Activation of RGS9-1GTPase Acceleration by Its Membrane Anchor, R9AP*

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The GTase-accelerating protein (GAP) complex RGS9-1-Gβ5 plays an important role in the kinetics of light responses by accelerating the GTP hydrolysis of Gα, in vertebrate photoreceptors. Much, but not all, of this complex is tethered to disk membranes by the transmembrane protein R9AP. To determine the effect of the R9AP membrane complex on GAP activity, we purified recombinant R9AP and reconstituted it into lipid vesicles along with the photon receptor rhodopsin. Full-length RGS9-1-Gβ5 bound to R9AP-containing vesicles with high affinity (Kd < 10 nM), but constructs lacking the DEP (dishevelled/EGL-10/pleckstrin) domain bound with much lower affinity, and binding of those lacking the entire N-terminal domain (i.e. the dishevelled/EGL-10/pleckstrin domain plus intervening domain) was not detectable. Formation of the membrane-bound complex with R9AP increased RGS9-1 GAP activity by a factor of 4. Vesicle titrations revealed that on the time scale of phototransduction, the entire reaction sequence from GTP uptake to GAP-catalyzed hydrolysis is a membrane-delimited process, and exchange of Gα between membrane surfaces is much slower than hydrolysis. Because in rod cells different pools exist of RGS9-1-Gβ5 that are either associated with R9AP or not, regulation of the association between R9AP and RGS9-1-Gβ5 represents a potential mechanism for the regulation of recovery kinetics.

Timely deactivation of G protein α subunits is a key element of responses to the stimulation of G protein-coupled receptors. It plays an especially important role in fast cellular responses such as those of vertebrate photoreceptors. In the rod and cone cell outer segments, the recovery phase of light responses depends on the presence of a GTase-accelerating protein (GAP)1 complex RGS9-1-Gβ5 (1–4). Whether and how the GAP activity of this complex is regulated is unknown.

RGS9-1 contains multiple functional domains, including an RGS domain that is responsible for its GAP activity (3), a G protein γ subunit-like domain for Gβγ binding (4, 5), an N-terminal domain that includes a DEP (dishevelled/EGL-10/pleckstrin) domain (6) and an intermediate domain (7), and a C-terminal domain that is unique to RGS9-1 among all of the RGS proteins (8). All of these domains have been found to participate in the regulation of GAP activity and substrate specificity (9–11). The inhibitory PDEγ subunit of the effector complex RGS9-1 and the catalytic core of RGS9-1 and enhances RGS9-1 GAP activity (12–14) in vitro, but it is not clear how this enhancement is accomplished in a physiological setting in which tight PDEγ binding to PDE6 catalytic subunits blocks GAP enhancement (15–18).

Additional possible mechanisms for regulation include a light- and calcium-regulated phosphorylation (19, 20) of RGS9-1 and interactions with the recently discovered membrane anchor protein, R9AP (7, 21). R9AP is a 25-kDa protein that is selectively expressed in photoreceptor outer segments. Homologues are apparent in genomic sequence from mouse (GenBank™ accession number NW_000311), human (GenBank™ accession number NT_011196), rat (GenBank™ accession number AC128498), zebra fish (NCBI trace archive numbers 15766910 and 46042404), and puffer fish (GenBank™ accession numbers CAAB01004012 and CAAB01001872), and in expressed sequence tags from human (GenBank™ accession numbers AW302149 and BQ187216), mouse (GenBank™ accession numbers BB591662 and BU506122), Xenopus (GenBank™ accession number BG515592), and zebra fish (GenBank™ accession number BE015922). R9AP contains a single transmembrane α helix, and sequence analysis suggests structural similarity to the syntaxin family of proteins involved in membrane targeting (for a review on syntaxin family proteins, see Ref. 22). Biochemical assays and colocalization on co-expression in cell culture indicate that it acts as a membrane anchor for RGS9-1-Gβ5, which binds to the cytoplasmic domain of R9AP (21). The molar ratio of R9AP to RGS9-1 in bovine rod outer segments appears to be variable and was found to range from 0.4 to 0.8. Consistent with these ratios, co-immunoprecipitation experiments revealed that whereas all detectable R9AP in the retina is bound to RGS9-1-Gβ5, a distinct pool of RGS9-1-Gβ5a is not associated with R9AP. Here we describe experiments with recombinant R9AP reconstituted into lipid vesicles, which demonstrate that binding of RGS9-1-Gβ5 to membrane-anchored R9AP dramatically enhances its GAP activity toward Gαγ.

EXPERIMENTAL PROCEDURES

Buffers—Standard buffers were: GAPN buffer: 100 mM NaCl, 2 mM MgCl2, and 10 mM HEPES, pH 7.4; ConA buffer: 300 mM NaCl, 50 mM Tris-HCl, pH 7.0, 1 mM CaCl2, 1 mM MgCl2, and 1 mM MnCl2; lysis buffer: 300 mM NaCl and 25 mM Tris-HCl, pH 8.0; and high-salt buffer: 10 mM HEPES, 1 mM NH4Cl, and 2 mM MgCl2. For all these buffers, 1 mM dithiothreitol and ~20 mg/liter solid phenylmethylsulfonyl fluoride were added before use.

Protein Electrophoresis and Immunoblotting—SDS-PAGE and immunoblotting were carried out using standard protocols (23). Rabbit
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anti-RGS9-1c polyclonal antiserum was generated as described previously and was used at a dilution of 1:10,000. The secondary antibodies used were horseradish peroxidase-conjugated (Promega) anti-rabbit antibody, with detection by chemiluminescence using the ECL™ system (Amersham Biosciences).

Expression and Purification of R9AP—His-tagged bovine R9AP cytoplasmic domain (His-br9AP-ΔC, amino acids 1-212 previously named His-br95-ΔC) and His-tagged full-length murine R9AP (His-mr9AP) were expressed in Escherichia coli using plasmids and procedures described previously (21), and His-br9AP-ΔC was purified as described. For His-mr9AP purification, the cells were collected and lysed in lysis buffer by sonication on ice, and insoluble proteins including His-mr9AP were separated from soluble proteins by centrifugation at 24,000 × g in a Beckman TL100 rotor. The insoluble proteins were extracted with 4% sodium cholate (3a,7α,12α-trihydroxy-5β-cholanic acid, sodium salt, Sigma) in lysis buffer for ~30–60 min at 4 °C with gentle agitation, and the proteins in the supernatant were separated from the pellet by centrifugation. The extraction was repeated a total of 3–4 times, yielding >70% of total His-mr9AP extracted in soluble form. The detergent supernatants were pooled together, and His-mr9AP was purified by nickel nitrilotriacetic (Ni-NTA) superflow, Qiagen chromatography in 4% sodium cholate in lysis buffer according to the manufacturer’s protocol to obtain His-mr9AP of >95% purity.

Expression and Purification of RGS9-1 (G9a)—GST-tagged RGS9-1 (amino acids 1-484)G6, RGS9-1-NGD (amino acids 1-431)G6, and His-tagged RGS9-1-IGDC (amino acid 112–484)G6 complex were expressed and purified from S9 cells as described previously (9, 11). GST-tagged RGS9-1-D (amino acid 276–431) was expressed and purified from E. coli as described previously (14).

Rhodopsin Purification—Bovine ROS were purified as described previously (25). Rhodopsin was purified from bovine ROS according to the protocol described previously with modifications (26). Briefly, ROS membranes were extracted in the dark with high salt buffer twice to remove soluble protein contaminants and then washed with ConA buffer and pelleted. Pelleted membranes were solubilized in 4% sodium cholate in ConA buffer, and insoluble material was removed by centrifugation at 24,000 × g for 40 min at 4 °C. A membrane was immobilized on a nitrocellulose membrane by cross-linking in 0.05% glutaraldehyde in 250 mM NaHCO3 at room temperature for ~2 h to prevent loss of immobilized concanavalin A and then prepared as described previously (26). Rhodopsin was eluted with 300 mM α-methyl-mannoside in 4% sodium cholate in ConA buffer. The sealed column and 1.5-column volumes of elution buffer were gently agitated at 4 °C for 20–30 min, and then the eluted protein was drained from the column. This procedure was repeated twice, and the eluted proteins were pooled together and concentrated in a protein concentrator (Millipore) to obtain a final rhodopsin concentration (determined by dark absorbance at 500 nm) of 2–3 mg/ml.

Vesicle Reconstitution—Lipids used for vesicle reconstitution were t-rhodopsin (brain and porcine-sodium salt), t-α-phosphatidylcholine and (egg and chicken), t-α-phosphatidylethanolamine (egg and chicken) from Avanti Polar Lipids. Lipids were mixed at a ratio of 3:1, unsaturated phosphatidylcholine and (egg and chicken), L-dimyristoylphosphatidylcholine and (egg and chicken), and L-β-phosphatidylethanolamine (egg and chicken), L-phosphatidylcholine:phosphatidylethanolamine:phosphatidylinositol–5=35:15:50 in chloroform, dried under argon flow, and redissolved in 4% sodium cholate in lysis buffer to a final concentration of 20 mg/ml. In the lipid-only vesicles (see below) and R9AP vesicles that were used in binding assays, rhodamine-labelled phosphatidylethanolamine (Molecular Probes Inc., N-(6-tetramethylrhodaminehexanocarbomyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylenammonium salt) was added in the lipid mixture to a final concentration of 0.5% (w/w) to facilitate the visualization of the lipids. Desalted lipids were then mixed with purified t-rhodopsin (rhodopsin, His-mr9AP, or rhodopsin and His-mr9AP) at a lipid-to-protein (w/w) ratio of 20:40±1 and dialyzed against GAPT buffer (100 × volume) for ~120 h with buffer changes every 20–24 h (27). Under these conditions, the majority of the proteins were incorporated into the vesicles. After dialysis, samples were collected and centrifuged at 4 °C in a Beckman TL100 rotor, remove aggregated lipids and lipids, and the vesicles in the supernatant were concentrated by one of the following two methods. For the purpose of vesicle binding assays, R9AP vesicles were pelleted by ultracentrifugation at ~88,000 × g to collect easily pelleted vesicles, and vesicles that stayed in the supernatant were discarded. Pelleted vesicles were resuspended in GAPT buffer, and concentration was adjusted extrusion through a 26-gauge needle. As a control for the binding assays, lipid-only vesicles without protein were made similarly in parallel. For the purpose of GAP assays the vesicles were concentrated in a protein concentrator (Millipore) to achieve the maximum yield. Lipid concentrations of the samples were determined by measuring the total phosphate concentrations (8). The molar vesicle concentrations were calculated using the following formula: molar concentration of vesicles = (formal concentration of phospholipids × V/H4πr2), where s is the average surface area of a phospholipid headgroup in Å2 (70 Å2), and r is the average vesicle radius in Å. We used r = 3.7 Å, which was derived from least squares fitting of the data in Fig. 4. This value is consistent with the appearance of the vesicles in electron micrographs (Fig. 1A, r = 240 ± 50 Å, n = 150). The rhodopsin concentrations were determined by measuring the differential absorbance at 500 nm (using extinction coefficient 46,600 M−1 cm−1) of the sample dissolved in 1.5% lauryldimethylammonium oxide before and after illumination. The R9AP concentrations were determined by densitometry of Coomasie-stained SDS-PAGE gels using bovine serum albumin as standard.

Electron Microscopy—The reconstituted vesicles in solution (vesicle concentration ~200–300 nm) were frozen across the holes of 400-mesh carbon-coated holey grids by rapid plunging into liquid ethane (29, 30). Electron microscopy was performed on a JEOL1200EX equipped with a Gatan liquid nitrogen specimen holder for 100 keV. Images were recorded at a nominal magnification of ×40,000 with a dose of ~20 electrons per Å onto Kodak SO-163 photographic film developed for 12 min in Kodak D19 developer at 20 °C. Micrographs were digitized using a Zeiss Phodis SCIA microdensitometer at 14 μm/pixel.

Vesicle Binding Assay—Purified RGS9-1 proteins (in GAPT buffer) were first centrifuged at 88,000 × g for 40 min to remove aggregates. Supernatants were diluted to desired concentrations in GAPT buffer containing 0.2 mg/ml ovalbumin, mixed with R9AP vesicles and lipid-only vesicles in a volume of 300 μl, and incubated with gentle shaking at 4 °C for ~3 h. 150 μl of the reaction mixture was then transferred to a new polypyrrole membrane (Beckman) to separate unbound proteins from bound proteins by pelleting the vesicles at 88,000 × g for 40 min. The pelleted vesicles were clearly visible as a pink pellet at the bottom of the tube because of the rhodamine tag. The final concentrations in the binding reactions were RGS9-1 proteins (GST-RGS9-1–G6, His-RGS9-1–G6, GST–RGS9–LexiG6, or GST–RGS9–LexiG6–1-D), 0.1 μM; R9AP (total concentration), 3.0 μM (lipid as an indication of vesicle concentration, measured by phosphate concentration), 0.8 mM. Equal proportions of the starting binding reactions, the supernatant after the vesicles were pelleted, and the pelleted vesicles were loaded on SDS-PAGE, and the RGS9 proteins were detected by immunoblotting using anti-RGS9-1c antiserum.

Transducin Activation Assay—Transducin activation by rhodopsin vesicles was determined as described previously (31). Briefly, rhodopsin or rhodopsin and R9AP vesicles were diluted in GAPT buffer and mixed with purified transducin in a volume of 125 μl in dim red light. The reaction was initiated by the addition of 18 μl of [γ-32P]GTP (specific activity ~400–500 Ci/mol) to the mixture, and 18 μl of the reaction was removed and quenched on the filter membrane at the specified time points. The reactions were kept in dim red light until t = 3.0 min and were exposed to room light starting from t = 3.0 min until the end of the assays. The final concentrations in the assays were 1 mM rhodopsin, 0.5 μM transducin, and 2.5 μM [γ-32P]GTP.

Single Turnover GTPase Activity Assay—Single turnover GTPase assays were carried out as described previously (9, and full-length His6-tagged PDEγ (expressed and purified from E. coli) was added to enhance RGS9-1 GAP activity. Briefly, rhodopsin, rhodopsin and R9AP vesicles, or urea-washed ROS membranes were mixed with purified transducin and RGS9-1 proteins in GAPT buffer and incubated on ice for ~90–30 min. GTP hydrolysis was initiated by the addition of [γ-32P]GTP to the above mixture and was quenched by adding 5% trichloroacetic acid at various times. Phosphate released from hydrolyzed GTP was determined by activated charcoal assay. Final concentrations in the reactions using the reconstituted vesicles were 0.1 μM RGS9-1 proteins and 0.2 μM recombinant His6-PDEγ; final concentrations in the reactions using urea-washed ROS were 72 μM rhodopsin, 1.0 μM transducin, and 1.0 μM recombinant His6-PDEγ. The first order rate constant for GTP hydrolysis (kcat) was obtained by the fitting of data to single exponentials, and the rate constants were plotted as mean ± S.D. In the reactions with urea-washed ROS, we used ROS membranes washed with either 4 M urea (32) or 6 M urea (7). Similar results were obtained on these two membranes. The difference was a slightly decreased kcat/Km value of RGS9-1–G6, on the membranes treated with 6 M urea that was probably caused by denaturation of some endogenous R9AP.
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RESULTS AND DISCUSSION

Reconstitution of R9AP and Rhodopsin Vesicles—Because R9AP has a transmembrane domain close to its C terminus, it is expressed as an insoluble protein in both E. coli and Sf9 cells (data not shown). Because the yield of E. coli expression (~10 mg/liter) is much better than that of Sf9 expression (~0.5 mg/liter), we used detergent extractions to purify R9AP expressed in E. coli. We tested both sodium cholate and dodecyl maltoside for their performance in extracting R9AP because of their compatibility with rhodopsin purification (26, 33), and we found that R9AP is readily extractable from the E. coli-expressed proteins by both detergents with comparable efficiency (data not shown). We therefore chose to use sodium cholate for our experiments for its lower cost.

Membrane Anchoring of RGS9-1 by R9AP—We have shown previously (21) that R9AP directly interacts with the RGS9-1 N-terminal domain. Here we tested whether RGS9-1 can be anchored to lipid vesicles by R9AP through this interaction (Fig. 2) by incubating recombinant RGS9-1 domain proteins with R9AP vesicles and removing the vesicles by centrifugation. We found that both the RGS9-1-NGD (amino acids 1–431) complex bound to the R9AP vesicles, whereas RGS9-1-IGDC (amino acids 112–484) complex bound much more weakly, and RGS9-1-D (amino acids 276–431) did not bind at all. The binding to lipid vesicles (Fig. 2, lane 6) was very weak. Therefore, R9AP is sufficient to anchor RGS9-1 to the membranes by interacting with its N-terminal domain, and both the dishevelled/EGL-10/pleckstrin (DEP) domain and the intermediate domain of RGS9-1 are important for the binding, consistent with our previous findings (21). By titrating in full-length RGS9-1-Gβ5 over a fixed concentration of R9AP vesicles in binding assays, we further determined that ~40% of the total R9AP on the vesicles was able to bind RGS9-1-Gβ5 (data not shown), suggesting that R9AP assumed a nearly random orientation in the vesicles during reconstitution.

Membrane Anchoring of RGS9-1-1-Gβ5 Complex by R9AP—Weisberg et al. (7) recently reported that association of the RGS9-1-Gβ5 complex with high affinity sites on ROS membranes dramatically enhanced its GAP activity. To test the enhancement of GAP activity caused by membrane anchoring of the RGS9-1-Gβ5 complex by R9AP, we measured the GAP activity of RGS9-1-Gβ5 complex on reconstituted vesicles with defined protein compositions. We reconstituted purified rhodopsin with and without recombinant full-length R9AP in lipid vesicles and compared the GAP activity of RGS9-1 on these two vesicles. We found that the activity of RGS9-1 increased ~3-4-fold on the rhodopsin and R9AP vesicles above that on the rhodopsin-only vesicles. The increase in activity occurred on...
Enhancement of RGS9-1 GAP Activity by R9AP membrane anchoring. A, GTP hydrolysis rates for G\textsubscript{t} reconstituted on vesicles containing rhodopsin only or rhodopsin and R9AP, in the presence of GST-RGS9-1-G\textsubscript{b\alpha}\ (RGS9) or GST-RGS9-1-D (RGS9-1-D), were measured as described in the text. Final concentrations in the assays were: rhodopsin, 4.0 \mu\text{M}; G\textsubscript{t}, 0.5 \mu\text{M}; RGS9-1 proteins, 0.1 \mu\text{M}; R9AP, 1.2 \mu\text{M}; vesicles, 96 nm (formal molar concentration of phospholipids divided by average number of phospholipids per vesicle). RGS9-1 GAP activity (\Delta \kappa_{\text{max}}) on each vesicle was calculated as \Delta \kappa_{\text{max}} = \kappa_{\text{max}} (of G\textsubscript{t} in the presence of RGS9-1) - \kappa_{\text{max}} (of G\textsubscript{t}). B, GTP hydrolysis rates on rhodopsin-only vesicles in the absence or presence of 0.1 \mu\text{M} GST-RGS9-1-G\textsubscript{b\alpha}\ (RGS9) and in the absence or presence of 0.5 or 2.0 \mu\text{M} His-bR9AP-3C (R9AP-3C) were measured by single turnover assays. Other protein and vesicle concentration were the same as in A.

To determine the maximal enhancement of GAP activity by the R9AP-containing vesicles, we performed a vesicle titration, holding the concentrations of RGS9-1-G\textsubscript{b\alpha}\ constant. Under these conditions, the GAP activity of RGS9-1-G\textsubscript{b\alpha}\ is expected to increase when the fraction of RGS9-1-G\textsubscript{b\alpha}\ associated with R9AP increases. However, as the vesicle concentration increases at a constant RGS9-1-G\textsubscript{b\alpha}\ concentration, a potential counteracting effect also occurs when the number of vesicles approaches and exceeds the number of RGS9-1-G\textsubscript{b\alpha}\ complexes. In these conditions, an increase in the number of vesicles increases the probability of G\alpha\prim\textsubscript{r}GTP formation on a vesicle lacking RGS9-1-G\textsubscript{b\alpha}\. Therefore, if G\alpha\prim\textsubscript{r}GTP exchange between vesicles is very slow on the time scale of GTP hydrolysis, then at higher vesicle concentrations the apparent GAP activity must decline. Moreover, if G\textsubscript{t} and RGS9-1-G\textsubscript{b\alpha}\ bind to rhodopsin- and R9AP-containing vesicles independently of one another, the apparent GAP activity should decline in a way predicted by Poisson statistics. As shown in Fig. 4A, the behavior observed corresponds precisely to these expectations. The effect of titration in R9AP vesicles is biphasic, with GAP activity enhancement increasing at low concentrations and decreasing at higher vesicle concentrations. We were able to model the experimental results by theoretical calculations on the basis of the above considerations and another assumption that the dissociation coefficient (K\textsubscript{d}) between RGS9-1 and R9AP on the vesicles is much lower than the concentrations used in the experiments (i.e. all of the RGS9-1 could be anchored by available R9AP). The calculated -fold enhancement agreed very well with the experimental data (Fig. 4A, fitted curve), supporting the validity of the assumption and giving a maximum GAP enhancement of 4-fold. The declining activity at higher vesicle concentra-

The full-length RGS9-1-G\textsubscript{b\alpha}\ complex that binds to R9AP but not on the RGS9-1-D protein that does not bind R9AP (Fig. 3A). Furthermore, the increase in activity was not merely because of the interaction between the R9AP-soluble domain and RGS9-1, because the addition of R9AP-soluble domain to rhodopsin-only vesicles had no effect on RGS9-1 activity (Fig. 3B). Therefore, we conclude that membrane anchoring of RGS9-1 by R9AP enhances RGS9-1 GAP activity, probably at least in part simply by localizing and orienting RGS9-1 on the vesicles.

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Enhancement of RGS9-1 GAP Activity by R9AP activity, when appropriately corrected for differences in conditions, seems likely to reflect the value attained in intact rod cells.

In addition to establishing the ability of R9AP membrane anchoring to stimulate GAP activity of RGS9-1, the results presented here using the highly efficient bacterial expression and reconstitution method we have developed will facilitate determination of the role of specific lipids and other molecules of ROS membranes in regulating GAP activity and, possibly, structural studies of the GAP complex.

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REFERENCES

2 M. E. Sowa and T. G. Wensel, unpublished data.
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