transcription/translation products were compared with those of α_s-WT in various biochemical assays. After reconstitution of translation products into purified S49 cyc-membranes (which lack endogenous α_s), α_s-Q170A was efficient at stimulating adenylyl cyclase in the presence of GTPγS or activated receptor (the β-adrenergic agonist isoproterenol plus GTP), with a response similar to or slightly greater than that of α_s-WT (Table 1). In contrast, the ability of α_s-Q170A to stimulate adenylyl cyclase in the presence of AlF_4^- was decreased significantly. For comparison, previously published results for α_s-R258A (expressed as percent of α_s-WT) also are shown (18). Although the two mutants respond similarly to GTPγS and AlF_4^-, receptor-mediated activation is markedly reduced in α_s-R258A but is normally maintained in α_s-Q170A. Sucrose density gradient experiments demonstrated that both mutants are stable for 1 h at the same temperature that the adenylyl cyclase assays were performed (30°C; ref. 18 and data not shown). Therefore, thermolability is unlikely to explain the decreased function of these mutants in the adenylyl cyclase assay.

We next examined the ability of AlF_4^- or GTPγS to protect α_s-Q170A from trypsin digestion, which measures the ability of

Table 1. Adenylyl cyclase stimulation by α_S-Q170A [pmol cAMP/ml translation product/15 min (percent of WT)]

α_S	GTP, 100 μM	Isoproterenol, 10 μM, + GTP, 100 μM	GTPγS, 100 μM	AlF_4^{-b*}
WT	25 ± 10	233 ± 5	259 ± 21	225 ± 12
Q170A	29 ± 6	246 ± 24 (106 ± 11)	305 ± 4 (118 ± 10)	113 ± 9 (50 ± 5)
R258A†		(24 ± 3)	(132 ± 6)	(62 ± 9)

In vitro transcription/translation products were mixed with purified cyc- membranes and were assayed for adenylyl cyclase stimulation at 30°C. Results are expressed as the mean ± SD, (σ_{n-1}) of triplicate determinations and are representative of three independent experiments. The levels of expression of α_S-WT and -Q170A were similar as determined by *in vitro* translation with [^{35}S]methionine, SDS/PAGE, and phosphorimaging. Background values determined from mock transcription/translation reactions (in pmol cAMP/ml translation medium/15 min; GTP, 39 ± 4; isoproterenol/GTP, 46 ± 1; GTPγS, 58 ± 2; and AlF_4^-; 68 ± 8) were subtracted from each determination.

*10 mM NaF, 10 μM $AlCl_3$, and 100 μM GDP.

†The results for α_S-R258A (shown here as percent of α_S-WT) have been published (18) and are shown here for comparison with α_S-Q170A.

each agent to bind to α_s and to induce the active conformation (27). When α_s attains the active conformation, specific arginine residues within the switch 2 region become inaccessible to trypsin digestion (9), leading to the generation of a partially protected, 38-kDa digestion product. α_s-WT was well protected by AlF_4^- or GTPγS at temperatures up to 37°C (Fig. 1). α_s-Q170A also was well protected by GTPγS at 37°C. Consistent with the results of the adenylyl cyclase assays, AlF_4^- was less effective than GTPγS in protecting α_s-Q170A from trypsin digestion, particularly at higher temperature (37 vs. 30°C; Fig. 1). Moreover, the ability of AlF_4^- to protect α_s-Q170A was restored by addition of excess GDP in the incubation. These results are similar to those obtained previously for α_s-R258A (18) and suggest that both mutants have a decreased ability to bind GDP, particularly at higher temperatures.

To directly determine the rate of GDP release in the basal state, bovine α_s-WT and -Q170A, each with a C-terminal hexahistidine tag, were purified from *E. coli*, and the time course of GTPγS binding to each was examined. The rate of GTPγS binding has been shown to be limited by the rate of GDP dissociation, and the experimentally determined values of these two rates are essentially identical (18, 28, 29). The k_{app} for GTPγS binding at 20°C was 0.05 min^{-1} for α_s-WT versus 0.23 min^{-1} for α_s-Q170A (Fig. 2), indicating that the Q170A substitution leads to a significantly increased rate of GDP release (as reflected in the rate of GTPγS binding). Similar results were reported for α_s-R258A, with a k_{app} of 0.36 min^{-1} (18). α_s-Q170A would be predicted to be more thermolabile because a greater proportion will have no bound guanine nucleotide, as has been shown for α_s-R258A (18) and for other mutants with decreased affinity for guanine nucleotide (22, 30). Sucrose density gradient experiments of *in vitro* translates as previously performed for α_s-R258A (18) demonstrated that, in fact, α_s-Q170A is more thermolabile but can be stabilized in the presence of excess guanine nucleotide and is capable of interacting with $\beta\gamma$ dimers (data not shown).

Impaired Receptor-Mediated Activation of α_s-R258A is Associated with Increased Intrinsic GTPase Activity. The Arg^{258}-Gln^{170} interdomain interaction is critical for maintaining the GDP-bound basal state because disruption of this interaction by substituting either residue increases GDP release, which leads to decreased activation by AlF_4^- and increased thermolability. However, the fact that activation by isoproterenol plus GTP is unaffected by the Gln^{170} substitution suggests that this interdomain interaction is not important for receptor-mediated activation and that substitution of Arg^{258} impairs receptor-mediated activation by another mechanism.

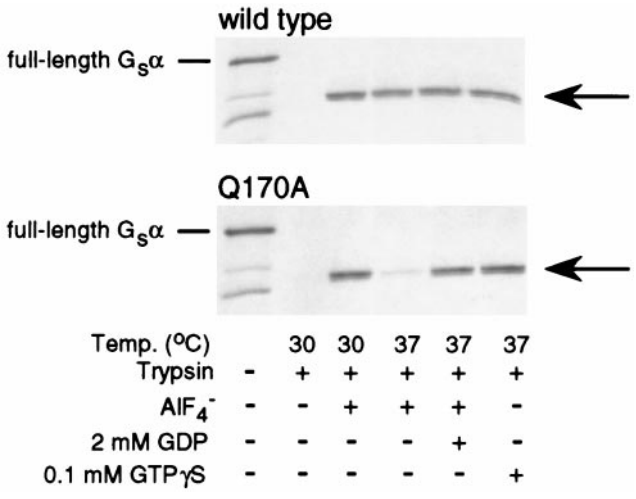

FIG. 1. Trypsin protection of [^{35}S]methionine-labeled *in vitro* translates of α_s-WT (*Upper*) and α_s-Q170A (*Lower*). In each gel, the full-length undigested α_s (52 kDa) is shown in the first lane, and complete digestion in the absence of activators is shown in the second lane. The next four lanes show the results after preincubation at the temperatures and in the presence of various agents indicated below. The smaller products in the left lane are attributable to initiation of protein synthesis at downstream methionine codons. G_s, stimulatory G protein.

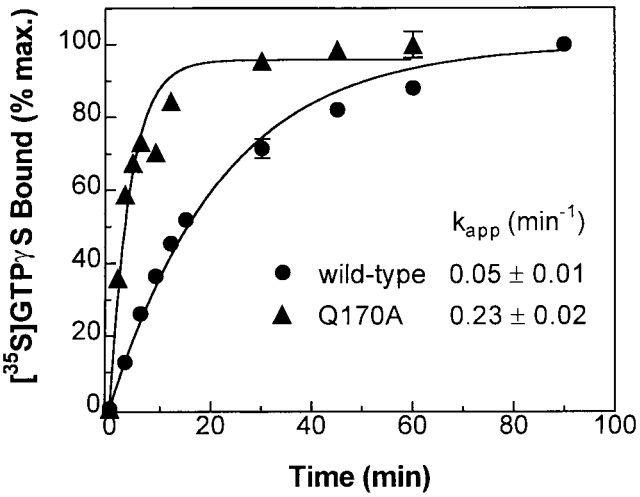

FIG. 2. Time course of GTPγS binding to purified α_s. Bovine α_s-WT and -Q170A, each with a C-terminal hexahistidine extension, were purified from *E. coli*, and the rate of GTPγS binding for each was determined. α_s-WT (●) and -Q170A (▲) were incubated with 1 μM [^{35}S]GTPγS (≈10,000 cpm/pmol) at 20°C for varying times, and the amount of bound GTPγS was determined (18). Each data point is the mean ± SD of triplicate determinations. The apparent on rates for GTPγS (k_{app}) are shown (mean ± SE of six experiments).

One possible mechanism for defective receptor-mediated activation is an increased rate of GTP hydrolysis leading to more rapid inactivation. Based on the fact that Arg^{258} interacts indirectly (through Glu^{50}) with Arg^{201}, a residue critical for catalyzing the hydrolysis of bound GTP to GDP (see Fig. 4 and *Discussion*), we hypothesized that impaired receptor-mediated activation of α_s-R258A might be caused by an increase in its intrinsic GTPase activity. To test this directly, the catalytic rates of GTP hydrolysis for purified α_s-WT, -R258A, and -Q170A were determined in a single turnover assay (Fig. 3). After loading each with [^{32}P-γ]GTP in the absence of magnesium, the GTPase reaction was initiated by the addition of magnesium and unlabeled GTP, and the rate of phosphate release was measured. At 0°C, the intrinsic catalytic rate of GTP hydrolysis by α_s-R258A (k_{cat} 7.0 ± 1.3 min^{-1}) was 14-fold higher than that by α_s-WT (k_{cat} 0.5 ± 0.1 min^{-1}). In contrast, the intrinsic GTPase activity of α_s-Q170A (k_{cat} 0.3 ± 0.1 min^{-1}) was similar to that of α_s-WT. This is consistent with the results of the adenylyl cyclase stimulation assays because, for α_s-R258A, increased GTPase activity is associated with reduced stimulation of adenylyl cyclase by receptor agonist (isoproterenol plus GTP) whereas for α_s-Q170A, both receptor-mediated activation and the GTPase activity are normal.

DISCUSSION

It has been suggested that interactions between Arg^{258} in the GTPase domain of α_s and residues within the helical domain are important for both guanine nucleotide binding and receptor-mediated activation based on the observations that both are impaired in α_s-R258A (18) and that replacing the α_s helical domain with the helical domain of α_{i2} corrects the receptor-mediated activation defect of α_s-R258A (20). The GTPγS-α_s crystal structure reveals that, amongst the helical domain residues, Arg^{258} most closely interacts with the conserved residue Gln^{170} (18). To further examine the importance of

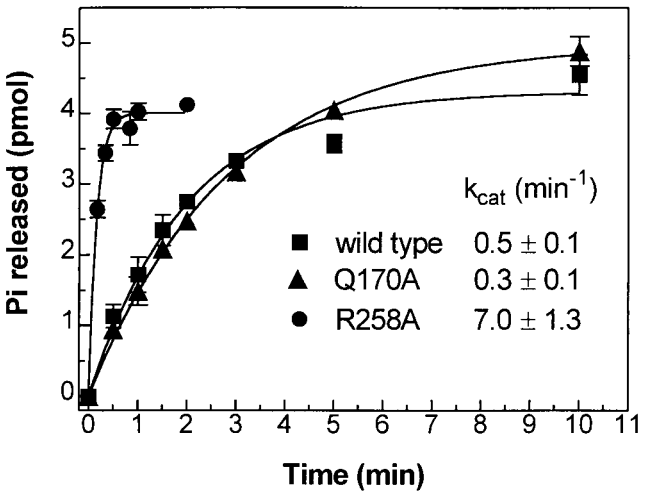

FIG. 3. Single turnover GTP hydrolysis rate of purified α_s isoforms. GTP hydrolysis of α_s-WT (■), α_s-Q170A (▲), and α_s-R258A (●) at 0°C is shown as the amount of phosphate (Pi) released as a function of time. The calculated catalytic rates (k_{cat}) also are shown. Each data point represents the mean ± SE of three experiments. Baseline counts at time zero ranged from 30 to 55% of maximum counts released. The majority of baseline counts is attributable to free inorganic phosphate contamination of the stock [^{32}P-γ]GTP.

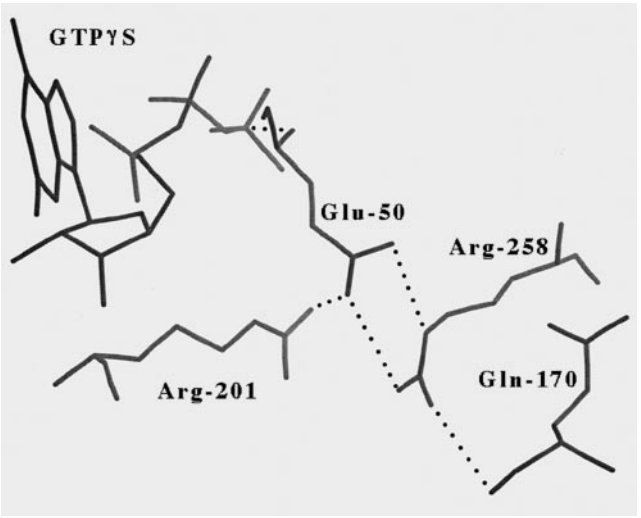

FIG. 4. Detailed view of interactions between the side chains of Arg^{258} in switch 3, Glu^{50}, and Arg^{201} based on the crystal structure of GTPγS-α_s. The interaction between the side chain of Arg^{258} and the backbone oxygen of Gln^{170} also is shown. Hydrogen bonds are shown as dotted lines. The figure was generated with LOOK 3.0 (Molecular Applications Group, Palo Alto, CA) by using coordinates for the short form of bovine GTPγS-α_s [Protein Data Bank accession code 1AZT (14)], although the numbering in the figure corresponds to the long form of α_s.

interdomain interactions in G protein function, we decided to disrupt the Arg^{258}–Gln^{170} interaction from the opposite side by mutating the Gln^{170} to alanine. This mutant, like α_s-R258A, had an increased rate of GDP release associated with increased thermolability and decreased activation by AlF_4^-. This provides further evidence that the interdomain interaction acts as a "lid" over the cleft that contains the guanine nucleotide binding pocket and that disruption of this interaction from either side opens the cleft, leading to increased GDP release in the basal state.

Although the substitution of Gln^{170} appears to disrupt the interdomain interaction, based on its effects on guanine nucleotide binding, this substitution has no effect on receptor-mediated activation because α_s-Q170A was able to normally stimulate adenylyl cyclase in the presence of isoproterenol plus GTP. This suggests that the interdomain interactions (or at least the ones involving Gln^{170}) are not critical for receptor-mediated activation and that the receptor activation defect in α_s-R258A is caused by the disruption of interactions between Arg^{258} and other α_s residues.

Examination of the GTPγS-α_s crystal structure reveals that Arg^{258} also interacts with Glu^{50} within the GTPase domain and that Glu^{50} interacts with Arg^{201} (Fig. 4). Arg^{201} is critical for catalyzing the hydrolysis of bound GTP to GDP, and mutations of this residue lead to constitutive activation because of a markedly reduced intrinsic GTPase activity (31). It is conceivable that disruption of the interaction between Arg^{258} and Glu^{50} might relieve constraints on Arg^{201}, which would allow Arg^{201} to adopt a more favorable position for catalyzing the hydrolysis of GTP. The resulting increased GTPase activity should lead to impaired receptor-mediated activation because the GTP-bound active state would be short-lived. To test this hypothesis, the intrinsic GTPase activity of α_s-R258A was measured directly in single turnover GTPase assays and was shown to be 14-fold higher than that of α_s-WT. The fact that the GTPase activity of α_s-Q170A measured in the same assay was normal demonstrates that the altered GTPase activity of α_s-R258A was specific for the Arg^{258} substitution and was not caused by disruption of the interaction between Arg^{258} and the helical domain.

Defects in receptor-mediated activation may result from decreased receptor-catalyzed nucleotide exchange, inability to attain the active conformation or interact with effector, or an increased rate of GTP hydrolysis leading to more rapid inactivation. The ability of α_s-R258A to attain the active conformation and interact with effector is demonstrated by the fact that the mutant can normally activate adenylyl cyclase in the presence of GTPγS (18) or when the Arg^{201} residue also is mutated to block hydrolysis of bound GTP (20). Impaired receptor-catalyzed nucleotide exchange may result from inability of the α-subunit to interact with receptor or $\beta\gamma$ or inability of the receptor-G protein interaction to stimulate guanine nucleotide exchange. We have demonstrated that α_s-R258A is capable of binding to $\beta\gamma$ (18). An α_s mutant created by Grishina and Berlot (20) with substitution of four switch 3 residues, including the R258A substitution, has somewhat increased apparent affinity for the β-adrenergic receptor, and it is therefore unlikely that the single R258A substitution has a major effect on receptor–G protein interactions. In their study (20), the above mutant (which includes the R258A substitution) has increased guanine nucleotide exchange in the absence of isoproterenol [consistent with our findings for α_s-R258A (18)] but has only minor changes in the rate of guanine nucleotide exchange in the presence of isoproterenol. It is therefore unlikely that the R258A substitution has a major effect on receptor-catalyzed guanine nucleotide exchange. In addition, deletion of the entire switch 3 region of transducin has no effect on $\beta\gamma$ binding or receptor-catalyzed guanine nucleotide exchange (15). Although it is possible that the α_s-R258A mutant has minor defects in receptor-catalyzed guanine nucleotide exchange, given our results and those described above, we feel that the receptor-mediated activation defect of α_s-R258A is caused primarily by a markedly increased GTPase activity. Evidence that increased GTPase rates can lead to decreased receptor-mediated activation is provided by the effects of regulators of G protein signaling proteins (which increase the GTPase rate of α-subunits) on receptor signaling (32).

Grishina and Berlot (20) showed that the receptor activation defect of α_s-R258A can be corrected by replacing the α_s helical domain with that of α_{i2}. Because, in α_{i2}, alanine is the residue in the position that corresponds to Arg^{258} in α_s, they concluded

(20) that replacing the α_s with the α_{i2} helical domain restores interdomain interactions in α_s-R258A and that this corrects the receptor activation defect. Although this is possible, the fact that we can interrupt an important interdomain interaction in α_s-Q170A (confirmed by its effects on GDP binding) without affecting receptor-mediated activation makes this explanation less likely. Although interactions between Arg^{258} and other helical domain residues might be important for receptor-mediated activation, the only helical domain residues that make direct contacts with Arg^{258} are identical in α_s and α_{i2}. It is also possible that substitution of the helical domain corrects the increased GTPase activity of α_s-R258A. It is interesting to note that substituting the α_{i2} helical domain into α_s-WT leads to constitutive activation (19, 20), which could result from decreased GTPase activity.

This is an example in which receptor signaling is impaired by a mutation that activates the intrinsic GTPase activity of a heterotrimeric G protein; mutations that lead to increased GTPase activity have been identified in the small guanine nucleotide binding proteins EF-Tu (33) and ras (34). This underscores the need to examine the GTPase activity when assessing the function of mutant G proteins with impaired receptor-mediated activation. Finally, these studies demonstrate that identification and analysis of naturally occurring G protein mutations in patients can lead to significant advances in our understanding of how G proteins function.

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