G protein-coupled receptors (GPCRs) are seven transmembrane domain proteins that transduce extracellular signals across the plasma membrane and couple to the heterotrimeric family of G proteins. Like most intrinsic membrane proteins, GPCRs are capable of oligomerization, the function of which has only been established for a few different receptor systems. One challenge in understanding the function of oligomers relates to the inability to separate monomeric and oligomeric receptor complexes in membrane environments. Here we report the reconstitution of bovine rhodopsin, a GPCR expressed in the retina, into an apolipoprotein A-I phospholipid particle, derived from high density lipoprotein (HDL). We demonstrate that rhodopsin, when incorporated into these 10 nm reconstituted HDL (rHDL) particles, is monomeric and functional. Rhodopsin-rHDL maintains the appropriate spectral properties with respect to photoactivation and formation of the active form, metarhodopsin II. Additionally, the kinetics of metarhodopsin II decay is similar between rhodopsin in native membranes and rhodopsin in rHDL particles. Photoactivation of monomeric rhodopsin-rHDL also results in the rapid activation of transducin, at a rate that is comparable with that found in native rod outer segments and 20-fold faster than rhodopsin in detergent micelles. These data suggest that monomeric rhodopsin is the minimal functional unit in G protein activation and that oligomerization is not absolutely required for this process.

G protein-coupled receptors (GPCRs) are an important class of cell surface receptors representing the third largest gene family in the human genome (1). They recognize a wide variety of extracellular stimuli (e.g. light, tastes, odors, hormones, neurotransmitters, and cytokines, etc.) leading to direct activation of intracellular and membrane-associated G proteins that in turn regulate various downstream effectors (e.g. adenylyl cyclase, phospholipase Cβ, and ion channels, etc.) (2). This diversity highlights the ability of nature to generate specificity in signal detection and propagation, and thus makes GPCRs optimal targets for therapeutics.

To complicate the pharmacology of these targets, GPCRs associate as oligomers of two or more receptors in cellular membranes (3). The growing list of GPCRs that form either hetero- or homomeric complexes implies that oligomerization is important. However, an essential role of oligomerization for GPCR function has not been established.

Evidence that an isolated, monomeric GPCR in a biological membrane is fully capable of stimulating nucleotide exchange by G proteins has been difficult to demonstrate. Intuitively, detergent solubilization and monodispersion of membrane proteins into micelles should represent a suitable substitute for a phospholipid bilayer. However, mounting evidence suggests otherwise, as detergents can have profound deleterious effects on the structure and function of membrane proteins (4–6).

Here we report the isolation of bovine rhodopsin, a prototypical GPCR, in reconstituted HDL (rHDL) particles (Fig. 1). The HDL platform, or a similar membrane protein reconstitution system has been utilized for a variety of proteins, including the proton pump bacteriorhodopsin (8), cytochrome P-450 (9), the β2-adrenergic receptor (10, 11) and, more recently, rhodopsin (12). The HDL platform represents a dramatic improvement over detergent micelles for maintaining GPCRs in a monomeric, monodispersed, but more importantly, functionally intact state.

Here we report the isolation of bovine rhodopsin, a prototypical GPCR (13, 14), in reconstituted HDL (rHDL) particles (Fig. 1). We take advantage of the unique spectral qualities of rhodopsin to demonstrate that it exists as a monomer in rHDL and to follow its activity and behavior compared with rhodopsin in native membranes isolated from rod outer segments (ROS).
find that the rates of formation and decay of metarhodopsin II (meta II), the active form of the receptor, for monomeric rhodopsin are identical to those observed in ROS. We also show that monomeric rhodopsin is capable of stimulating rapid nucleotide exchange on Gt transducin, a photoreceptor-specific G protein, at rates comparable with those observed in ROS. These data show that monomeric rhodopsin is fully functional with regard to G protein activation and suggest that receptor oligomerization does not play an essential role in this process.

EXPERIMENTAL PROCEDURES

Expired human sera were generously donated by Dr. Bert La Du (University of Michigan). All lipids were purchased from Avanti Polar Lipids (Alabaster, AL). N-Dodecyl β-d-maltoside (DDM) was obtained from Dojindo Molecular Technologies (Gaithersburg, MD). Concanavalin A (ConA)-Sepharose, Triton X-100, and sodium cholate were purchased from Sigma. Frozen bovine retina were obtained from J. A. Lawson Co. (Lincoln, NE). All other reagents were of analytical grade.

**Purification of Human ApoA-I**—ApoA-I was purified from human serum essentially as described by Gan et al. (15). Briefly, clarified serum was diluted into a conditioning buffer (50 mM Tris-HCl, pH 8.0, 1 mM CaCl₂, 3 M NaCl, 5 mM EDTA) and added to an equal volume of Cibacron Blue F3GA-agarose (Sigma) equilibrated in 50 mM Tris-HCl, pH 8.0, 1 mM CaCl₂, 3 M NaCl, 5 mM EDTA (Buffer A). The slurry was stirred for 30 min and then batch washed by filtration through a Whatman No. 1 filter in a Büchner funnel five times with Buffer A or until the absorbance (A = 280 nm) of the filtrate was less than 0.025 OD. The resin was then washed with 2 additional volumes of Buffer A without NaCl (Buffer B). The residual cake was resuspended in an equal volume of Buffer B and transferred to a column. ApoA-I was then eluted with Buffer B containing 5 mM cholate, yielding fractions with purities typically between 80 and 90%. Peak fractions were pooled, concentrated, and then diluted 1:1 in 25 mM Tris-HCl, pH 8.0, 1 mM CaCl₂, 5 mM EDTA, 0.2% Triton X-100 and loaded on to a Q-Sepharose (GE Healthcare) column. The column was washed with 20 mM Tris-HCl, pH 8.0, 1 mM CaCl₂, 5 mM EDTA, 0.1% Triton X-100 (Buffer C) and eluted with a shallow linear gradient with Buffer C, containing 1 mM NaCl. Peak fractions (>95% purity) were buffer exchanged into 100 mM potassium acetate, pH 5.0, 1 mM EDTA, 0.1% Triton X-100 and applied to an SP-Sepharose column (GE Healthcare) equilibrated in 25 mM potassium acetate, pH 5.0, 1 mM EDTA, 0.1% Triton X-100 (Buffer D). The column was washed in Buffer D, and apoA-I was eluted with a linear gradient of Buffer D containing 1 mM NaCl. Residual contaminants were removed by size exclusion chromatography on a Superdex 200 column (GE Healthcare) in 20 mM Hepes-NaOH, pH 8.0, containing 100 mM NaCl, 1 mM EDTA, 20 mM cholate, at 4 °C. ApoA-I fractions were pooled and concentrated (~10 mg/ml), then dialyzed at 4 °C against 20 mM Hepes-NaOH, pH 8.0, 100 mM NaCl, 1 mM EDTA, 5 mM cholate, and stored at −80 °C until further use.

**Purification of Bovine Rhodopsin**—Rhodopsin was purified from ROS isolated from bovine eyes as described previously (16), a procedure that yielded rhodopsin with an A₂₈₀nm/A₅₀₀nm absorbance ratio of 1.63. ZnSO₄ was removed by dialysis in the presence of 0.1% DDM (final) to avoid precipitation of added cholate during the subsequent rHDL preparation.
In Vitro Reconstitution of HDL—HDL was reconstituted in vitro essentially as described by Jonas (17) with slight modifications. Palmitoyloleylophosphatidycholine (POPC) and palmitoyleoletoylophosphatidylglycerol were used in combination at a 3:2 molar ratio to mimic the zwitterionic environment of a cell membrane (18). Lipids were dried under argon (or nitrogen) from a chloroform solution and desiccated under vacuum for 30–60 min to remove residual chloroform. Then briefly, lipids were solubilized in 20 mM Hepes-NaOH, pH 8.0, 100 mM NaCl, 1 mM EDTA (Buffer A) containing 50 mM detergent (DDM or cholate) and added to purified rhodopsin. ApoA-I was then added and the mixture incubated for 1–2 h on ice. The final concentrations of the components were 1 µM rhodopsin, 24 mM detergent, 8 mM lipids, and 100 µM apoA-I. This mixture was added to Bio-Beads (Bio-Rad) to remove the detergents. Samples were stored on ice until used. All of the above procedures were performed under dim red light λ > 640 nm.

Analytical Size Exclusion Chromatography—Analytical size exclusion chromatography was performed on an HR10/30 column (GE Healthcare) packed with about 20 ml of Superdex 200 prepaarative resin (GE Healthcare) (Ve = 7 ml). The column was calibrated with thyroglobulin (molecular mass of 669 kDa, Stokes diameter 17.2 nm, elution volume (Ve) 9.1 ml, apoferritin (432 kDa, Stokes diameter 12.2 nm, Ve 10.6 ml), alcohol dehydrogenase (150 kDa, Stokes diameter 9.1 nm, Ve 13.2 ml), bovine serum albumin (66 kDa, Stokes diameter 7.2 nm, Ve 14.7 ml), and carbonic anhydrase (29 kDa, Stokes diameter 4 nm, Ve 17.8 ml). Samples were loaded in 100–500-µl static loops and run at a flow rate of 0.7 ml/min using the BioLogic DuoFlow system (Bio-Rad) at 4 °C. Column fractions, 200 µl each, were collected in a 96-well plate for further analysis. Chromatography of rhodopsin samples was performed under dim red light.

UV-visible Absorbance Assays—All UV-visible absorbance assays were performed in UV-transparent 96-well plates (Corning Glass) on a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA). The PathCheck™ feature was used for normalizing path lengths to 1 cm. Rhodopsin concentrations were determined by the absorbance change at λ = 500 nm before and after bleaching with 40,600 M⁻¹ cm⁻¹ used as the extinction coefficient (19). Rhodopsin photoactivation was achieved by illuminating samples for 15 s with a 500-watt halogen lamp affixed with a 495 nm long pass filter (Melles Griot, Rochester, NY).

Meta II Formation and Decay Spectra—Rhodopsin samples were adjusted to pH 6.0 and photoactivated as described above. Subsequent absorbance spectra were recorded at 0, 5, 10, 15, and 30 min. The meta II decay rate was measured by the tryptophan fluorescence (λeicitation = 295 nm and λemission = 330 nm) method of Farrens and Khorana (20). All measurements were performed with 10 mM rhodopsin in a buffer consisting of 10 mM BisTris-HCl, pH 6.0 (or 10 mM Tris-HCl, pH 8.0, where indicated), containing 100 mM NaCl, which favors the formation of the meta II state. Similar meta II decay measurements were made at pH 8.0 using a buffer containing 10 mM Tris-HCl, pH 8.0, and 100 mM NaCl. An LS 55 luminescence spectrophotometer (PerkinElmer Life Sciences) was used to measure the intrinsic fluorescence increase because of Trp residues that correlates with the decrease in the protonated Schiff base concentration (data not shown but consistent with Refs. 20–22). Rhodopsin+HDL or ROS membranes were bleached by a Fiber-Lite illuminator for 15 s immediately before the fluorescence measurements. Bleaching was carried out from a distance of 15 cm, to prevent heat accumulation, and a thermostat was applied to stabilize the temperature of the cuvette at 20 °C. Fluorometer slit settings were 2.5 nm at λ = 295 nm for excitation and 8.0 nm at λ = 330 nm for emission.

Purification of Rhodopsin+rHDL Particles by ConA-Sepharose Chromatography—Prior to use, the ConA-Sepharose resin was cross-linked with glutaraldehyde as described previously (23). The cross-linked resin was then poured into a column and pre-washed with 5 column volumes of 1 mM NaCl, 5 mM MgCl₂, 5 mM MnCl₂, 5 mM CaCl₂, and then equilibrated with 10 column volumes 20 mM Hepes, pH 7.2, 150 mM NaCl, 5 mM MgCl₂, 5 mM CaCl₂. The sample was then loaded onto the column and continuously passed through until at least 90% had bound as determined by A₂₈₀ (five passes were usually sufficient). The column was then washed with at least 10 column volumes of the equilibration buffer. Rhodopsin+rHDL particles were eluted with the equilibration buffer containing 200 mM α-methylmannosine. All procedures were performed at 4 °C in the dark.

Rhodopsin+HDL Purification by 1D4 Immunoaffinity Chromatography—Reconstituted rhodopsin+rHDL was loaded on a 1D4-coupled CNBr-activated Sepharose 4B column (Amersham Biosciences), equilibrated with a buffer consisting of 10 mM BisTris, pH 7.5, 100 mM NaCl, and 1 mM EDTA. The flow-through fraction was reloaded onto the same column two additional times. The beads were washed with the equilibration buffer until the absorbance at 280 nm was below 0.01. Purified rhodopsin+rHDL was eluted with 100 µM nonapeptide TETSQVAPA (from the rhodopsin C-terminal sequence) in the above buffer at room temperature. The purified rhodopsin concentration was determined using a Cary 50 (Varian) UV-visible spectrophotometer. All procedures were performed in the dark.

Rhodopsin:ApoA-I Molar Ratio—Rhodopsin:apoA-I ratios were determined from ConA-Sepharose or 1D4 affinity-purified rhodopsin+rHDL preparations using the known extinction coefficients for rhodopsin (40,600 M⁻¹ cm⁻¹ at λ = 500 nm) (19) and apoA-I (31,720 M⁻¹ cm⁻¹ at λ = 280 nm) (24) and the known 280–500 nm absorbance ratio of pure rhodopsin (ratio ~ 1.6).

Activation of Transducin—Photoactivation of Gt, was performed as described previously (25, 26). Rhodopsin concentrations were determined by the absorbance change at λ = 500 nm before and after bleaching using the extinction coefficient of 40,600 M⁻¹ cm⁻¹ for purified rhodopsin (19). Gt concentration was determined by Bradford assay (Bio-Rad). Purified, native bovine Gt, heterotrimer (Gtα, Gtβ₁, Gtγ₁) was added to rhodopsin in sonicated bovine ROS membranes before the measurement or rhodopsin+rHDL. Photoactivation was done at a Gt:rhodopsin ratio of 8:3:1 at a concentration of 250 nm and rhodopsin at 30 nm, within the linear range of fluorescence change and protein concentration. The measurement was performed in a buffer consisting of 20 mM BisTris-HCl, pH 6.0, containing 120 mM NaCl and 6 mM MgCl₂. The sample was bleached for 15 s with a Fiber-Lite using a long pass wavelength filter (λ > 490 nm) and...
followed by a 400-s incubation with continuous low speed stirring. Transducin activation was initiated by the addition of GTPyS (5 μM final). Activation was monitored by following the intrinsic fluorescence increase from Gzα using an LS 55 luminescence spectrophotometer (PerkinElmer Life Sciences) employing excitation and emission wavelengths of λ = 300 nm and λ = 345 nm, respectively, at 20 °C (21, 26, 27). No signals from rhodopsin without transducin were detected in control experiments (not shown).

Transmission Electron Microscopy (TEM)—Reconstituted HDL samples were placed on a carbon-coated copper grid and stained with 1% phosphotungstic acid, pH 6.5. Samples were imaged with a Philips CM-100 TEM operating at 60 kV. Purified (either ConA or 1D4 immunoaffinity) rhodopsin-rHDL particles were adsorbed for 10 s to parlodion carbon-coated copper grids rendered hydrophilic by glow discharge at low pressures. Grids were washed with double distilled water and stained with 0.75% uranyl formate. Electron micrograph images of rhodopsinin-rHDL particles were recorded with a Hitachi H-7000 TEM operated at 100 kV.

**RESULTS**

Photoactivation of rhodopsin results in the isomerization of its covalently bound ligand 11-cis-retinal (absorbance maximum, λ = 500 nm) to all-trans-retinal, creating meta II (λ = 380 nm), a spectroscopically distinct form (19). Successful reconstitution of rhodopsin into HDL particles was observed using a mixture of POPC and palmitoyloleoylphosphatidylglycerol at a 3:2 ratio, consistent with the notion that optimal photoisomerization to the meta II state requires unsaturated lipids of 16 carbons or greater (28). POPC alone was also used successfully (data not shown).

Incorporation of rhodopsin into rHDL, in the presence of excess rHDL, yielded particles with Stokes diameters of 10.5 nm based on detection at λ = 280 nm as assessed by size exclusion chromatography (SEC), whereas the peak at absorbance at λ = 500 nm eluted with an estimated diameter of 11 nm (Fig. 2a). No absorbance at λ = 500 nm was observed with empty HDL particles (not shown). Analysis of the peak SEC fraction by spectroscopy indicates that rhodopsin in rHDL is functional as it photoisomerized to the active meta II state (Fig. 2b).

**TABLE 1**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>A_{280}</th>
<th>A_{500}</th>
<th>[Rhodopsin]:[apoA-I]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>1</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Flow-through</td>
<td>0.57</td>
<td>0.02</td>
<td>0.035</td>
</tr>
<tr>
<td>Elute 2</td>
<td>0.12</td>
<td>0.04</td>
<td>0.47</td>
</tr>
<tr>
<td>Elute 3</td>
<td>0.07</td>
<td>0.02</td>
<td>0.47</td>
</tr>
<tr>
<td>Elute 4</td>
<td>0.04</td>
<td>0.01</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**FIGURE 2. Rhodopsin incorporated in rHDL and resolved by size exclusion chromatography is photoactivable.** a, purified bovine rhodopsin incorporated into rHDL particles was resolved on a Superdex 200 size exclusion column; fractions (200 μl) were collected and analyzed for λ = 280 nm (blue) and λ = 500 nm (red) absorbance. b, peak fraction from the gel filtration run was adjusted to pH 6.5, and UV-visible spectra were measured before and after photoactivation.

**FIGURE 3. Rhodopsin exists as a monomer in rHDL particles.** a, rhodopsin incorporated into rHDL particles was purified on a ConA-Sepharose column and subsequently resolved on a size exclusion column. Eluates were assayed for absorbance at λ = 280 nm (blue) and λ = 500 nm (red), and the rhodopsin:apoA-I ratio (green) was calculated as described under “Experimental Procedures.” b, TEM of negatively stained purified rhodopsin-rHDL particles showing their homogeneous discoidal shapes and monodispersal distribution.

It is important to note that removal of the detergent in the absence of lipid and/or apoA-I severely alters the integrity of rhodopsin. If either component is excluded, most of the rhodopsin precipitates and pellets after brief sedimentation in a microcentrifuge (16,000 × g for 2 min). Any rhodopsin that seemed not to sediment migrated in the void volume of the size exclusion column, suggesting that it formed large protein aggregates or was contained in proteoliposomes (data not shown). When both components were included, we routinely observed reconstitution efficiencies of 70–90%, based on absorbance at λ = 500 nm.

To determine the rhodopsin:rHDL stoichiometry, we utilized the known extinction coefficients of rhodopsin (at λ = 280 and λ = 500 nm) and apoA-I (at λ = 280 nm) to calculate the molar ratio of [rhodopsin]:[apoA-I] in purified rhodopsin-rHDL preparations. Rhodopsin-rHDL purified by either ConA-Sepharose (taking advantage of the glycosylated rhodopsin) (Table 1) or by 1D4 immunoaffinity chromatography (data not shown) and then resolved by SEC (Fig. 3a). The average [rhodopsin]:[apoA-I] molar ratio of the SEC peak fractions was determined to be 0.47 ± 0.05 (Table 1 and Fig. 3a). Note that the A_{280}:A_{500} ratio of rhodopsin is unaltered by reconstitution in phospholipids in comparison with detergent micelles (see supplemental Fig. 7). Taking into consideration **TABLE 1**

Rhodopsin exists as a monomer in rHDL particles. Rhodopsin-rHDL particles were resolved on a size exclusion column and then purified on a ConA-Sepharose column. The load, flow-through, and three serial elutions from the ConA-Sepharose column were assayed for absorbance at λ = 280 nm and λ = 500 nm. ConA-Sepharose column eluates were pooled and re-resolved on a size exclusion column in order to confirm that the size of the rhodopsin-rHDL particles remained the same before and after processing by the ConA-Sepharose column. The average A_{280}:A_{500} ratios of all fractions across the peak were calculated as described under “Experimental Procedures.”
that there are two apoA-I proteins per rHDL particle (17, 29), these data suggest that only one rhodopsin molecule is reconstituted per rHDL. The purified rhodopsin/rHDL complexes also had a Stokes diameter of 11 nm based on their elution volume from the SEC column (Fig. 3a). This rules out the possibility that larger rHDL particles were formed (i.e. two rhodopsins to four molecules of apoA-I, still in a 0.5:1 ratio). Moreover, TEM of the 1D4-immunopurified rhodopsin/rHDL complexes shows a monodispersed and homogeneous rHDL particle morphology with an estimated diameter of 13.7 ± 1.4 nm (n = 105; Fig. 3b). We attribute the slightly larger diameter compared with those estimated by SEC and the previously reported HDL sizes (30) to a commonly observed flattening artifact of particles by surface tension during drying of the stain (31, 32). Atomic force microscopy confirms that these particles are discoidal with a thickness of about 43 ± 4.7 Å, similar to that of a single phospholipid bilayer (33) (data not shown).

To assess the function of rhodopsin incorporated into rHDL, we performed UV-visible absorbance scans of these reconstitutions before and after photoactivation (Fig. 4, a and b). Exposure to light resulted in a rapid and almost complete conversion of the absorbance spectra of rhodopsin/rHDL from λ = 500 nm (rhodopsin) to λ = 380 nm (meta II). Over a time course of 30 min at pH 6.0 (Fig. 4a), meta II slowly decayed to both free all-trans-retinal (as observed in the slight red-shift of the λ = 380 nm peak) and meta III (as observed in the increase of the λ = 465 nm peak). This sequence is identical to that observed for rhodopsin in native ROS membranes (19) as well as for rhodopsin reconstituted in long chain phospholipid-containing vesicles (28, 34). Photoactivation under higher pH conditions (pH 8.0) was rapid; however, progression out of meta I (λ_max ~ 480 nm) appears slower (Fig. 4b), consistent with previously established biophysical properties of rhodopsin (35). Using the method of Farrens and Khorana (20), we determined that the time constant, τ, for the meta II decay for rhodopsin in rHDL was 19.2 ± 0.75 min (Fig. 4d), nearly identical...
to the decay rate for rhodopsin from ROS (20 ± 2.4 min) (Fig. 4c) and similar to previously observed values (25).

Unlike other members of the heterotrimeric G protein family, transducin (Gt) can be suspended in solution in the absence of detergent (36). Transducin activation may be accomplished by simply adding the heterotrimer to rhodopsin preparations, whether in ROS membranes or as a purified protein. Fig. 5a illustrates that activation of transducin by photoactivated rhodopsin from ROS membranes, at 8.3:1 Gt:rhodopsin ratio, occurred with a rate constant of \( \sim 1.7 \times 10^{-2} \text{ s}^{-1} \), comparable with previously reported values (22, 25). Strikingly, however, activation of monomeric rhodopsin in rHDL occurred at a similar rate (Fig. 5b). The rates of transducin activation in these preparations are markedly greater than we previously reported using detergent-solubilized rhodopsin in different oligomeric states (37). Titration with increasing \( \text{Gt:rhodopsin in rHDL} \) ratios raised the rate of activation to a maximal value of \( \sim 2.6 \times 10^{-2} \text{ s}^{-1} \) (Fig. 5c). Maximal rates were obtained at \( \text{Gt:rhodopsin in rHDL} \) ratios of \( \sim 12:1 \).

DISCUSSION

Receptor oligomerization has become the subject of considerable attention in the GPCR field. A plethora of studies using elegant approaches have provided descriptive evidence of receptor homo- and/or heterodimerization of visual receptors and many hormone receptors (38, 39). Comparatively fewer contributions, however, have provided evidence for functional roles of oligomerization. Targeting roles of receptor hetero-oligomers have been suggested for the GABA_\text{A}_1 and GABA_\text{B}_2 receptors (33, 40) or with the \( \alpha_{1\text{h}1}^\beta \) and \( \alpha_{1\text{d}1}^\beta \) adrenergic receptors (41). Biophysical and biochemical approaches have also suggested allosteric effects of receptor homo-oligomers of the metabotropic glutamate receptors (42) or leukotriene B\(_4\) receptors (43). In addition, a growing body of evidence suggests that most GPCRs exist as oligomers in cellular membranes, complexes that are found in the endoplasmic reticulum and Golgi apparatus and thus occur during receptor maturation (reviewed by Bulenger et al. (44)). Previously reported imaging data of native retinal disk membranes also suggest that certain receptor types, such as rhodopsin, are capable of organizing into higher order oligomers (45, 46). However, the question as to whether G protein activation is dependent on oligomerization has yet to be elucidated.

Data presented here suggest that activation of transducin by monomeric rhodopsin preparations is similar to oligomeric rhodopsin preparations in ROS membranes. These data are in apparent contradiction to our previously reported results using detergents to selectively solubilize oligomeric rhodopsin complexes (37). Our ability to observe faster rates of transducin activation by monomeric rhodopsin in rHDL particles compared with detergent likely reflects the combined effects of lipids on rhodopsin structure and on G protein incorporation. Moreover, the monomeric rhodopsin data are consistent with
early studies addressing single photon responses of rod cells, demonstrating that one activated rhodopsin per cell triggers phototransduction (47). The heterotrimeric transducin is acyl-modified on both the N terminus of G\textsubscript{t}\textalpha (myristoylation) and the C terminus of G\gamma (isoprenylation) subunits, and both these modifications have been shown to contribute to G protein interaction with rhodopsin (48, 49). Although many studies suggest that monomeric rhodopsin in detergent micelles represents the minimal functional unit (reviewed by Chabre and le Maire (50)), few of them report the isolation and characterization of monomeric rhodopsin in a membrane environment. Reconstitution of monomeric rhodopsin into a phospholipid bilayer such as rHDL therefore represents a significant advance in our understanding of the contributions of homo-oligomerization of rhodopsin and perhaps most GPCRs. 

We have recently reported the functional reconstitution of a monomeric \beta\textsubscript{2}-adrenergic receptor and demonstrated efficient G protein coupling (11). However, unlike this study, we could not take advantage of a direct comparison with the behavior of natively organized \beta\textsubscript{2}-adrenergic receptor oligomers. Likewise, a recent report by Bayburt et al. (12) used a similar approach to incorporate either one or two rhodopsins in rHDL-like particles termed nanodiscs. In that study rHDL-like particles containing two rhodopsin molecules were found to be half as effective in activating transducin as the monomeric form but still functioned at rates considerably faster than those observed in detergent micelles. The slower rates observed with the particles containing two rhodopsins suggest that only one rhodopsin molecule within the “dimer” is active, consistent with a pentameric model containing rhodopsin-G protein-rhodopsin, as described below. Although these data support this attractive model, the authors acknowledge that the slower observed transducin activation rates may be due to the formation of “antiparallel” dimers (i.e. N termini located on opposite sides of the phospholipid bilayer) within the nanodisc. Transducin coupling to antiparallel dimers may not be optimal under these conditions. Alternatively, two parallel rhodopsin monomers may be inserted, but steric effects may preclude efficient coupling of each monomer to transducin. Our current data suggest that oligomeric rhodopsin from ROS membranes activates transducin at comparable activation rates as monomeric rhodopsin in rHDL. Evidence from imaging studies demonstrated that native ROS membranes contain arrays of rhodopsin dimers that are arranged in a parallel fashion (51), suggesting that our preparations contain parallel dimers.

Monomeric G\textsubscript{t}-rhodopsin-rHDL data strongly suggest that whereas oligomerization occurs with rhodopsin and perhaps most GPCRs, the minimal G protein signaling unit is likely a monomer. These data represent a departure from our previously reported interpretation that rates of activation of transducin depend on rhodopsin oligomeric states (37). The simplest explanation for this discrepancy could be that the presence of phospholipids surrounding rhodopsin is critical for efficient precoupling and for fast G activation.

The simplest model of G protein-receptor stoichiometry that is consistent with the data presented here and with the notion of receptor oligomerization is that there is one G protein heterotrimer per receptor homodimer (illustrated in Fig. 6). Such a pentameric, rhodopsin-G protein complex was previously proposed by Filipek et al. (52), a model that has also been proposed for the GABA\textsubscript{A} receptor (53, 54), mGlu receptor (42), leukotriene B\textsubscript{2} receptor (55), and \alpha\textsubscript{1b}/\alpha\textsubscript{2A}-adrenergic receptor (41).

In summary, our data suggest that a reconstituted monomeric GPCR is capable of activating a G protein as efficiently as its native receptor oligomer and are consistent with previous biophysical models of transducin activation (reviewed in Ref. 50). Similar rates of transducin activation by rhodopsin-rHDL compared with ROS membranes suggest that oligomerization is not essential for G protein activation. Rather, GPCR oligomerization may contribute toward fine-tuning of photoreceptor responses, receptor stabilization, targeting, and desensitization. Indeed, the “double chalis” structure of arrestins infers that its interaction may be significantly enhanced by receptor oligomerization (56–58). The recruitment of arrestin to GPCRs and therefore the recruitment of various kinases (e.g. Src, Raf, and Erk) have been proposed to serve as an important, non-G protein-dependent function of the superfamily of seven transmembrane-spanning receptors (59). Future studies will undoubtedly reveal the underlying functions of oligomerization itself and the contributions it makes toward recruitment of important signaling partners.

Acknowledgments—We thank John Tesmer for critical discussion and Dorothy Sorenson in the University of Michigan Microscopy and Image Analysis Laboratory for assistance with the electron microscopy.

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Monomeric Rhodopsin in a Phospholipid Bilayer