Large-scale conformational rearrangement of the α5-helix of Gα subunits in complex with the guanine nucleotide exchange factor Ric8A

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Resistance to inhibitors of cholinesterase 8A (Ric8A) protein is an important G protein–coupled receptor (GPCR)-independent regulator of G protein α-subunits (Gα), acting as a guanine nucleotide exchange factor (GEF) and a chaperone. Insights into the complex between Ric8A and Gα hold the key to understanding the mechanisms underlying noncanonical activation of G-protein signaling as well as the folding of nascent Gα proteins. Here, we examined the structure of the complex of Ric8A with minimized Gαi (miniGαi) in solution by small-angle X-ray scattering (SAXS) and exploited the scattering profile in modeling of the Ric8A/miniGαi complex by steered molecular dynamics (SMD) simulations. A small set of models of the complex featured minimal clash scores, excellent agreement with the experimental SAXS data, and a large-scale rearrangement of the signal-transducing α5-helix of Gα away from its β-sheet core. The resulting interface involved the Gα α5-helix bound to the concave surface of Ric8A and the Gα β-sheet that wraps around the C-terminal part of the Ric8A armadillo domain, leading to a severe disruption of the GDP-binding site. Further modeling of the flexible C-terminal tail of Ric8A indicated that it interacts with the effector surface of Gα. This smaller interface may enable the Ric8A-bound Gα to interact with GTP. The two-interface interaction with Gα described here distinguishes Ric8A from GPCRs and non-GPCR regulators of G-protein signaling.

The resistance to inhibitors of cholinesterase 8 (Ric8)2 proteins are guanine nucleotide exchange factors (GEFs) and chaperones for G protein α-subunits (Gα) (1–4). The Ric8A isoform regulates a diverse subset of Gα subunits, including Gαq, Gαs, and Gα12/13, and in this capacity it is essential to multiple cellular signaling pathways, including asymmetric cell division and synaptic transmission (5–7). Ric8 selectively interacts with the GDP-bound state of Gα and induces release of GDP. A stable intermediate complex of Ric8 and nucleotide-free Gα is formed and persists until Gα binds GTP and dissociates from Ric8 (2, 8). Although the mechanism of Ric8A GEF activity is thought to be very different from that of G protein–coupled receptor (GPCR)-dependent activation of heterotrimeric G proteins (Gαβγ), one striking parallel has emerged (i.e. both Ric8A and GPCRs interact with the C termini of Gα, and transmission of the GPCR-induced activation signal involves the Gα α5-helix) (9–13). In particular, the largest conformational change in Gα is an outward translation with rotation of the α5-helix that disrupts the guanine ring binding loop β6-α5 of Gα (11). The first structural clues to the mechanism of Gα activation by Ric8A have been provided by the recent crystal structure of the complex of Ric8A with the C-terminal fragment of Gα corresponding to the α5-helix (10). Based on this structure, we modeled the complex of Ric8A with miniGαi and the full-length Gαi subunit (10). The key premise for the model was the observation that the steric overlap between Ric8A and Gα is markedly reduced when a GPCR-bound conformation of Gα was used in the modeling that involved superimposition of the α5-helix (10). The remaining clashes in the model were resolved with an assumption that Ric8A adopts an open conformation to accommodate the Ras-like domain (RD) of Gα. Indeed, the steered molecular dynamics (SMD) simulations with force applied to the Ric8A region that clashed with Gα readily yield such an “open” conformation (10). In this study, we examined the solution structure of the Ric8A/miniGαi complex by small-angle X-ray scattering (SAXS) to evaluate and/or refine this model. Unexpectedly, the experimental SAXS profile of the Ric8A/miniGαi complex revealed a very poor agreement with the theoretical SAXS profile of the model, necessitating its revision. We explored the possibility that the complex formation leads to conformational changes in Gα with SMD simulations where force is applied to the miniGαi α5-helix. Thus, we obtained a group of similar conformations of miniGαi that show no significant clashes in modeling of the Ric8A/miniGαi complex. Importantly, the resulting models are in excellent agreement with the experimental SAXS profile, and they feature large rearrangement of the Gα α5-helix.
Results

Analysis of the Ric8A/mini\(\alpha\) complex solution structure by SAXS

We utilized minimized \(\alpha\) lacking flexible parts of the protein, the helical domain (HD) and the N-terminal \(\alpha\)N-helix (\(\Delta\alpha\)N-\(\alpha\)N-G\(\alpha\)), in SAXS experiments to limit conformational uncertainty. A highly purified sample of the Ric8A1–492/\(\Delta\alpha\)N-mini\(\alpha\) complex was analyzed by size-exclusion chromatography (SEC)-SAXS (Fig. 1). Previously, we generated two models of the Ric8A1–492/mini\(\alpha\) complex that differ in the position of the distal C-terminal tail of Ric8A (10). Comparison of the theoretical SAXS profiles of the two corresponding Ric8A1–492/\(\Delta\alpha\)N-mini\(\alpha\) models 1 and 2 with the experimental SAXS profile revealed poor fits for both models (Fig. 2). We also evaluated the theoretical SAXS profile of the Ric8A1–452/\(\Delta\alpha\)N-mini\(\alpha\) model (previous SMD model) lacking residues 453–492 of Ric8A, which served as a template for models 1 and 2 (Fig. 2A). The quality of the fit (\(\chi^2\) value) remained poor, suggesting that the disagreement of the Ric8A1–492/\(\Delta\alpha\)N-mini\(\alpha\) models with the experimental SAXS data was not due to the incorrectly modeled residues 453–492 of Ric8A. The above-mentioned models of Ric8A1–492/\(\Delta\alpha\)N-mini\(\alpha\) were derived using the “open” conformation of Ric8A1–452 that resulted from an SMD simulation with force applied to the clashing part of the protein (10). In light of the disagreement of the model for the complex with the SAXS data, we re-examined the validity of “open” conformation of Ric8A by reverting to its conformation observed in the crystal structures (10, 14). The crystal structure conformation leads to an improbable model in which the two proteins extensively clash (“clash” model) (Fig. 2A). However, the theoretical SAXS profile of the clashing model of Ric8A1–452/\(\Delta\alpha\)N-mini\(\alpha\) complex agreed with the experimental data somewhat better than the models with the “open” conformation of Ric8A (Fig. 2B). Thus, the “open” conformation of Ric8A is not supported by the experimental SAXS profile of the Ric8A1–492/\(\Delta\alpha\)N-mini\(\alpha\) complex.

SAXS-directed modeling of the Ric8A/mini\(\alpha\) complex indicates rearrangement of the \(\alpha\)5-helix

To avoid clashes, and barring the “open” conformation of Ric8A, conformational changes more extensive than the GPCR-induced changes would have to occur in \(\alpha\). To simulate the forces that act on the \(\alpha\)5-helix upon binding of Ric8A, we conducted an SMD simulation of \(\Delta\alpha\)N-mini\(\alpha\) that was further truncated by 5 N-terminal residues with conformational ambiguity (\(\Delta\alpha\)N-mini\(\alpha\)). The 12-ns SMD trajectory yielded 300 conformations of \(\Delta\alpha\)N-mini\(\alpha\) (Fig. 3A). Models of the Ric8A1–452/\(\Delta\alpha\)N-mini\(\alpha\) complex were constructed for each of the SMD conformations of \(\Delta\alpha\)N-mini\(\alpha\). Top SMD models of the Ric8A1–452/\(\Delta\alpha\)N-mini\(\alpha\) complex were selected based on the combinations of low clash score and agreement with the experimental SAXS profile (low \(\chi^2\) value) (Fig. 3, B and C). Compared with the starting “clashing” model (clash score 100.1, \(\chi^2 = 13.4\)), the top six SMD models displayed markedly improved clash scores (<25) and \(\chi^2\) values (<2.0) (Fig. 3, E and F). All of these models were similar and featured a large hinge-like movement of the \(\alpha\)5-helix away from the \(\beta\)-sheet core of the RD (Fig. 3D). This movement allowed positioning the \(\alpha\)5-\(\beta\)-sheet on top of the C-terminal portion of Ric8A1–452 and minimized clashes between the two proteins (Fig. 3E). Residual clashes in the top SMD models were readily eliminated with energy minimization of the model structures.
ACCELERATED COMMUNICATION: Complex of Ric8A with Go

Discussion

The analysis of the structure of the Ric8A1–492/ΔαN-miniGo complex in solution by SAXS reported in this study led us to propose a new model of this complex. The starting point in our modeling lies in the established location of the Go α5-helix with respect to Ric8A (10). Although all known conformations of Go produce extensive clashes with Ric8A when modeled according to the position of the α5-helix, the clashes are less severe using the GPCR-bound conformation. To avoid steric overlap, additional conformational changes must take place either in Ric8A, Go, or both. The previously proposed model hypothesized conformational changes in Go that are similar to those induced by agonist-bound GPCRs and additional changes in the armadillo core domain of Ric8A that lead to a more stretched “open” conformation (10). Such a model appeared to be the most parsimonious, as the transition of the Go subunit to its GPCR-bound conformation is well understood, and conformational flexibility of the N- and C-terminal parts of armadillo-fold proteins has been described (11, 20–23).

However, the experimental scattering profile of the Ric8A1–492/ΔαN-miniGo complex revealed strong disagreement with the theoretical profile of the model based on the “open” conformation of Ric8A, thereby refuting it. Accordingly, we explored the possibility of further conformational changes in Go that extend beyond those induced by GPCRs using the SMD simulation mimicking potential Ric8A-induced changes in Go. Remarkably, low clash scores and low χ2 values converged in a small set of models with similar SMD conformations of ΔαN-miniGo. The key feature of these Go conformations is a large dislocation of the Go α5-helix, whereby the latter becomes
completely detached from the hydrophobic β-sheet core of Ga. The Ga β-sheet core is then stabilized by interactions with the C-terminal part of the Ric8A armadillo domain.

Two routes have been shown to transmit GPCR-induced conformational changes from the Ga α5-helix to the GDP binding site: disruption of the guanine nucleotide-binding loop β6-α5 and rearrangement of the interfaces between α5, α1, and β2-β3 that causes destabilization of the phosphate-binding β1-α1 loop (11, 21, 22). The model of the Ric8A/Ga complex suggests a major disruption of the β6-α5 loop, and a disordering of the β1-α1 loop can be predicted as well.

The modeling of the flexible C-terminal tail of Ric8A (residues 453–492) in complex with Ga provides further insight into the mechanism of Ric8A GEF and chaperone activities, as this region is critical for both activities of the protein (9, 10, 15). Interestingly, whereas our SAXS analyses argue against significant conformational changes in the core armadillo domain of Ric8A, they support a large conformational change in the flexible distal portion of the Ric8A C-terminal tail on binding of Ga. The SAXS analysis of the Ric8A/Ga complex reveals a smaller $R_g$ value than that for the apo-Ric8A (10), which is indicative of immobilization of the extended floppy tail into a more compact conformation. Such immobilization occurs when, according to the favored cluster of models, the C-terminal α-helix of Ric8A interacts with the groove between the switch II region and α3-helix of Ga. This is a conformation-sensitive surface that Ga subunits utilize to bind multiple partners. At this surface, GaGTP binds effector molecules, whereas GaGDP interacts with GoLoco/GPR proteins or guanine-nucleotide exchange modulators, such as GIV/Girdin (24–29).

Our model and existing biochemical evidence suggest two not mutually exclusive roles for the distal C-terminal tail of Ric8A, Ric8A452–492. The C-tail of Ric8A may serve as a “hook” that helps to hold Ga while the α5-helix is being pulled away from the β-sheet core. Thus, it may facilitate transition to

Figure 3. Modeling with steered molecular dynamics simulation. A, root mean square deviation (RMSD) plot for the 12-ns SMD of ΔΔαN-miniGa, B, clash scores for the models of the Ric8A1–452/ΔΔαN-miniGa complex were calculated using MolProbity. C, $\chi^2$ values for the fits of theoretical SAXS profiles of models to the experimental SAXS data. Among the top six SMD models of ΔΔαN-miniGa (D) and the corresponding models of the Ric8A1–452/ΔΔαN-miniGa complex (clash score < 25, $\chi^2$ value < 2) (E), models a and b featured the lowest $\chi^2$ value (1.31) and clash score (13.5), respectively. F, fits of the theoretical SAXS profiles of the models a and b to the experimental SAXS profile of the Ric8A1–492/ΔΔαN-miniGa complex.
a stable intermediate complex of Ric8A with nucleotide-free Gα. Another role, and possibly the key role of the Ric8A C-tail is to promote binding of GTP to Gα. Compared with GPCRs, the interface of Ric8A with Gα is more extensive, and the disruption of the nucleotide-binding site is more severe. It then becomes imperative to stabilize the structural elements of Gα involved in the interaction with the γ-phosphate of GTP, which would enable GTP binding. By interacting with the conformation-sensitive switch II/α3-helix region, the C-tail of Ric8A may nudge the switch II region and its α2 loop toward the GTP γ-phosphate binding position with cooperative changes in the switch I region, all of which would promote binding of GTP.

In summary, this study suggests a novel and unusual type of interface between Gα and its GPCR-independent GEF. The main interface involves the 5-helix and the 3-sheet core of Gα that interact extensively with the concave surface of the Ric8A armadillo core and its C-terminal part, respectively. This interface is made possible by a large-scale dislocation of the Gα 5-helix. A second smaller interface is between the distal C-terminal tail of Ric8A and the effector surface of Gα. The two interfaces on the opposite sides of Gα appear to be a unique feature of Ric8A as a GEF, and they may be critical to its function as a Gα chaperone.

**Experimental procedures**

**Protein expression and purification**

Bovine Ric8A1–492 was expressed and purified as described previously (10). Sequence encoding ΔαN-miniGαi (which

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Figure 4. Models of the Ric8A1–492/ΔαN-miniGαi complex. Shown are three representative FloppyTail models from each of the clusters, cluster 1 (A), 2 (B), and 3 (C). The switch II region of Gαi is shown in cyan; the modeled distal C-terminal tail of Ric8A (residues 453–492) is shown in magenta, gray, or light green. D, model of the Ric8A1–492/Gαi complex. Arrows, HD (gray) and RD domains and the αN-helix of Gαi. The distal C-terminal tail of Ric8A in a representative conformation from cluster 1 is shown in magenta. E, parameters of the FloppyTail models of the Ric8A1–492/ΔαN-miniGαi complex from clusters 1–3 (mean ± S.D.).

Figure 5. DENSS electron density map of the Ric8A1–492/ΔαN-miniGαi complex. A, the pairwise distance distribution function P(r) was calculated from the SAXS data using GNOM (Dmax = 107 Å) and served as an input into the DENSS reconstruction. B, Fourier shell correlation (FSC) curve for the DENSS reconstruction estimates resolution at 32 Å. C, electron density is shown as volume colored according to density (bar, electron density values in $α$ from 2 to 200 (blue to red)). Shown is a representative model of the Ric8A1–492/ΔαN-miniGαi complex from cluster 1 aligned with the DENSS map. D, DENSS electron density envelope contoured at 2σ and aligned with the model in C.
starts with Go14, residue Glu-28 following Met) was amplified from the miniGo4 vector (10). ΔαN-miniGo4 was cloned into the modified pET21a vector containing an N-terminal His6 tag followed by the B1 domain of streptococcal protein G (GB1) tag and a tobacco etch virus cleavage site. The ΔαN-miniGo4 construct was expressed and purified as described previously for miniGo4. The His6-GB1 tag was removed from ΔαN-miniGo4 by adding tobacco etch virus protease in a 1:50 molar ratio to the protein eluted from cobalt-nitrilotriacetic acid resin. The sample was incubated overnight at 4 °C and purified by SEC using a HiLoad 16/600 Superdex 75-pg column equilibrated with 20 mM Tris-HCl (pH 8.0) buffer containing 150 mM KCl, 10% glycerol, 20 mM MgSO4, 10 μM GDP, and 1 mM TCEP.

For the small-angle X-ray scattering data collection, Ric8A1–492/ΔαN-miniGo4 complex was prepared by mixing Ric8A with ΔαN-miniGo4 at a 1:1.5 molar ratio. The complex was purified by SEC using a Superdex 200 10/300 GL (GE Healthcare) column equilibrated with 20 mM Tris-HCl (pH 8.0) buffer containing 150 mM KCl, 5% glycerol, and 1 mM TCEP. This procedure removed excess ΔαN-miniGo4, ensuring 1:1 stoichiometry of the complex.

SAXS

SAXS data were collected at the Bio-CAT beamline 18-ID-D at the Advanced Photon Source (Argonne, IL) using an in-line SEC-SAXS configuration (30) with a Superdex 200 column (GE Healthcare). A 250-μl volume of 10 mg/ml sample in 20 mM Tris, 150 mM KCl, 5% glycerol, 1 mM TCEP, pH 7.5, buffer was loaded onto the column at a flow rate of 0.9 ml/min. SAXS data were collected as described previously (10) and deposited in the Small Angle Scattering Biological Data Bank (ID SASDGB86). BioXTAS RAW and ATSAS 2.8 were used for SAXS data reduction and analysis (31, 32). The pair distribution function was calculated with GNOM, and an ab initio electron density map was calculated from the SAXS data with DENSs (19, 32).

SMD simulations

SMD simulations were performed on a homology model of miniGo4 lacking conformationally ambiguous residues from the N-terminal αN-helix and αN-β1 loop (ΔΔαN-miniGo4, which starts with Go14, residue Glu-33). The structure of GPCR-bound miniGo4 served as a template for the homology model (PDB entry 5G53) (33). The structure file for ΔΔαN-miniGo4 was prepared using VMD (34) and the plugin QwikMD (35). The simulations employed the NAMD molecular dynamics package (36) and the CHARMM36 force field (37) as described previously (10). The SMD simulations (38) of constant velocity stretching (SMD-CV protocol) employing a pulling speed of 2.5 Å/ns and a harmonic constraint force of 7.0 kcal/mol/Å2 were performed for 12.0 ns. In this step, SMD was employed by harmonically restraining the positions of ΔΔαN-miniGo4 corresponding to Glu-33 and Val-34 of Go14, and moving second restraint residues corresponding to Go14 residues 329–354. The force direction was defined by the axis between the center of mass of Ric8A atoms that clash with ΔΔαN-miniGo4, and the center of mass of the Go4 α5-helix.

 Modeling of the Ric8A1–492/ΔΔαN-miniGo4 complex

The FloppyTail model of Ric8A1–492 was generated previously (10). The SMD trajectory yielded 300 conformations of ΔΔαN-miniGo4. Models of the Ric8A1–492/ΔΔαN-miniGo4 complex were constructed for each of these conformations by superimposition of the α5-helix of ΔΔαN-miniGo4 with the Go4 α5-helical residues from the structure of the Ric8A1–492/MBP-Go4,327–350 complex (PDB entry 6N8S). Clash scores for the complex models were calculated using MolProbity to identify models with the least steric clash (39). The Crysol program was used to generate and compare fits of theoretical SAXS profiles of the models to experimental SAXS data (χ2 values) (40). Top models of the Ric8A1–492/ΔΔαN-miniGo4 complex were energy-minimized using YASARA Structure 18.2.7.

 FloppyTail modeling

The model of the Ric8A1–492/ΔΔαN-miniGo4 complex with the best fit to the experimental SAXS data was selected among the top six models with low clash scores and low χ2 values as a template for the FloppyTail simulations (16) of the Rosetta software suite. Residues Ric8A453–492 in extended conformation were added to the Ric8A1–492/ΔΔαN-miniGo4 model. Residues 471–490 were kept helical, based on the secondary structure prediction (10). Also, the N terminus of ΔΔαN-miniGo4 in the complex was extended by 5 residues to that of ΔαN-miniGo4 using YASARA. The FloppyTail protocol was similar to that described previously (10). Two experimental linear distance constraints of 30 Å for the previously identified cross-linked pairs Ric8A-K488-miniGo4-K122, and Ric8A-K462-miniGo4-K212 were used during the simulation (10). In addition to energy scores from Rosetta and χ2 values, surface distances between cross-linked residues were used in model selection. The solvent-accessible surface distances were calculated using Jwalk as a more accurate estimate for the feasibility of cross-linking between two residues (41). Models of the Ric8A1–492/ΔΔαN-miniGo4 complex were aligned with the DENSSE ab initio electron density map using UCSF Chimera (42). The model of the Ric8A1–492/Go4 complex was generated by superimposition of the Go4 structure (PDB 6CMO) (43) lacking the α5-helix with ΔΔαN-miniGo4.

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