Structure of the Regulator of G Protein Signaling 8 (RGS8)-Gαq Complex
MOLAR BASIS FOR Gα SELECTIVITY*

Received for publication, December 22, 2015, and in revised form, January 8, 2016. Published, JBC Papers in Press, January 11, 2016, DOI 10.1074/jbc.M115.712075

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Regulator of G protein signaling (RGS) proteins interact with activated Gα subunits via their RGS domains and accelerate the hydrolysis of GTP. Although the R4 subfamily of RGS proteins generally accepts both Gαq and Gαq11 subunits as substrates, the R7 and R12 subfamilies select against Gαq11. In contrast, only one RGS protein, RGS2, is known to be selective for Gαq11. The molecular basis for this selectivity is not clear. Previously, the crystal structure of RGS2 in complex with Gαq revealed a non-canonical interaction that could be due to interfacial differences imposed by RGS2, the Gα subunit, or both. To resolve this ambiguity, the 2.6 Å crystal structure of RGS8, an R4 subfamily member, was determined in complex with Gαq. RGS8 adopts the same pose on Gαq as it does when bound to Gαγ3, indicating that the non-canonical interaction of RGS2 with Gαq is due to unique features of RGS2. Based on the RGS8-Gαq structure, residues in RGS8 that contact a unique α-helical domain loop of Gαq were converted to those typically found in R12 subfamily members, and the reverse substitutions were introduced into RGS10, an R12 subfamily member. Although these substitutions perturbed their ability to stimulate GTP hydrolysis, they did not reverse selectivity. Instead, selectivity for Gαq seems more likely determined by whether strong contacts can be maintained between α6 of the RGS domain and Switch III of Gαq, regions of high sequence and conformational diversity in both protein families.

Many transmembrane signaling events are transduced inside the cell by heterotrimeric G proteins, which are activated by cell surface G protein-coupled receptors. G protein-coupled receptors stimulate the exchange of bound GDP for GTP on the Gα subunit, which then separates from the Gβγ subunits and interacts with downstream effectors (1). After hydrolyzing GTP to GDP, the Gα subunit is deactivated and is rapidly sequestered by Gβγ. In biological processes, such as the visual response, deactivation of Gα has been observed at much faster rates than those measured for isolated Gα subunits in vitro (2, 3). This discrepancy helped lead to the discovery of a family of GTPase-activating proteins (GAPs),2 now known as regulator of G protein signaling (RGS) proteins (4–6). RGS proteins contain a conserved helical domain called the RGS domain that directly binds to the three switch regions (SwI–III) of the Gα subunit and stabilizes them in a transition state conformation (7).

RGS domains are divided into four subfamilies based on sequence homology and substrate preference: RZ, R4, R7, and R12 (8). All utilize Gαq/11 subunits as substrates, although some RZ members seem selective for Gαq subunits (9). A recent study using surface plasmon resonance indicated that the RGS domains that belong to the R7 and R12 subfamilies bind weakly or not at all to Gαq, whereas the RZ and R4 subfamilies tend to interact with both Gαq and Gαq11 (10). The exception is RGS2, an R4 subfamily member that is uniquely selective for Gαq11 (11). The underlying molecular mechanism dictating RGS selectivity has not been fully answered, and past research has focused mainly on RGS2 in part because of its strong link to hypertension and cardiac hypertrophy via its regulation of Gαq signaling in vivo (12–16). RGS2 can be altered to enhance its selectivity for Gαq11 by making mutations at three positions unique to RGS2 (Cys<sup>106</sup>, Asn<sup>184</sup>, and Glu<sup>191</sup>) that interact with the G protein, primarily near the Sw1 region (11). These residues are conserved as Ser, Asp, and Lys, respectively, in other RGS proteins. The crystal structure of an RGS2 mutant with conversion of these three residues to their equivalents in other RGS domains (RGS2<sup>30DKS</sup>) in complex with Gαq confirmed that the mutant binds in the same canonical orientation as observed in other RGS-Gα<sub>q</sub> complexes (17).

Subsequently, the structure of wild-type RGS2 in complex with Gαq revealed a distinct binding mode (18). Because this was also the first RGS domain-Gαq complex to be structurally characterized, it was unclear whether the significant tilt in the orientation of the RGS domain with respect to Gα was due to sequence differences in either RGS2 or Gαq. Therefore, before one can address the molecular basis for why some RGS proteins select against Gαq11 subfamily members, structures of conventional R4 subfamily members in complex with Gαq need to be determined.

Herein, we show that RGS8, an R4 subfamily member, binds to Gαq in a manner similar to how other RGS proteins bind
Gα10 indicating that the distinct pose of RGS2 is driven by its unique switch binding residues. We investigate unique contacts formed between RGS8 and the Ga α-helical domain and demonstrate that although residues in the α-helical domain modulate GAP activity, the chief determinant of selectivity is found elsewhere, most likely SwIII. These results clarify the molecular determinants of RGS domain selectivity and, by extension, how RGS proteins impact signaling pathways in vivo.

**Experimental Procedures**

Protein Expression and Purification—All constructs encoding RGS variants were confirmed by sequencing on both strands. Residues 42–173 spanning the RGS domain of RGS8 were expressed using the pQTEV vector (a kind gift from Dr. R. Neubig, Michigan State University). Residues 22–157 spanning the RGS domain of RGS10 were expressed using a pLIC-SGC1 vector obtained from the Structural Genomics Consortium (10). After cleaving the N-terminal His6 tag with tobacco etch virus protease from RGS8 and RGS10, the exogenous sequence QSM is left on the N terminus.

For RGS8 variants, 1 liter of BL21 Rosetta cells grown in Terrific Broth was induced with 100 μM isopropyl-1-thio-β-D-galactopyranoside at 20 °C. The cells were pelleted by centrifugation at 3500 × g for 15 min and then resuspended in Buffer A (20 mM HEPES, pH 8.0, 100 mM NaCl, 10 mM β-mercaptoethanol), supplemented with 7.6 μM leupeptin, 360 mM lima bean trypsin inhibitor, 1 mM PMSF, and 0.1 mM EDTA before being homogenized with a Dounce. Cells were then lysed using an EmulsiFlex-C3 homogenizer (Avestin). Cell debris was pelleted by centrifugation at 40,000 rpm (185,500 g) in a Type 45 Ti fixed angle rotor (Beckman-Coulter). The supernatant was passed over a 5-ml Ni-NTA affinity column pre-equilibrated with Buffer A (20 mM HEPES, pH 8.0, 500 mM NaCl, and 10 mM β-mercaptoethanol), followed by 100 ml of Buffer A with 150 mM imidazole, pH 8.0, and then dialyzed into Buffer A.

Elution of the His6-tagged Ga11 was verified using SDS-PAGE and pooled and concentrated to 7.5–9 mg/ml.

The insect cell vector pFastBacHT expressing an N-terminally truncated variant of Mus musculus Gαq spanning residues 35–359 (ΔN-Gαq) was described previously (18). For purification, 6 liters of High Five™ cells (BTI-TN-5B1-4) expressing ΔN-Gαq were pelleted at 3000 × g for 20 min. The pellet was then resuspended in Buffer A (20 mM HEPES, pH 8.0, 100 mM NaCl, 10 mM β-mercaptoethanol, and 10 mM GDP, pH 8.0), 7.6 μM leupeptin, 360 mM lima bean trypsin inhibitor, 1 mM PMSF, 0.1 mM EDTA, and 3 mM MgCl2. Cells were then homogenized, lysed, and pelleted as described for RGS8. The supernatant was then passed through a Ni-NTA agarose affinity column pre-equilibrated with Buffer A supplemented with 1 mM MgCl2. The column was washed with 100 ml of Buffer A plus 1 mM MgCl2, followed by 100 ml of Buffer A plus 1 mM MgCl2 and 10 mM imidazole, pH 8.0, and then eluted with 25 ml of Elution Buffer (Buffer A with 1 mM MgCl2 and 150 mM imidazole, pH 8.0). Ga11-R183C was produced as described previously (19).

The Escherichia coli vector pQE60 expressing a C-terminal, His6-tagged Ga11, spanning residues 1–354 was provided courtesy of Dr. Barry Kreutz (University of Illinois at Chicago). Expression was carried out as described previously (20). Purification was similar to RGS8, with the following exceptions. The Lysis Buffer was 50 mM HEPES, pH 8.0, 1 mM EDTA, 2 mM DTT, 0.1 mM PMSF, 7.6 μM leupeptin, and 360 mM lima bean trypsin inhibitor. Buffer A was 50 mM HEPES, pH 8.0, and 2 mM DTT. After washing the Ni-NTA affinity column with Buffer A, the elution step was performed using Elution Buffer (50 mM HEPES, pH 8.0, 2 mM DTT, and 150 mM imidazole, pH 8.0). The eluate was then loaded onto an UnoQ anion exchange chromatography column (Bio-Rad) pre-equilibrated with Buffer A and eluted using a gradient of 0–250 mM NaCl in Buffer A. The integrity of Ga11 was confirmed by visualizing trypsin digests on SDS-PAGE as described previously (21).

**Purification of the RGS8-Gαq Complex**—Purified ΔN-Gαq was incubated with 30 μM AlCl3, 10 mM NaF, and 1 mM MgCl2 in a buffer also containing 10 μM GDP, pH 8.0, 20 mM HEPES, pH 8, 100 mM NaCl, and 2 mM DTT. It was then mixed with purified RGS8 in a 1:1 molar ratio based on the RGS8 concentration determined using a NanoDrop™ ND-1000 spectrophotometer, and the ΔN-Gαq concentration was determined using Bradford reagent. The proteins were incubated together for 30 min on ice before loading onto tandem Superdex 200 10/300 GL (GE Life Sciences) gel filtration columns equilibrated with 20 mM HEPES, pH 8.0, 100 mM NaCl, 2 mM DTT, 10 μM GDP, pH 8.0, and 1 mM MgCl2. Fractions shown to contain 1:1 complex by SDS-PAGE were then concentrated to 5–7 mg/ml.

**Crystallization and Cryoprotection**—Crystals were grown in VDX plates (Hampton Research) using hanging drop vapor diffusion on glass cover slides. The RGS8-Gαq complex (6.6 mg/ml) was mixed 1:1 with well solution to a final volume of 1 μl and suspended over 1 ml of well solution. Octahedral crystals grew in 2 weeks at 4 °C using a well solution containing 0.1 M ammonium acetate, 0.1 M Bis-Tris, pH 5.5, and 11% PEG 8000. Crystals were harvested by adding several μl of cryoprotectant (20 mM HEPES, pH 8.0, 100 mM Bis-Tris, pH 5.4, 200 mM ammonium acetate, 15% PEG 8000, 200 mM NaCl, 1 mM DTT, and 30% 2-Mercaptoethanol) andonto Superdex 200 10/300 GL (GE Life Sciences) gel filtration columns equilibrated with 20 mM HEPES, pH 8.0, 100 mM NaCl, 2 mM DTT, 10 μM GDP, pH 8.0, and 1 mM MgCl2. Fractions were then concentrated to 5–7 mg/ml.
Structure/Function of the RGS8-\(\alpha_q\) Complex

50 \(\mu M\) GDP, pH 8.0, 20 \(\mu M\) AlCl₃, 10 mm NaF, and 5 mm MgCl₂ in 0.5-\(\mu l\) increments to the drop containing the crystal. The crystal was then transferred into 100% cryoprotectant and moved stepwise through mixtures of cryoprotectant plus glycerol until a final glycerol concentration of 24% (v/v). The crystal was then suspended in a nylon loop and frozen in liquid nitrogen.

Data Collection, Processing, and Model Building—X-ray diffraction data were collected at the Life Sciences Collaborative Access Team (LS-CAT) beamline 21-ID-D at the Advanced Photon Source (APS). Reflection intensities were integrated and scaled using HKL2000, and initial phases were determined by molecular replacement using Phaser and structures of \(\Delta N-\alpha_q\) from Protein Data Bank (PDB) entry 4EKD and RGS8 from PDB entry 2ODE as search models. Manual model building in Coot was alternated with TLS refinement with local non-crystallographic symmetry restraints in REFMAC5. Coordinates and structure factors were deposited with the Protein Data Bank as entry 5DO9. Figures were generated using The PyMOL Molecular Graphics System, Version 1.5.0.4 (Schrödinger, LLC).

GAP Assays—3 and 4 mg/ml stocks of \(\alpha_q\)-R183C and \(\alpha_q\), respectively, were incubated for 10 min with 10 mm EDTA, and then diluted to 0.3 \(\mu M\) final concentration in 300 \(\mu l\) of Incubation Buffer (50 mm HEPE, pH 8.0, 1 mm DTT, 1 mm EDTA, 100 \(\mu g/\mu l\) albumin, 5.5 mm CHAPS, 5% glycerol, and 37.5 \(\mu M\) ammonium sulfate) plus 33.3 \(\mu Ci/ml\) \([\gamma-\text{32P}]\)GTP (PerkinElmer, EasyTide) and enough cold GTP, pH 8.0, to make the total GTP concentration 6.25 \(\mu M\). The reaction was then incubated at room temperature for 3 h (\(\alpha_q\)-R183C) or 30 min (\(\alpha_q\)). Samples were buffer-exchanged into fresh Incubation Buffer using a pre-equilibrated Micro Bio-Spin™ chromatography column (Bio-Rad) and stored on ice for the duration of the assay. Each assay was initiated by adding 20 \(\mu l\) of the buffer-exchanged \(\alpha_q\) subunit to a tube containing 100 nm RGS protein in 180 \(\mu l\) of Assay Buffer (20 mm HEPE, pH 8.0, 80 mm NaCl, 1 mm DTT, 1 mm EDTA, 0.9 mm MgCl₂, 1 mm cold GTP, pH 8.0, 10 \(\mu g/\mu l\) albumin, and 0.2% w/v cholate) on ice. 40-\(\mu l\) aliquots of the reaction were quenched at various time points by vortexing with 750 \(\mu l\) of ice-cold Quenching Buffer (10 mm sodium phosphate, pH 2.0, and 5% (w/v) activated charcoal). The quenched reaction was spun for 25 min at 6500 \(\times g\) at 4 °C. Afterward, 200 \(\mu l\) of the supernatant was added to 3 ml of MicroScint™ 40 scintillation mixture (Perkin-Elmer) and read on a liquid scintillation counter instrument measuring \(32P\) cpm. Each RGS variant was tested in triplicate in three separate experiments. Data were processed in Prism 6 using a non-linear exponential fit with a time lag for \(\alpha_q\) or a straight line fit for \(\alpha_q\)-R183C.

Sequence Alignment and Structure Comparisons—Human RGS sequences from UniProt were aligned using Clustal Omega at the European Molecular Biology Laboratory-Europpean Bioinformatics Institute (22). RMSD calculations were performed using Superpose from the CCP4 software suite (23, 24). Calculation of buried surface area for complexes was performed using PISA (25).

Results

Crystal Structure of the RGS8-\(\alpha_q\) Complex—To determine whether the altered pose of RGS2 on \(\alpha_q\) was due to the unique switch interface residues Cys106, Asn184, and Glu191 of RGS2 or to unique residues in the switch regions of \(\alpha_q\) relative to \(\alpha_q\), the crystal structure of the RGS domain of RGS8, an R4 subfamily member selective for both \(\alpha_q\) and \(\alpha_q\), was determined in complex with N-terminally truncated \(\alpha_q\) (\(\Delta N-\alpha_q\)). RGS8 was used because it readily crystallizes, and the structure of its complex with \(\alpha_q\) was previously reported (10). The final structure was refined to 2.6 Å spacings (Table 1, Fig. 1A). Residues 42–173 of RGS8 with two N-terminal exogenous residues are visible in all complexes, as well as residues 38–350 of \(\Delta N-\alpha_q\). Three complexes of RGS8-\(\alpha_q\) exist in the asymmetric unit, with their overall RMSD in Ca positions varying by less than 0.6 Å. Comparisons with the RGS8-\(\alpha_q\) and RGS2-\(\alpha_q\) \(\alpha_q\) complexes give RMSD values of 0.9 Å for 432 and 426 Ca atoms, respectively, whereas comparison with the RGS2-\(\alpha_q\) complex gives an RMSD of 1.2 Å for 439 Ca atoms. This indicates that RGS8 binds \(\alpha_q\) in a manner most similar to how RGS proteins have previously been shown to bind \(\alpha_q\) subfamily members (Fig. 1B). Thus, the unique substitutions (Fig. 2A) in the \(\alpha_q\)-binding interface of RGS2 are primarily responsible for its altered pose when bound to \(\alpha_q\).

Molecular Basis for RGS Subfamily Selectivity—Next, the structures of the RGS8-\(\alpha_q\) and RGS8-\(\alpha_q\) complexes were

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**TABLE 1**

Crystallographic and refinement statistics for the RGS8-\(\alpha_q\) complex

| RGS8-\(\alpha_q\) |
|-------------|
| Data collection |
| X-ray source | APS 21 ID-D |
| Wavelength (Å) | 1.0383 |
| \(D_{\text{max}}\) (Å) | 30.0–2.60 (2.64–2.60) |
| Space group | C121 |
| Cell dimensions |
| a, b, c (Å) | 1740.9, 95.9, 112.9 |
| β (°) | 94.3 |
| Total reflections | 211,145 |
| Unique reflections | 56,869 |
| \(R_{\text{rel}}\) (%) | 12.5 (58.5) |
| Completeness (%) | 99.3 (92.3) |
| (I/σI) | 7.6 (4.1) |
| Redundancy | 3.7 (3.6) |
| \(CC_{\text{ref}}\) | (71.5) |

**Refinement**

| |
|---|
| Refinement resolution (Å) | 30.0–2.60 (2.66–2.60) |
| Total reflections used | 54,036 (3705) |
| RMSD bond lengths (Å) | 0.013 |
| RMSD bond angles (°) | 1.584 |
| Estimated coordinate error (Å) | 0.219 |
| Ramachandran plot |
| Favorable (%) | 98.35 |
| Outliers (%) | 0.00 |
| \(R_{\text{work}}/R_{\text{free}}\) | 17.8/22.5 (27.0/31.0) |
| Protein atoms | 11,092 |
| Ligand atoms | 102 |
| Solvent molecules | 231 |
| Average B-factor (Å²): |
| Protein | 48.2 |
| Ligand | 27.1 |
| Solvent | 37.7 |
| Wilson B factor (Å²) | 37.5 |
| MolProbity score | 1.39 (100th percentile) |

| PDB entry | 5DO9 |

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\(a\) \(CC_{\text{ref}}\), Pearson correlation coefficient. 
**Structure/Function of the RGS8-G\(_{\alpha_q}\) Complex**

FIGURE 1. Structure of RGS8 in complex with G\(_{\alpha_q}\) reveals a canonical tilt. A, the 2.6 Å crystal structure of the RGS8 GAP domain in complex with ΔN-G\(_{\alpha_q}\). The αB-αC loop exhibits structural differences between G\(_{\alpha}\) and G\(_{\alpha_q}\) that could dictate the selectivity of RGS proteins. RGS8 is cyan, G\(_{\alpha_q}\) is yellow, the three switch regions are red, GDP is black, AlF\(_4^-\) is green, Mg\(^{2+}\) is orange, and the αB-αC loop of G\(_{\alpha_q}\) is shown in pink (PDB code 2ODE). B, RGS2 adopts a unique tilt when bound to G\(_{\alpha_q}\). The G\(_{\alpha}\) subunits of the RGS2-G\(_{\alpha_q}\) complex (PDB code 4EKD), RGS8-G\(_{\alpha}\) complex (PDB code 2ODE), and RGS8-G\(_{\alpha_q}\) complex (PDB code 5DO9) were superimposed to compare the position of the RGS domain in each complex. G\(_{\alpha_q}\) is shown in yellow, G\(_{\alpha}\)-bound RGS8 is shown in pale cyan, G\(_{\alpha_q}\)-bound RGS8 is shown in orange, and RGS2 is shown in dark blue.

compared to identify RGS domain contacts with G\(_{\alpha}\) that are distinct between the G\(_{\alpha_{10}}\) and G\(_{\alpha_{q1}}\) subfamilies. The structural element that differs most is the αB-αC loop in the α-helical domain. In the RGS8-G\(_{\alpha_q}\) complex, the αB-αC loop is less ordered when compared with the RGS8-G\(_{\alpha}\) complex based on temperature factors, but extends closer to the G\(_{\alpha}\) protein, which creates additional buried surface area (Fig. 1A). In fact, RGS2 seems to exploit this surface to maintain greater buried accessible surface area with G\(_{\alpha_q}\) (2050 Å\(^2\)) than does RGS8 (1900 Å\(^2\)), or than does RGS8 in complex with G\(_{\alpha}\) (1650 Å\(^2\)). RGS residues that would contact this loop exhibit sequence heterogeneity among the various RGS subfamilies (Fig. 2A). The R4 family has a conserved Glu-Lys dyad in the α7 helix (RGS8 residues 155–156), whereas RGS10, an R12 member, has Lys-Tyr (residues 131–132) (Figs. 2A and 3, A and B). Superposition of the G\(_{\alpha}\) subunits in the RGS8-G\(_{\alpha_q}\) (Fig. 3A) and RGS10-G\(_{\alpha_q}\) (Fig. 3B) complexes suggests that charge repulsion and/or steric hindrance by this dyad could discourage binding of R12 family members to G\(_{\alpha_q}\), as there is a charge reversal in the first position and introduction of a bulkier Tyr residue for Lys in the second (Fig. 3C). In comparison, modeling Glu-Lys for the Lys-Tyr dyad of RGS10 anticipates no overt issues with G\(_{\alpha}\) binding (Fig. 3D). R7 subfamily members instead have a Lys-(Lys/Ser) dyad (Fig. 2A). The subfamily-specific sequences of these dyads could therefore contribute to G\(_{\alpha}\) selectivity. In support of this hypothesis, a previous study found that mutating these positions contributes to differences in GAP activity of various R4 family members on G\(_{\alpha}\) (26).

**Functional Analysis of the α-Helical Domain Interface**—The aforementioned α-helical domain interface was tested by site-directed mutagenesis followed by single turnover GTPase assays using [γ-\(^{32}\)P]GTP (Table 2, Fig. 4). RGS8-Glu\(^{155}\) and/or Lys\(^{156}\) were converted to their analogous residues in RGS10 (Lys and Tyr, respectively). Complementary mutations were introduced in RGS10, mutating Lys\(^{131}\) and/or Tyr\(^{132}\) to Glu and Lys, respectively. If G\(_{\alpha}\) selectivity for G\(_{\alpha_q}\) subunits was achieved via ionic repulsion with the α-helical domain of G\(_{\alpha_q}\), then mutation at the first position (E155K in RGS8, K131E in RGS10) would result in a selectivity switch. Selectivity achieved through steric pressure would be potentially altered by mutation at the second position (K156Y in RGS8, Y132K in RGS10). If both steric and charge were necessary to affect a selectivity switch, then both point mutations (E155K/K131E in RGS8 and K131E/Y132K in RGS10) would be required.

Wild-type G\(_{\alpha_{11}}\) and the slow-hydrolyzing mutant G\(_{\alpha_{11^-}}\)R183C were used as substrates for each RGS variant. As expected, wild-type RGS8 showed robust GAP activity on both G\(_{\alpha_{11}}\) (Fig. 4A) and G\(_{\alpha_{11^-}}\)R183C (Fig. 4B), whereas wild-type RGS10 only showed GAP activity on G\(_{\alpha_{11}}\) (Fig. 4C, Table 2). All three mutants of RGS8 retained their activity on G\(_{\alpha_{11}}\), but also retained wild-type, if not higher, activity on G\(_{\alpha_{11^-}}\)-R183C. The RGS10 double mutant and K131E single mutant also retained activity on G\(_{\alpha_{11}}\). Interestingly, the Y132K mutant did not. None of the RGS10 mutants showed GAP activity on G\(_{\alpha_{11^-}}\)-R183C. These results indicate that the RGS domain dyad that contacts the αB-αC loop is not responsible for RGS10 being inactive on G\(_{\alpha_q}\) as RGS10 and RGS8 mutants had no increase or loss, respectively, in selectivity for G\(_{\alpha_{11^-}}\)-R183C.

**Discussion**

RGS proteins range from being relatively small proteins with little more than the RGS domain, to complex multi-domain entities with multiple signaling domains. However, even in simple RGS proteins such as RGS2, RGS4, and RGS8, the regions outside the RGS domain can play important roles such as targeting these enzymes to membranes, G protein-coupled receptors, or effector enzymes (27–30). Thus, when one considers the selectivity of an RGS protein for a particular G\(_{\alpha}\) signaling pathway, there are many levels at which this can occur. However, the most fundamental aspect of selectivity is imposed by the direct interaction of the RGS domain with G\(_{\alpha}\) to promote acceleration of GTP hydrolysis. Consequently, this study focused solely on the interaction of the RGS domain found in
Structure/Function of the RGS8-\(\alpha_q\) Complex

**A**

| RGS8  | RGS2  | RGS3  | RGS4  | RGS5  | RGS6  | RGS7  | RGS8  | RGS9  | RGS10 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL | APREVNIDFQTITKMLDKSVKQHFLDRQKDRHGRQYTFEDAQEHIYKLMKSDSYPRFVGFEDYQL |

**R4**

- RGS8
- RGS2
- RGS3
- RGS4
- RGS5
- RGS6
- RGS7
- RGS8
- RGS9
- RGS10

**R7**

- RGS8
- RGS2
- RGS3
- RGS4
- RGS5
- RGS6
- RGS7
- RGS8
- RGS9
- RGS10

**R12**

- RGS8
- RGS2
- RGS3
- RGS4
- RGS5
- RGS6
- RGS7
- RGS8
- RGS9
- RGS10

*FIGURE 2. Sequence conservation of RGS8 residues in α6 and α7 suggests selectivity mechanisms.* A, sequence alignment of the α6–9 regions of the R4, R7, and R12 subfamilies. Alignments were performed with Clustal Omega using human sequences. Residue positions important in \(\alpha_q\) activity for individual \(\alpha_q\) subunits remained unclear.

RGS proteins with \(\alpha_q\) and \(\alpha_q\) subunits. Moreover, previous studies have shown that isolated RGS domains exhibit selectivity for individual \(\alpha\) subfamilies (10).

Previous structural analysis of the RGS2 complex with \(\alpha_q\) suggested that RGS2 has a distinct tilt relative to the \(\alpha\) subunit (Fig. 1B) that allows it to bury more surface area with \(\alpha_q\) than it could with \(\alpha_q\). Moreover, the conformationally flexible α6 helix of RGS2 allows it to maintain optimal contacts with SwIII, despite the unique pose of the RGS domain (18). When the Cys106, Asn186, and Glu191 interface residues are mutated to their equivalents in other RGS proteins in RGS2, it can bind to \(\alpha_q\) in a canonical fashion (17), but does not lose activity against \(\alpha_q\), suggesting that interactions with Sw1 are not directly responsible for \(\alpha_q\) selectivity (11, 17). It was further demonstrated that altering interactions between the α7 helix of the RGS2 domain and the α-helical domain of \(\alpha_q\) can dramatically promote or inhibit GAP activity (18), but the molecular basis for selectivity against \(\alpha_q\) observed in other RGS subfamilies remained unclear.

In this work, it was shown that an R4 family member, RGS8, binds to \(\alpha_q\) in a canonical fashion, permitting a more precise comparison of the interactions between RGS proteins and these two \(\alpha\) subfamilies. The tilt of RGS2 in complex with \(\alpha_q\) can thus be attributed to interfacial differences dictated by unique interfacial residues in RGS2. Two regions, in particular the αB-αC loop of the α-helical domain, stand out as a potential selectivity determinant. The G protein α-helical domain has previously been shown in some instances to be a major determinant of GAP activity, and there are sequence signatures unique to each RGS subfamily that interact with this domain (18, 31). Although a selectivity switch was not achieved in our study, the GAP assay results are consistent with RGS activity being enhanced or inhibited by interactions with the α-helical domain. Interestingly, the RGS10 Y132K mutant, creating a Lys-Lys dyad in α7, did not retain activity for \(\alpha_q\), but could be rescued by the addition of the K131E mutation. The disadvantage of having a Lys-Lys dyad may be due to electrostatic repulsion between the adjacent positions or with the α-helical domain. However, it seems clear that the α-helical domain is not a major \(\alpha\) selectivity determinant because no substitution in this interface could promote activity on \(\alpha_q\) by RGS10 (Table 2).

Instead, the evidence now points toward SwIII, which interacts with the N-terminal end of the RGS α6 helix, as being the
primary determinant of selectivity, as suggested in Ref. 10. In SwIII, the side chain of \( \alpha_{q} \)-Asp \(^{243} \) stacks with the side chain of RGS8-Phe \(^{125} \). The analogous residue in \( \alpha_{q} \), Glu \(^{238} \), cannot make this interaction because the backbone of its SwIII is positioned differently. Phe is shared by several other R4 family members at this position, but not by any R7 or R12 members (Fig. 2A). R4 RGS domains typically also have a basic residue, e.g. RGS8-Arg \(^{128} \), that is positioned to form a hydrogen bond with a backbone carbonyl of another SwIII residue in \( \alpha_{q} \). However, due to the poor sequence conservation and conformational heterogeneity of this region in R12 family members (R12 subfamily members also have a 1-residue deletion in the \( \alpha_{q} \) helical region), it is not possible to easily test this hypothesis because conversion of SwIII contacts in RGS8 to those found in RGS10, and vice versa, is not possible by simple substitution.

Regardless, this model does not explain how RGS proteins in the R7 and R12 subfamilies retain activity for \( \alpha_{q} \), if they fail to make productive interactions with SwIII. These subfamilies may have optimized interactions in other contact regions (e.g. \( \alpha_{A} \) and other regions in the \( \alpha_{q} \) \( \alpha \)-helical domain), as has been shown for some R4 family members in determining their relative activity on members of the \( \alpha_{q} \) subfamily (26).
hypothesis is supported by the increased GAP activity observed for RGS variants that have RGS10 substitutions in α7, and decreased GAP activity for RGS10 variants with RGS8 substitutions (Table 2). This result is consistent with prior studies that have likewise probed positions in α7 and shown them to modulate RGS domain interactions and GAP activity (18, 26, 32). Another possible explanation might be found in the S11 interface. Goq has Pro185, whereas Go has Lys180. The Goq-Lys180 side chain buries more surface area with the RGS protein when compared with Pro185 in Goq. Hence, if the interactions with S11 are not strong, RGS proteins may be less active against Goq as a result of less buried surface area with S11. Indeed the specific activity of RGS4 is ~10 times lower than wild-type when using the Goq-K180P variant as a substrate (33).

In summary, the rules that dictate RGS domain selectivity for a given Gα subunit are complex. They involve leveraging beneficial versus negative interactions at different points of contact with the Gα subunits, as well as the ability of individual RGS proteins to undergo induced fit when required (18). However, the structure-function analysis reported here still points to the interface. Goq modulate RGS domain interactions and GAP activity (18, 26, 34). Conversely, AlF4−-activated Goq-P185K can be pulled down by RGS2 in significantly greater amounts than wild-type Goq (32).

Author Contributions—V. G. T. and J. J. G. T. designed the experiments, determined the x-ray structure, and prepared the manuscript. V. G. T. and P. A. B. purified the complex. P. A. B. crystallized the complex. V. G. T. purified proteins for, and performed and analyzed the GAP assays. All authors approved the final paper.

Acknowledgments—Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract Number DE-AC02-06CH11357. Use of the LS-CAT Sector 21 was supported by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor (Grant 085P1000817). This research used the DNA Sequencing Core of the Michigan Diabetes Research and Training Center supported by DK20572. We thank the LS CAT staff members at Argonne Photon Source for their help with data collection and Dr. Jennifer Cash for assistance with crystal structure determination.

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