Dynamics of Arrestin-Rhodopsin Interactions:  
ACIDIC PHOSPHOLIPIDS ENABLE BINDING OF ARRESTIN TO PURIFIED RHODOPSIN IN DETERGENT

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running title: dynamics of arrestin-rhodopsin interactions

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We report acidic phospholipids can restore the binding of visual arrestin to purified rhodopsin solubilized in n-dodecyl-β-D-maltopyranoside (DM). We used this finding to investigate the interplay between arrestin binding and the status of the retinal chromophore ligand in the receptor binding pocket. Our results show that arrestin can interact with the late photoproduct Meta III and convert it to a Meta II-like species. Interestingly, in these mixed micelles, the release of retinal and arrestin is no longer directly coupled as it is in the native rod disk membrane. For example, up to ~50% of the retinal can be released even though nearly all the arrestin remains bound to the receptor in a long-lived complex. We anticipate this new ability to study these proteins in a defined, purified system will facilitate further structural and dynamic studies of arrestin-rhodopsin interactions.

The integral membrane protein rhodopsin (Rho2) enables the conversion of light to nerve signals in the rod cells, resulting in dim-light vision (1-3). In the dark, the chromophore (11-cis retinal) is linked to Rho at Lys296 by a protonated Schiff-base (λmax~500 nm). Light absorption isomerizes the chromophore to all-trans retinal. Within milliseconds two photoproducts evolve, Meta I (λmax~480 nm) and Meta II (λmax~380 nm), which are in a pH and temperature-sensitive equilibrium. Meta II’s ability to bind and activate the G-protein transducin (4) is terminated in several ways. Meta II can decay through hydrolysis of the Schiff-base linkage and release of retinal, a process that takes ~1 minute at physiological temperature and pH (5). Alternatively, Meta II can decay to the long-lived retinal storage photoproduct Meta III (λmax~470 nm), in which the Schiff-base is intact and protonated (6-9). Finally, Rho signaling can be blocked through a series of protein-protein interactions that involve phosphorylation of Rho’s C-terminal tail by Rho kinase and binding of the protein arrestin (10).

Earlier, we found these inactivation mechanisms are related. That is, arrestin and retinal release appear to be directly linked events – both are described by similar activation energies and arrestin slows the rate of retinal release ~2-fold at physiological temperatures. Intriguingly, we also found a fraction of the arrestin remains bound to ROS*-P long after “active” Meta II decay (5).

In the present work, we have expanded upon these studies and made several surprising discoveries. First, we find adding phospholipids restores arrestin binding to purified Rho*-P solubilized in dodecylmaltoside (DM). Second, we clearly establish in the mixed micelle system that arrestin interacts with the post-Meta II photodecay product Meta III. Finally, we find that arrestin and retinal release appear to be unlinked in mixed micelles, with the acidic phospholipids PS, PI, and PA showing a more pronounced effect than the neutral phospholipids PC and PE (Figure 1 shows a scheme of the different lipid head-groups). Intriguingly, with the acidic phospholipids, arrestin dissociation is nearly completely inhibited, yet ~half of the retinal is released, with the remainder trapped in the binding pocket.

EXPERIMENTAL PROCEDURES

Materials - Frozen bovine retinas were purchased from Lawson and Lawson, Inc. (Lincoln, NE), and GBX red light filters were from Eastman Kodak Co. 11-cis retinal was a generous gift from Rosalie Crouch (Medical University of South Carolina and National Eye Institute). Biomax centrifugal concentrators (10 kDa cutoff) were obtained from Millipore (Bedford, MA), and...
monobromobimane was purchased from Molecular Probes (Eugene, OR). Cuvettes were purchased from Uvonics (Plainview, NY), and band-pass filters and long-pass filters were obtained from Oriel (Stratford, CT). Acrylamide / bisacrylamide solution (37.5:1) and microcolumns were purchased from Bio-Rad. Concavalin A Sepharose, HiTrap Heparin and HiTrap Q pre-packed columns were obtained from Amersham (Piscataway, NJ). Spectroscopic-grade buffers were from USB Corporation (Cleveland, OH). Asolectin was purchased from Fluka (Buchs, Switzerland), and n-dodecyl-β-D-maltopyranoside (DM) was from Anatrace (Maumee, OH). Purified phospholipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), L-α-phosphatidylinositol (PI, from soy) and 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) were obtained from Avanti Polar Lipids (Alabaster, AL). All other chemicals and reagents were purchased from Sigma.

Buffers - Buffer A: 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8mM NaH₂PO₄, 2 mM CaCl₂, 2 mM MgCl₂ and 2mM MnCl₂, pH 6.0. Buffer B: 20 mM HEPES, 2 mM CaCl₂, 2 mM MgCl₂, 2 mM MnCl₂, pH 6.5. Buffer C: 20 mM HEPES, pH 7.4. Buffer D: 10 mM Tris-HCl, 2 mM EDTA, 100 mM NaCl, pH 7.5. Buffer E: 10 mM Tris-HCl, 2 mM EDTA, pH 7.0. Buffer F: 10 mM Tris-HCl, 2 mM EDTA, pH 8.5. Buffers A, B, and C were supplemented with 0.1 mM PMSF, and Buffers D, E, and F were supplemented with 1 mM DTT and protease inhibitor cocktail (Sigma, for bacterial cell extracts) immediately before use.

Preparation of Rod Outer Segments and Purification of Rho - ROS and highly phosphorylated ROS were prepared from bovine retinas as described previously (5).

Rho was purified using Con A. Briefly, ROS containing 1 mg of Rho was solubilized in 14 mL of Buffer A containing 1% DM (mixing for 30 min, 4°C) and then clarified by centrifugation (40,000 x g, 30 min). The supernatant was added to Con A sepharose (400 μl settled beads, equilibrated with Buffer A plus 0.1% DM) and incubated overnight at 4°C while mixing. Washing occurred batch-wise: the beads were pelleted using a clinical centrifuge (2,000 RPM, 3 min), the supernatant was removed, fresh buffer was added, and the beads were mixed for 10 min (4°C). The beads were washed three times with 15 mL Buffer A plus 0.1% DM, three times with Buffer B plus 0.1% DM, and two times with Buffer C plus 0.05% DM. After transferring the beads to a micro-column, Rho was eluted with Buffer C plus 0.05% DM and 0.3 M methyl α-D-mannopyranoside. Rho concentration was ascertained by absorbance at 500 nm (ε = 40,800 liters cm⁻¹ mol⁻¹), and aliquots were snap-frozen and stored at -80°C. After thawing, Rho samples were centrifuged at 100,000 x g for 20 min and the concentration was reassessed before use in experiments.

Construction, Expression, and Purification of Arrestin - The bovine visual arrestin cDNA with a single glycine inserted at residue 2 (a generous gift from V.V.Gurevich) was cloned in the pET15b vector (Invitrogen) for bacterial expression. Mutant constructs W194F and I72C/W194F were created using PCR, and the constructs were verified by DNA sequencing.

Arrestin was expressed in Escherichia coli BL21(DE3) cells and purified as described with some modifications (11,12). A single colony was used to inoculate 400 mL of LB plus ampicillin (100 μg/mL) and grown while shaking at 37°C overnight. This culture then split between four flasks (each containing 1 liter LB + ampicillin) and grown at 30°C while shaking. Upon reaching an OD₅₉₅ of 0.6, the cultures were induced with 30 μM IPTG and grown for an additional 16-20 hours. Cells were harvested by centrifugation (6,000 x g, 15 min), resuspended in cold Buffer D, and lysed by two-passes through a French Press (20,000 PSI). The lysate was cleared by centrifugation (27,000 x g, 30 min). Ammonium sulfate was added to a concentration of 0.32 g/mL, and the precipitated protein was collected by centrifugation (27,000 x g, 30 min). The pellet was resuspended in Buffer E and centrifuged again before being dialyzed overnight against Buffer E plus 0.1 M NaCl. The dialyzed lysate was loaded onto a HiTrap Heparin column (20 mL) equilibrated with Buffer E plus 0.1 M NaCl. The column was washed with ~200 mL of Buffer E plus 0.1 M NaCl. After elution by a linear gradient 0.1 M to 0.5 M NaCl, the arrestin-containing fractions were determined by SDS PAGE, pooled, and dialyzed overnight against Buffer F plus 0.1 M NaCl. The dialyzed fractions were loaded onto a HiTrap Q column (5 mL) equilibrated with Buffer F. The dialyzed fractions were diluted 1:10 with buffer F while loading onto the column. The loaded column was washed with 50 mL of Buffer F, and arrestin was eluted with a
two-step gradient: 0 M to 0.1 M and 0.1 M to 0.5 M NaCl. The arrestin-containing fractions were pooled and concentrated to \(\sim 2.5\) mg/mL, snap-frozen, and stored at -80°C. The purity of the recombinant arrestin was \(>95\%\) as ascertained by SDS PAGE, and the yield was typically 5 to 6 mg of arrestin.

Labeling of Arrestin - Arrestin samples were labeled with monobromobimane as described previously (5), except that no His-tag selection was used. Briefly, arrestin samples were buffer-exchanged (5 mM MES, 150 mM NaCl, pH 6.5) and concentrated, and monobromobimane was added in 10-fold molar excess to arrestin. After a 3-hour incubation at room temperature, the majority of the free label was removed by ultrafiltration (Millipore Biomax). The labeled arrestin was then passed over a size exclusion column (500 µl, Sephadex G-15) to remove trace free label and buffer exchanged into 20 mM HEPES, 150 mM NaCl, pH 7.4. The labeling efficiency was calculated as described previously (5); recombinant arrestin I72C/W194F labeled at ~92% efficiency; recombinant arrestin W194F labeled at less than 2% efficiency. No free label contamination was detected in the labeled arrestin samples. Centrifugal pull-down analysis (5) showed arrestin W194F and bimane-labeled I72C/W194F to have essentially the same affinity for Rho*-P as WT arrestin (data not shown).

Preparation of Mixed Micelles – For experiments using mixed micelles of ROS phospholipids and DM, ROS containing 50 µM Rho-P was solubilized in 1% DM. The membranes were dispersed in the detergent by continuous sonication (25°C, 2 minutes, Branson 1210), followed by centrifugation at 100,000 x g for 15 minutes to pellet the insoluble material. This stock of solubilized ROS-P was diluted into buffer containing different amounts of DM, and samples were sonicated briefly and incubated at 25°C for one hour to allow equilibration of the micelles.

For experiments using asolectin, an appropriate volume of 1% DM was added to a portion of powdered asolectin to yield a stock of 1% asolectin/DM. The solution was passed multiple times through a fine needle to disperse the phospholipids, and was clarified by centrifugation (100,000 x g, 20 min) before use. For all experiments, DM and asolectin stocks were diluted in 20 mM HEPES, 150 mM NaCl, pH 7.4 to give a final DM concentration of 0.02%. For molarity calculations of asolectin, an average phospholipid molecular weight of 750 g/mole was assumed.

For experiments using purified phospholipids, a measured volume of the chloroform stock corresponding to 1 mg of phospholipid (as supplied from Avanti) was dispersed into a glass test-tube, and the chloroform was evaporated with a continuous stream of argon. The dried lipid film was then resuspended in a volume of 1% DM in buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) by vortexing and multiple freeze-thaw cycles to give a final phospholipid concentration of 5 mM. All phospholipids suspensions were stored in the dark and handled under argon to avoid lipid oxidation, and lipid stocks were clarified by centrifugation (30,000 x g, 10 min) before use.

Fluorescence Spectroscopy - All steady-state and time-resolved fluorescence measurements were made as described previously (5). The tryptophan-less arrestin mutant W194F was used, for it contributes less background while measuring opsin tryptophan fluorescence in samples of Rho and arrestin. Since asolectin exhibits some intrinsic fluorescence, background controls were measured and subtracted from appropriate fluorescence spectra.

UV-Visible Absorbance Spectroscopy - All UV-visible absorption spectra were recorded with a Shimadzu UV-1601 spectrophotometer using a bandwidth of 2 nm. For the photodecay experiments, the absorbance of 1 µM of Rho-P (120 µl) was recorded in the dark after base-lining with the appropriate buffer. The sample was photoactivated using a 150-watt fiber optic light source (>495 nm) for 20 seconds, and spectra were subsequently recorded every 90 seconds for 120 min. The presence of Schiff-base was ascertained by the addition of 5 µl of 0.8 N H₂SO₄.

NaBH₄ Reduction and V8 Proteolysis of Rho - Reduction of the Schiff-base in Rho with NaBH₄ results in the fluorescent n-retinylidene opsin species \((\lambda_{ex}: 340\) nm, \(\lambda_{em}: 480\) nm) (13,14). Samples of 3 µM Rho-P, with or without 6 µM arrestin, solubilized in 0.02% DM, 0.02% DM and 0.02% asolectin, or 0.02% DM and 100 µM of purified phospholipid (20 µl) were photo-activated using a 150-watt fiber optic light source (>495 nm) for 20 seconds and allowed to decay at 20°C in the dark. After 120 minutes, 5 µl of 1% NaBH₄ (made fresh in water) was added to each sample. After 10 minutes, 15 µl of 1 M sodium phosphate (pH 7.0) was added, each sample was split into two 20 µl aliquots, and 5 µl of 4.8 µM V8 protease was added.
to half the samples. Proteolysis occurred for 30 minutes at room temperature. To assess the amount of Schiff-base present in Meta II Rho immediately after activation, 1% NaBH₄ was added to Rho-P (0.02% DM) in the dark. The sample was then photo-activated at 4°C and immediately processed as described above. Bands were resolved by 15% Tris-Tricine SDS PAGE, and gels were soaked in 30% methanol before visualization. The n-retinylidene opsin was excited with a short wave UV source (Alpha-Innotech FluoroChem 5500 gels-doc), and the fluorescent bands were detected by a CCD camera (535 ± 50 nm cut-off filter; 10 minute exposure). AlphaEase FC software was used to quantify the fluorescence of the bands.

**RESULTS**

Our results here indicate: 1) phospholipids are required to enable Rho-arrestin interactions in detergent micelles, 2) in mixed DM/phospholipid micelles, arrestin interacts with Meta III and converts it to a Meta II-like species, and 3) arrestin release is significantly inhibited from Rho*-P in mixed micelles containing acidic phospholipids, while half of the retinal is trapped in the binding pocket. Details are given below.

**Arrestin has reduced affinity for DM-purified Rho*-P** – The binding of arrestin I72B to Rho*-P in native membranes results in an increase (~40%) and blue-shift (~15 nm) in fluorescence (Figure 2A). However, when Rho-P is purified from ROS and solubilized in DM, the fluorescence changes are dramatically reduced, indicating a loss of arrestin binding (Figure 2B).

**Asolectin stimulates arrestin binding to DM-purified Rho*-P** - Adding asolectin (a mixture of phospholipids often used in Rho reconstitution (15)), to DM-purified Rho-P restores I72B binding: the fluorescence increases ~70% and blue-shifts ~15 nm after photo-activation (Figure 2C).

**Dilution of ROS lipids with detergent inhibits arrestin binding to Rho*-P** – We determined the effect of decreasing the apparent concentration of native ROS lipids on arrestin binding using DM (Figure 2D). Rather than purify ROS lipids and add them back to purified Rho-P, we fully-solubilized native ROS-P membranes and then added this mixture to buffer containing low to high concentrations of DM (0.02% to 1.0%). In this way, the relative Rho/ phospholipid ratio was preserved, but the number of micelles into which the phospholipids could segregate was increased. This process ensured that minimal oxidation or damage occurred to the ROS lipids. A similar approach has previously been used in control experiments involving ROS lipids (16).

When this DM-solubilized ROS-P is added to 0.02% DM (micelle concentration ~3µM⁻¹), arrestin can bind, indicated by the ~70% increase and 15 nm blue-shift in fluorescence. However, at higher DM concentrations, binding is dramatically inhibited (Figure 2D). In 1% DM (micelle concentration ~150 µM), arrestin I72B’s fluorescence increases only ~17% and blue-shifts less than 2 nm after light-activation. Assuming the ROS phospholipids distribute evenly to all micelles, we interpret this result to reflect DM’s effect of solubilizing ROS phospholipids away from Rho. Although DM might directly inhibit arrestin binding, the addition of sufficient amounts of exogenous phospholipids, even at high DM concentrations, can rescue arrestin binding (see Figure 2E, inset).

~50 phospholipids per Rho-P are required for arrestin binding – We quantified the phospholipid effect by titrating asolectin in samples of DM-purified Rho-P (Figure 2E). These studies indicate that ~0.015% asolectin, or ~200 µM phospholipid, is required to achieve maximal arrestin binding under conditions where there is roughly one Rho per micelle. This corresponds to ~67 phospholipids per Rho. Interestingly, this is similar to the Rho-phospholipid ratio in the ROS, where Rho composes half the volume of the tightly-stacked membranous organelles (17-19). When 10-fold more DM is present, correspondingly more phospholipid is required (0.1% to 0.12% asolectin) (Figure 2E, inset). Again, this value corresponds to ~50 phospholipids per micelle.

**Some retinal and arrestin release is inhibited in mixed micelles** – We used a fluorescence dual rate assay (5) to investigate the various dynamics of arrestin and retinal release. This assay monitors release of retinal as an increase in opsin’s tryptophan fluorescence (14), while simultaneously measuring dissociation of arrestin I72B as a decrease in bimane fluorescence. We determined the maximal and minimal fluorescence values possible for each process (the “plateaus”) by adding hydroxylamine, a compound which cleaves the Schiff-base and converts all remaining photoproducts to opsin and free retinaloxime.

Figures 3A, 3C, 3E compare retinal and arrestin release from Rho*-P in pure DM micelles or mixed micelles containing ROS phospholipids or
asolectin. The half-lives ($t_{1/2}$) of retinal and arrestin release, and the relative levels of retinal trapping and arrestin binding, are given in Tables 1 and 2. The data are briefly summarized below.

**Mixed DM/ROS phospholipid micelles** – In these samples, retinal release from Rho*-P ($t_{1/2} \sim 15$ min) is slowed compared to intact native membranes by a factor of two (5). Adding hydroxylamine at the end of decay causes only a slight increase in fluorescence (Figure 3A). In the presence of arrestin, retinal release is slowed by a factor of $\sim 1.5$ ($t_{1/2} \sim 23$ min), and slightly more retinal appears to be trapped ($\sim 5\%$).

Binding of arrestin I72B in these samples causes an increase ($\sim 50\%$) in bimane fluorescence, and the fluorescence decreases over time as arrestin dissociates at slightly slower rate than retinal release ($t_{1/2} \sim 28$ min) (Figure 3A). The fluorescence plateaus at $\sim 20\%$ of the starting state intensity, indicating some arrestin remains bound. The addition of hydroxylamine returns the fluorescence to the starting state level. This pronounced residual post-Meta II binding is very similar to what we previously observed in native membranes (Table 2) (5).

**Pure DM micelles** - Retinal is released from these samples with a $t_{1/2} \sim 8$ min, and the tryptophan fluorescence increases a further $\sim 20\%$ upon the addition of hydroxylamine (Figure 3C). Arrestin does not significantly affect either the rate of retinal release ($t_{1/2} \sim 9$ min) or the fluorescence plateau, probably because little binding occurs. This sample shows only a small increase ($\sim 12\%$) in bimane fluorescence (Figure 3C). We attribute the small amount of arrestin binding to DM-purified Rho-P to residual ROS phospholipids that are not removed during purification. Residual phospholipids are extremely difficult to remove with detergents (20-22).

**Mixed DM/asolectin micelles** – The most striking effects are observed for these samples (Figure 3E). Remarkably, in the presence of arrestin, opsin’s tryptophan fluorescence plateaus at $\sim$half that seen in the absence of arrestin. Addition of hydroxylamine causes a dramatic increase in the opsin fluorescence to the same level as seen without arrestin.

Arrestin binding to Rho*-P in these samples causes a $\sim 50\%$ increase in bimane fluorescence, and the rate of arrestin release ($t_{1/2} \sim 23$ min) is $\sim 70\%$ slower than retinal release (Figure 3E). The bimane fluorescence plateaus at $\sim$half of the starting state intensity, and hydroxylamine returns the fluorescence to the starting state level.

**Arrestin blocks Meta III formation** – We also assessed the effect of the mixed micelles and arrestin on Rho-P photodecay by UV-visible absorption spectroscopy. In each type of micelle, dark-state Rho-P exhibits a characteristic absorption maximum at 500 nm that shifts to 380 nm after light-activation (Figures 3B, D, and F, upper panels). Over time, the 380 nm absorbance decreases while the absorbance between 440 nm and 480 nm increases. This latter increase can be due to both the appearance of Meta III (470 nm) and release of retinal from the binding pocket to form adducts with phospholipids (440-450 nm).

After photodecay, we looked for the presence of retinal Schiff-base by acidifying the samples. A protonated retinal Schiff-base adduct absorbs at 440 nm, and this property can be used to detect retinal attached to opsin or linked to phospholipid. These measurements showed very little Schiff-base present for photo-decayed Rho*-P in DM micelles (Figure 3D, upper panel). In contrast, photo-decayed Rho*-P in DM/ROS phospholipid and DM/asolectin mixed micelles gives a broadened red-shifted absorbance (Figures 3B and 3F, upper panels) that is typical for retinal adducts with PE (data not shown).

Not surprisingly (since it shows limited binding) arrestin does not effect the photodecay absorption spectra of Rho*-P in DM micelles (compare upper and lower panels of Figure 3D). In contrast, arrestin causes substantial changes to the spectra of Rho*-P in mixed DM/ROS phospholipid and in DM/asolectin micelles (Figure 3B and 3F). Both the absorbance loss at 380 nm, as well as the increase between 440 nm and 480 nm, is inhibited. Acidification produces a 440 nm absorbance peak indicative of protonated Schiff-base. Together, these data show that arrestin stabilizes Rho*-P as a Meta II-like 380 nm absorbing species in which retinal is covalently attached by a Schiff-base.

**Arrestin converts Meta III to spectral Meta II** – Clearly, arrestin can prevent Meta III formation (see above). We next tested if Meta III could interact with arrestin directly. We added arrestin to Rho*-P 27 minutes after photoactivation (20°C) and saw a substantial decrease at 470 nm (Meta III) and increase at 380 nm (Figure 4A). Acidification indicates this 380 nm species is characterized by an intact Schiff-base (Figure 4A, inset). Most importantly, addition of arrestin clearly speeds the depletion of Meta III (Figure 4B). The residual
absorbance at 475 nm is likely due to retinal-phospholipid adducts formed in these mixed micelles before the addition of arrestin.

Acidic phospholipids enhance arrestin binding and inhibition of retinal release – We next assessed the effect of different phospholipids. In these experiments, we used the dioleoyl form (except for PI, which contained a mixture of fatty acids). Experiments were carried out with a standard 100 µM phospholipid, because in our hands, this was the maximal concentration at which all phospholipids were soluble in 0.02% DM.

Retinal release data with the different phospholipids are summarized in Table 1. The neutral phospholipids PC and PE show no significant difference in retinal trapping with or without arrestin. The absorbance data confirm this result (Figure 5). In contrast, significant differences in the ability of arrestin to trap retinal are seen using the acidic phospholipids PS, PI and PA. With these lipids, ~half of the retinal appears to be trapped when arrestin is present, and the absorbance spectral changes are significantly reduced. Interestingly, arrestin appears to only affect the level at which opsin tryptophan fluorescence plateaus after photo-activation, not the rate of retinal release.

The effect of the different types of phospholipids on arrestin I72B binding and release to DM-purified Rho*-P is summarized in Table 2. Qualitatively, arrestin binding is only slightly enhanced by PC and PE but is significantly enhanced by PS, PI and PA (Figure 5). However, we are reluctant to read too much into the relative increases in arrestin I72B fluorescence. The bimane probe is sensitive to the polarity of its environment (23-25), and we are unsure of how the different phospholipid head-groups might alter the fluorescence intensity of the probe.

We also tested the effects of docosahexanoic acid (DHA)-conjugated phospholipid, since the ROS membrane is unusually enriched in this highly unsaturated long fatty acid (17,21,26). However, there were no significant differences in the data using this lipid compared to the dioleoyl forms (data not shown).

Biochemical evidence that arrestin traps retinal as a Schiff-base adduct – We used the plateau level of tryptophan fluorescence to quantify the amount of trapped retinal, as has been described by Hofmann and coworkers (Figure 6A and 6B) (6). We also developed an independent assay to measure the amount of trapped retinal, which exploits the fact that reducing the retinal Schiff-base with NaBH₄ forms a fluorescent species, n-retinylidene opsin (13,14). This new assay quantifies the amount of trapped retinal in Rho*-P in different micelles by NaBH₄-reduction two hours after photoactivation, in the presence or absence of arrestin (Figure 6C). Subsequent SDS PAGE enables the separation the n-retinylidene opsin from possible retinal-phospholipid adducts, and quantification of the fluorescent bands is plotted relative to the total amount of retinal Schiff base present in Meta II Rho*-P immediately after photobleach (Figure 6D).

The two methods report very similar amounts of retinal trapping (compare Figures 6B and 6D). In pure DM micelles, ~20% of the original retinal population is attached to opsin after photodecay. This residual retinal may represent Meta III, as well as retinal which is attached to peripheral lysines on Rho*-P after release from the binding pocket. In the presence of arrestin, the amount of fluorescence is not significantly changed.

Why is the trapping in DM/asolectin mixed micelles (~10%) lower than in DM alone? Presumably, PE in asolectin competes with lysine residues for retinal (27). This conclusion is supported by the presence of a low molecular-weight fluorescence species seen on the gel (data not shown). Significantly, arrestin causes ~60% of the retinal to remain attached to opsin (lane 5). In data not shown, V8 proteolysis analysis shows that most of this retinal (~80%) is attached to fragment F2 (residues 240-338, which contains Lys to which retinal is attached in dark-state and Meta II Rho). These results confirm the fluorescence and absorption data in Figure 3: in the presence of asolectin phospholipid, arrestin traps ~half the retinal, presumably at Lys²⁹⁶.
In mixed micelles containing the purified phospholipids, very little retinal is trapped by arrestin in the presence of PC or PE. In contrast, PS, PI, and PA allow significantly more retinal to be trapped when arrestin is present.

Although generally the two methods for quantifying trapped retinal show excellent agreement, samples with PS are unusual—they show more retinal trapped as measured by tryptophan fluorescence (~57%) than that measured by NaBH₄-reduction (~22%). We are not sure of the cause of this discrepancy, but PS may somehow inhibit the reduction reaction.

**DISCUSSION**

We find that acidic phospholipids can restore arrestin binding to DM-purified Rho*-P. This discovery opens the door for studying arrestin-Rho interactions in a soluble mixed micelle system. Mixed micelles, since they are chemically defined, can be extremely helpful in the study of membrane proteins (28-31). For example, they enable the purification of stable opsin from transfected COS cells (32,33) and increase the affinity of transducin for Rho (34). Mixed micelles exhibit similar advantages for studying arrestin, and we used our new system to carry out a more detailed examination of the interplay between arrestin and the retinal chromophore in Rho. The implications of our findings are discussed below.

**Arrestin stabilizes spectral Meta II**—Our studies show that in mixed micelles containing asolectin, PS, PI or PA, arrestin inhibits ~50% of the retinal release from Rho*-P. We confirmed this result using two independent methods: the tryptophan fluorescence retinal release assay and a new NaBH₄-reduction/SDS PAGE method. Our results demonstrate that arrestin traps this retinal in a Schiff-base linked form with a 380 nm absorbance (Figures 3F and 5), with the retinal most likely still attached to Lys²⁹⁶ (data not shown). Cleaving the Schiff-base retinal linkage with hydroxylamine releases both the trapped retinal and arrestin from Rho*-P in mixed micelles (Figures 3E and 5). Thus we conclude arrestin can stabilize Meta II in a long-lived arrestin-Rho complex.

**Arrestin converts Meta III to spectral Meta II**—Previously, we found arrestin can still bind photo-decayed Rho*-P in ROS membranes, even though no significant Meta II remains (5). However, due to light-scattering issues, we were unable to spectrally identify the nature of the post-Meta II photoproduct to which arrestin was binding. Because our mixed micelle system is optically clear, we can now firmly establish that arrestin can interact with Meta III and convert it to a species with Meta II-like characteristics (Figure 4). The fast conversion of Meta III to Meta II may explain why very little Meta III forms when arrestin is present (Figures 3 and 5). An alternative explanation is that arrestin favors Meta II at the expense of Meta I (35), which is the precursor to Meta III (8).

Why do phospholipids affect arrestin binding to Rho*-P?—It is unlikely that phospholipid-dependent effects on Rho photochemistry (16,36-38) explain the results presented here. The mixed micelles used in this study do not greatly affect the Meta/II equilibrium, as the spectra of Rho*-P in the various mixed micelles are similar to those in pure DM micelles (see Figure 5).

One possibility is that arrestin interacts directly with the phospholipids. Rho is certainly intimately associated with its surrounding phospholipids—it immobilizes ~25 phospholipids and induces a reorganization of these lipids upon light-activation (39,40). In fact, Rho and ROS phospholipids interact so tightly that it is very difficult to remove all lipids during Rho purification with detergents, or even with organic solvents (20-22). Our results may suggest that phospholipids compose a significant portion of the Rho-arrestin interface, and when phospholipids are removed from Rho-P by purification in detergent, the relative affinity decreases and arrestin binding is severely diminished.

Interestingly, our results may also imply that negative charges found on the phospholipid headgroups are involved in a high-affinity interface between arrestin and Rho*-P. In data not shown here, we have found that free fatty acid or water-soluble short-chain acidic lipids, like dioctanoyl-phosphoserine, can enhance arrestin binding to DM-purified Rho*-P. Conversely, we also find that high concentrations of salt can inhibit arrestin binding to Rho*-P, either in mixed micelles or native membranes. Together, these results imply that ionic interactions may be involved in arrestin binding. However, it is difficult to interpret the effect of salt, as it may alter the micelle structure or interactions with the phosphorylated tail and cytoplasmic loops of Rho*-P.

However, it is also possible that the phospholipids help stabilize a spectrally silent Rho...
conformation that interacts more tightly with arrestin. For example, Sakmar and coworkers suggest that the phospholipid PS induces helical structure in Rho’s Helix 8 (41). Thus, arrestin may not bind to DM-purified Rho*-P because Helix 8’s structure is perturbed. Addition of acidic phospholipids may restore the helical structure and enable arrestin binding.

Some arrestin and retinal release appear unlinked in mixed micelles – Surprisingly, we find the rates of retinal and arrestin release differ in mixed phospholipid/detergent micelles, in contrast to our previous findings with ROS membranes (5). The magnitude of the difference is dependent on the type of phospholipid. In mixed micelles containing ROS phospholipids, arrestin is released only ~20% slower than retinal; in micelles containing pure PC or PE, arrestin is released ~40% slower. Remarkably, in the presence of acidic phospholipids PS, PI and PA, arrestin is released at least ten-times slower than retinal. We can rule out the possibility that this effect is merely due to non-specific interaction of arrestin with the micelle, based on our retinal trapping data (discussed below).

What could explain this anomaly? Is it related to the observation that adding detergent to membrane bound Rho also affects retinal release and uptake, or “ligand channeling”? Hofmann and coworkers have proposed that a secondary retinal binding site is formed by Rho’s Helix 8 (42-44), and detergent may disrupt the interaction of Helix 8’s palmitoyl anchors with the membrane. Since we find arrestin and retinal release are decoupled in detergent, our results may imply a role for ligand channeling in arrestin release. Interestingly, the Subramaniam laboratory has found addition of detergent to membrane extracts of Drosophila rhodopsin appears to decouple arrestin release and the rates of regeneration in the photocycle (45).

Note that the rates of arrestin and retinal release are more similar in micelles containing native ROS lipids than those containing purified lipids. Perhaps some “special ingredient” present in the ROS might be responsible for coordinating these two events. ROS phospholipids are composed of approximately 45% PC, 42% PE and 13% PS (17,26), and these phospholipids are enriched in the highly unsaturated long fatty acid docosahexaneoic acid (22:6n-3) (DHA) (17,21,26). The ROS membrane also contains a substantial amount of cholesterol (12-14%) (46,47).

Perhaps the most intriguing anomaly in our results is that, while most of the arrestin (~90%) remains bound using acidic phospholipids, only about half of the retinal is trapped. The cause of this discrepancy is not likely due to the presence of a large amount of nonphosphorylated Rho, since our samples, at ~6 phosphates per Rho (5), are near the maximum of 7 (48).

An intriguing (but highly speculative) possibility is that arrestin forms a long-lived complex when it interacts with a dimer of Rho*-P, in which the retinal is able to dissociate from only one dimer partner. This possibility has obvious important implications for the function of arrestin and the structure of the complex (49). Interestingly, conditions that disfavor the association of Rho with native lipids, such as addition of high concentrations of DM to native membranes, inhibit Rho dimerization (50). Perhaps arrestin binding is dependent on Rho dimerization, which is in turn dependent on the presence of phospholipid. We hope to address this possibility in future experiments.

Acidic phospholipids promote stable complexes of arrestin and Rho*-P – The fact that long-lived Rho*-P/arrestin complexes can form in mixed micelles containing acidic phospholipids opens up new possibilities for studying the arrestin-Rho complex. At low temperature (8°C) our assay indicates that arrestin and Rho*-P in DM/PA mixed micelles form an extremely long-lived complex with a t_{1/2} of ~85 hours (data not shown). As seen at 20°C (Figure 5), we note that arrestin traps ~half of the retinal at low temperature. This finding should help enable long-lived complexes useful in crystallization, NMR, electron microscopy, or cross-linking experiments.

Possible implications of these results in the visual cycle – In bright light conditions, up to half of the rhodopsin in the human retina is estimated to be converted to Meta III (9). In a recent review, Lamb and Pugh hypothesized that arrestin is necessary to remove this Meta III to allow regeneration during dark adaptation, since patients who lack functional arrestin develop a form of stationary night blindness called Oguchi disease (2,51). Our results presented here support this hypothesis: we find that phosphorylated Meta III is bound by arrestin and stabilized as a Meta II-like species. Arrestin-bound Meta II may be more accessible to the regeneration machinery than Meta III (52), and arrestin may be necessary to block Meta III-induced transducin signaling (53).
Finally, we note that visual arrestin’s interaction with phospholipid may represent another level of regulation. For example, PS, which undergoes a light-dependent translocation to the cytoplasmic side of the membrane (39), may interact specifically with arrestin in the native ROS, as it appears to do with transducin (54). Intriguingly, phosphoinositide has been implicated in the trafficking of Drosophila visual arrestin and recruitment of β-arrestin to clathrin coated pits, although significant binding of phosphoinositide to visual arrestin has not been reported (55,56). The molecular details of arrestin’s interaction with ROS phospholipids will be the focus of future study.

REFERENCES


FOOTNOTES

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2 The abbreviations used are: Rho, rhodopsin; Rho-P, phosphorylated rhodopsin; Rho*, light-activated rhodopsin; Meta I, metarhodopsin I; Meta II, metarhodopsin II; Meta III, metarhodopsin III; ROS, rod outer segment, or wild-type rhodopsin in native membranes; GPCR, G-protein coupled receptor; DM, n-dodecyl-β-D-maltopyranoside; PC, 1,2-dioleyl-sn-glycero-3-phosphocholine; PE, 1,2-dioleyl-sn-glycero-3-phosphoethanolamine; PS, 1,2-dioleyl-sn-glycero-3-phosphoserine; PI, L-α-phosphatidylxinositol; PA, 1,2-dioleyl-sn-glycero-3-phosphate; λmax, wavelength of maximum absorption; λex, wavelength of excitation; λem, wavelength of emission; PMSF, phenylmethylsulphonylfluoride

3 This calculation assumes that the aggregation number of 132 DM/micelle (57) is not dramatically changed by the presence of ROS phospholipids.
Our conditions were well above the CMC of DM, which is 0.006\% at 0.2 M NaCl (58). All experiments were carried out in a fully soluble system, since samples could be centrifuged at 100,000 x g for one hour with no loss of Rho-P absorbance.

Note that our results do not rule out the possibility that the retinal may have migrated to a secondary binding site, perhaps near Helix VIII, as has been proposed to occur during ligand channeling (43).

Note that in our studies with mixed micelles, we define Meta III as a rhodopsin photoproduct absorbing at 470 nm. We have not formally established that this photoproduct is identical to the Meta III that forms in membranes (6-9).

FIGURE LEGENDS

FIGURE 1. Structural models of rhodopsin, arrestin and the detergents and lipids used in this study. A) Model showing rhodopsin (dark gray) and its hypothetical dimer partner (light gray) in a membrane bilayer (49). The location I72 on arrestin is indicated by a back sphere at the site of the α-carbon. Inset - The structure of monbromobimane, which was attached to a mutant cysteine residue at site I72 (not to scale with the protein models). Models were created as described previously (5). B) Structure of the detergent n-dodecyl-β-D-maltopyranoside (DM). C) The structure of 1,2-dioleoyl-sn-glycero-3-phospholipid (left) and the various head-groups used in this study (X, right). The full-names of the phospholipids are given in the footnote.

FIGURE 2. Phospholipids are required for arrestin binding to DM-purified Rho*-P. Fluorescence of bimane-labeled arrestin I72C/W914F (I72B) in the presence of: A) Rho-P in native membranes (ROS-P), B) purified Rho-P solubilized in 0.02\% DM, and C) purified Rho-P solubilized in mixed micelles (0.02\% DM and 0.02\% asolectin). Spectra show before (solid trace) and after (dashed trace) light-activation. D) The effect of increasing DM concentrations on I72B binding to solubilized Rho*-P in the presence of native ROS phospholipids. A scheme of how the samples were created is shown above. The dark-state and light-activated (+hν) spectra at each DM concentration are labeled below. In each experiment, 1µM labeled arrestin and 2 µM Rho-P were used. E) The relative fluorescence of 1µM I72B at 470 nm (dark) and 456 nm (after light-activation) was measured in the presence of a 3-fold excess of DM-solubilized purified Rho-P (0.02\% DM) and increasing amounts of asolectin. The experimental scheme is shown above. The average from three independent experiments is plotted below, and the error bars represent the standard error. The inset shows an example of the same experiment performed in 0.2\% DM. All experiments were carried out at 20°C in standard buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) with 380 nm excitation.

FIGURE 3. Arrestin inhibits retinal release and Meta III formation in mixed micelles. The effect of arrestin on retinal release and Meta III formation was investigated by fluorescence and absorbance spectroscopy. A) Retinal release measured from solubilized Rho-P in mixed micelles of DM (0.02\%) and native ROS phospholipids (prepared as described in Figure 2). The assay monitored the increase in opsin tryptophan fluorescence (330 nm) in the absence (dark blue) or presence of a 2-fold excess of arrestin I72B (light blue). The sample was photo-activated at t=0, and 10 mM hydroxylamine was added at t=210 minutes. Arrestin binding and release was also monitored (456 nm, green trace) during the same experiment. B) The absorbance of solubilized Rho-P in DM/ROS phospholipid mixed micelles was observed in the dark (red) and after photo-activation (blue), both in the absence (upper panel) and presence (lower panel) of a 2-fold excess of arrestin. Spectra were recorded every 90 seconds after photo-activation (black spectra) for 120 minutes (the last spectrum is orange). The amount of retinal Schiff-base remaining at the end of the experiment was assessed by adding H2SO4 (green spectrum). Experiments in C, D, E and F were carried out as described for (A) and (B), except that purified Rho-P in pure DM micelles (C and D) or DM/asolectin mixed micelles (E and F) was used, and 10 mM
hydroxylamine was added at 120 min (C and E). In each experiment, 1 µM Rho-P in 20 mM HEPES, 150 mM NaCl, pH 7.4, 0.02% DM ± 0.02% asolectin was used (20°C).

**FIGURE 4.** Arrestin converts Meta III to spectral Meta II. Rho-P in mixed micelles was photoactivated (<495 nm) and after 27 minutes, either arrestin or an equal volume of buffer (control) was added to the cuvette, and absorbance spectra were recorded every 90 seconds. A) Difference spectra representing the conversion of ~470 nm absorbance to ~380 nm absorbance after the addition of arrestin. The base line spectrum (the first spectrum of Rho*-P after the addition of buffer) was subtracted from spectra which had been recorded at 1, 2.5, 4, 5, 17.5, 50 and 90 minutes after the addition of arrestin. The first and last spectra are labeled. Inset – The increased 380 nm absorbance is most likely due to an increase in Meta II, since acidification at the end of the experiment yields more of the 440 nm species (indicating Schiff-base) compared to the control in which no arrestin was added. B) Addition of arrestin caused a decrease in Meta III absorbance (475 nm) over time. The arrow marks the time at which buffer (closed circles) or arrestin (open circles) was added. The data points represent the average from three independent experiments, and the error bars represent the standard error. In each experiment, 1 µM Rho-P in 20 mM HEPES, 150 mM NaCl, pH 7.4, 0.02% DM, 0.02% asolectin was used (20°C), and arrestin was added to a final concentration of 1.5 µM.

**FIGURE 5.** Arrestin binding is strongly enhanced by acidic phospholipids. Fluorescence and absorption spectroscopy of DM-solubilized Rho-P in the presence of PC, PE, PS, PI, or PA (100 µM) is presented as shown in Figure 3. Data obtained in the absence of phospholipid is shown for reference (DM). Fluorescence – Retinal release in the absence (dark blue) or presence (light blue) of a 2-fold excess of arrestin I72B was monitored (330 nm). Arrestin binding and release was also monitored during the same experiment (green trace, 456 nm). The sample was photo-activated at t=0, and 10 mM hydroxylamine was added at t=120 minutes. Absorbance - Spectra of DM-solubilized Rho-P in the presence of difference phospholipids (100 µM) were measured as described in Figure 3, in the absence or presence of two-fold excess of arrestin. In each experiment, 1µM Rho-P in 20 mM HEPES, 150 mM NaCl, pH 7.4, 0.02% DM was used (20°C).

**FIGURE 6.** Arrestin enhances trapping of retinal as a Schiff-base adduct on Rho*-P. A) Trapped retinal, expressed as “% of max fluorescence”, can be quantified from the plateau of Rho*-P’s tryptophan fluorescence and the increase that occurs after the addition of hydroxylamine. This scheme illustrates how this is calculated. B) Average change in Rho*-P’s tryptophan fluorescence that occurred after the addition of hydroxylamine, calculated as described above in (A). The analysis was carried out on the experiments described in Figures 3, Figure 5, and Table 1. The data represent two independent experiments ± S.E. C) Retinal trapping can also be measured by reduction with NaBH₄ followed by SDS PAGE. The fluorescence of the bands indicates the amount of retinal covalently attached to opsin. Briefly, Rho-P solubilized in 0.02% DM (lanes 2 and 3), 0.02% DM and 0.02% asolectin (lanes 4 and 5), or 0.02% DM and 100 µM of the various purified phospholipids (lanes 6-15), without or with a two-fold excess of arrestin, was photo-activated, allowed to decay at 20°C in the dark, NaBH₄-reduced after 120 minutes, and then subjected to SDS PAGE. The total amount of Schiff-base linked retinal was quantified by comparison to samples in which Meta II was reduced immediately after bleach (lanes 1 and 16). The fluorescence of the gels is shown in the upper panels and the Coomassie-stained gels are shown in the lower panels. D) Plot of the average quantified fluorescence from four independent experiments as shown in (C), and error bars represent the S.E. The fluorescence was measured as described in the Experimental Procedures and is expressed as a percent of the total Meta II fluorescence (% max fluorescence). All bands were compared to a Meta II control sample run on the same gel.
Table 1. Effect of arrestin on the rate of retinal release and amount of trapped retinal\(^a\)

| membrane or micelle | no arrestin | | + arrestin | |
|---------------------|-------------|------------------------|------------------------|
|                     | \( t_{1/2} \) retinal release (min) \(^b\) | % retinal trapped \(^c\) | \( t_{1/2} \) retinal release (min) \(^b\) | % retinal trapped \(^c\) |
| native membranes    | 7.5 ± 0.3   | N/D *                 | 8.9 ± 0.7              | N/D *                 |
| DM micelles         | 7.9 ± 0.3   | 19 ± 1                | 8.6 ± 0.2              | 20 ± 2                |
| DM / asolectin micelles | 12.3 ± 0.8   | 5 ± 0                 | 13.2 ± 0.7             | 52 ± 4                |
| DM / PC             | 12.0 ± 0.9  | 14 ± 2                | 14.0 ± 0.2             | 16 ± 1                |
| DM / PE             | 12.4 ± 0.8  | 1 ± 0                 | 14.0 ± 0.3             | 3 ± 1                 |
| DM / PS             | 12.6 ± 1.1  | 6 ± 3                 | 12.8 ± 0.3             | 58 ± 4                |
| DM / PI             | 13.2 ± 1.4  | 17 ± 3                | 13.6 ± 0.1             | 49 ± 1                |
| DM / PA             | 8.8 ± 0.6   | 5 ± 1                 | 11.1 ± 0.6             | 60 ± 3                |

\(^a\) Values are derived from experiments described in Figures 3 and 5 and, for the native membrane sample, from previously published work (5). Values represent the average ± the S.E. from two independent experiments (20°C, pH 7.4).

\(^b\) Single-exponential rates were measured and determined as described in the Experimental Procedures and converted to \( t_{1/2} \) values (\( t_{1/2} = \ln 2/k \), where \( k \) is the rate constant in min\(^{-1}\)). In each experiment, 1 \( \mu M \) Rho-P ± 2 \( \mu M \) arrestin I72B was used, except for the native membrane sample, where 2 \( \mu M \) Rho-P ± 4 \( \mu M \) arrestin was used.

\(^c\) The percent of trapped retinal was calculated from the Rho-P tryptophan fluorescence retinal release data (330 nm) as follows: \( \frac{(c-a)}{c} \times 100 \), where \( a \) is the \( \frac{(F/F_0)}{1} \) value before the addition of NH\(_2\)OH, and \( c \) is the \( \frac{(F/F_0)}{1} \) value after the addition of NH\(_2\)OH (see Figure 6A for more details).

* Not determined, because of secondary fluorescence effects observed to occur when NH\(_2\)OH is added to native membranes.
Table 2. Arrestin I72B fluorescence changes due to Rho*-P binding and release

<table>
<thead>
<tr>
<th>membrane or micelle</th>
<th>arrestin binding (F/F₀) – 1 (456 nm)</th>
<th>arrestin plateau (F/F₀) – 1 (456 nm)</th>
<th>% residual fluorescence b</th>
<th>t₁/₂ of arrestin release (min) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>native membranes</td>
<td>0.42 ± 0.08 *</td>
<td>0.09 ± 0.02 *</td>
<td>21</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td>native membranes solubilized by DM</td>
<td>0.5 ± 0.1</td>
<td>0.1 ± 0.01</td>
<td>20</td>
<td>28.0 ± 0.7</td>
</tr>
<tr>
<td>DM micelles</td>
<td>0.12 ± 0.1</td>
<td>0.03 ± 0.02</td>
<td>25</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>DM / asolectin micelles</td>
<td>0.5 ± 0.2</td>
<td>0.25 ± 0.02</td>
<td>50</td>
<td>23.4 ± 0.4</td>
</tr>
<tr>
<td>DM / PC</td>
<td>0.28 ± 0.2</td>
<td>0.06 ± 0.01</td>
<td>21</td>
<td>19.5 ± 1.3</td>
</tr>
<tr>
<td>DM / PE</td>
<td>0.24 ± 0.1</td>
<td>0.07 ± 0.01</td>
<td>29</td>
<td>19.8 ± 0.6</td>
</tr>
<tr>
<td>DM / PS</td>
<td>0.68 ± 0.1</td>
<td>0.62 ± 0.04</td>
<td>91</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>DM / PI</td>
<td>0.62 ± 0.1</td>
<td>0.55 ± 0.01</td>
<td>89</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>DM / PA</td>
<td>0.93 ± 0.1</td>
<td>0.87 ± 0.07</td>
<td>93</td>
<td>&gt; 120</td>
</tr>
</tbody>
</table>

a Values are derived from experiments described in Figures 3 and 5 and, for the native membrane sample, from (5) and unpublished work. Values represent the average ± the S.E. from two independent experiments (20°C, pH 7.4).
b Determined from the average fluorescence intensity of arrestin I72B 120 min after light-activation (or 200 min for DM-solubilized ROS-P) divided by the average intensity immediately after light-activation.
c Single exponential rates were measured and determined as described in the Experimental Procedures and converted to t₁/₂ values (t₁/₂ = ln2/k, where k is the rate constant in min⁻¹). In each experiment, 1 µM Rho-P and 2 µM arrestin I72B was used, except for the native membrane sample, where 2 µM Rho-P and 4 µM arrestin was used. Rates of arrestin release were measured during the same experiment in which retinal release was measured for Table 1.

* The fluorescence intensity of arrestin I72B in the presence of native membranes is complicated by the variable amount of scatter generated by different membrane preparations.
FIGURE 1

A rhodopsin

B n-dodecyl-β-D-maltopyranoside

C X - Head groups
- H PA
- CH₂-CH₂-NH₃ PE
- CH₂-CH₂-N(CH₃)₃ PC
- COO⁻ PS
- CH₂-CH-NH₃⁺ PI

1,2-dioleoyl-sn-glycero-3-phospholipid
FIGURE 3
FIGURE 4

A

\[ \Delta \text{Absorption} \]

0.004

0.002

0.000

-0.002

-0.004

350 400 450 500 550

Wavelength (nm)

1 min

90 min

B

\[ \Delta \text{ABS 475 nm} \]

0.006

0.004

0.002

0.000

0 20 40 60 80 100 120

Time (min)

no arrestin

+ arrestin

arrow
FIGURE 6

A

\[
\left( \frac{c - a}{c} \right) \times 100 = \text{% max fluorescence}
\]

B

tryptophan fluorescence

\begin{tabular}{c|c|c}
& no arrestin & + arrestin \\
\hline
DM & & \\
aso & & \\
PC & & \\
PE & & \\
PS & & \\
PI & & \\
PA & & \\
\end{tabular}

C

Arr

\begin{tabular}{c|c|c|c|c|c|c|c|c|c}
& Meta II & DM & aso & PC & PE & PS & PI & PA & Meta II \\
\hline
fluorescence & & & & & & & & & \\
coomassie & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\
& 10 & 11 & 12 & 13 & 14 & 15 & 16 & 17 & 18 \\
\end{tabular}

D

n-retinylidene fluorescence

\begin{tabular}{c|c|c|c|c|c|c|c|c|c}
& no arrestin & + arrestin & Meta II \\
\hline
DM & & & \\
aso & & & \\
PC & & & \\
PE & & & \\
PS & & & \\
PI & & & \\
PA & & & \\
\end{tabular}
Dynamics of arrestin-rhodopsin interactions: Acidic phospholipids enable binding of arrestin to purified rhodopsin in detergent
Martha E. Sommer, W. Clay Smith and David L. Farrens

J. Biol. Chem. published online January 20, 2006

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