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Rhodopsin Reconstituted into a Planar-Supported Lipid Bilayer Retains Photoactivity after Cross-Linking Polymerization of Lipid Monomers

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The visual photoreceptor, bovine rhodopsin (Rho), has been reconstituted into a planar-supported lipid bilayer (PSLB) composed only of a polymerizable lipid. The finding that the photochemical activity of Rho is unaffected by cross-linking lipid polymerization is unprecedented and has important implications for development of molecular devices based on reconstituted transmembrane proteins (TMPs). TMPs, particularly ion channels and G-protein-coupled receptors (GPCRs), play key roles in transport and signal transduction and are important pharmacological targets. Many TMPs, including Rho, have been isolated and reconstituted into artificial membranes, including PSLBs,[1] which maintain TMP activity as well as prevent nonspecific adsorption of nontarget proteins.[2,3] However, since biomembranes composed of natural lipids are associated solely via noncovalent interactions, their instability may present a significant obstacle to their use in TMP-based devices (e.g., biosensors).[2,4] One strategy is polymerization of synthetic lipid monomers to form robust bilayers.[2] However, it is unclear if extensive lipid cross-linking can be achieved without adversely affecting the activity of incorporated TMPs. The conventional view is that the membrane must be highly fluid to maintain TMP activity.[1a,b]

Rhodopsin[5] is a model for GPCRs and has been used to explore the effects of lipid composition and bilayer structure on TMP activity.[6,7] Studies of Rho and bacteriorhodopsin reconstituted into poly(lipid) vesicles have been reported.[8] UV photopolymerization of vesicles composed of diacetylenic lipids was found to inactivate both Rho and bacteriorhodopsin. Rhodopsin activity can be maintained by reconstituting it into vesicles composed of polymerized and fluid domains;[8] however, this approach undermines the strategy of using lipid cross-linking to achieve bilayer stability. Although cross-linked poly(lipids) can withstand exposure to destabilizing conditions (drying, surfactants, solvents), fluid lipid domains generally do not.[2,4,9]

Here Rho was reconstituted into a PSLB composed only of 1,2-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine (bis-SorbPC).[9] Irradiation with UV light initiates bis-SorbPC polymerization, yielding a cross-linked, structurally stabilized bilayer.[2,9] We have utilized plasmon-waveguide resonance (PWR) spectroscopy[10] to characterize Rho in PSLBs. PWR is highly sensitive to the optical properties (complex refractive index, thickness) of thin films deposited on the resonator surface and has been used to detect mass density and conformational changes accompanying protein−lipid and protein−ligand interactions occurring at or within PSLBs, including photoactivation of Rho reconstituted in fluid PSLBs.[7,11]

The procedure used to form a PSLB across an orifice in a Teflon sheet separating the resonator surface from the aqueous volume of the PWR cell has been described.[7,11] Rho[12] was reconstituted into the PSLB by introducing small aliquots of octylglucoside-solubilized receptor into the PWR cell, which contained 10 mM phosphate buffer (pH 5.5).[13] Lipids were polymerized by directing UV light from a mercury lamp[2] through a port in the PWR cell for 45 min. A band-pass filter (Hoya U-330, 330±80 nm) was used to remove visible light (>450 nm) that irreversibly photoactivates Rho (see Supporting Information, SI). Under these conditions, ≥95% polymerization of bis-SorbPC was achieved, based on attenuation of the monomer UV absorbance (see SI). Cross-linking enhanced the resistance of the PSLB to solubilization by Triton X-100, whereas in the absence of UV irradiation, Triton X-100 addition caused significant changes in the PWR spectrum, attributable to PSLB dissolution (see SI).

After polymerization, the PSLB containing Rho was exposed to saturating yellow light (YL, >500 nm).[7] PWR spectra were collected after each exposure (∼5 s each) until no further change was observed (typically ≤3 exposures; elapsed time ≤5 min). Illuminating Rho with YL causes isomerization of the retinal chromophore, initiating a thermal decay leading to a metastable equilibrium between two intermediates, metarhodopsin I and II (MI and MII).[5−7] Formation of MII is accompanied by a conformational change that triggers G-protein (transducin) binding and activation. As substantiated previously in comparative studies of YL activation of Rho,[7] the magnitude of the conformational change detected by PWR parallels the extent of MII formation monitored by flash photolysis; thus PWR provides a quantitative measure of Rho photoactivity.

Figure 1 shows typical *s*-polarized PWR spectra acquired at each stage of an experiment with poly(bis-SorbPC); Table 1 lists *p*- and *s*-polarized shifts in PWR reflectance minima for several types of PSLBs (see SI for for *p*-polarized spectra and a larger compilation of shift data). Deposition of bis-SorbPC on the resonator surface is accompanied by shifts of ∼110 (*p*) and ∼80 (*s*) mdeg, which are consistent with formation of a single lipid bilayer.[10] Reconstitution of Rho into the PSLB induces further resonance angle shifts of ∼70 (*p*) and ∼50 (*s*) mdeg. These shifts are due to the increase in membrane refractive index and thickness that occurs upon Rho insertion, which displaces water and forces redistribution of lipids into the Gibbs border surrounding the bilayer.[7,11] Both PSLB formation and Rho insertion produce *p*-polarized shifts that are larger than the corresponding *s*-polarized shifts. This anisotropy reflects a uniaxial distribution of molecular orientations for both lipids and Rho about the membrane surface normal.[7,10,11,14] UV

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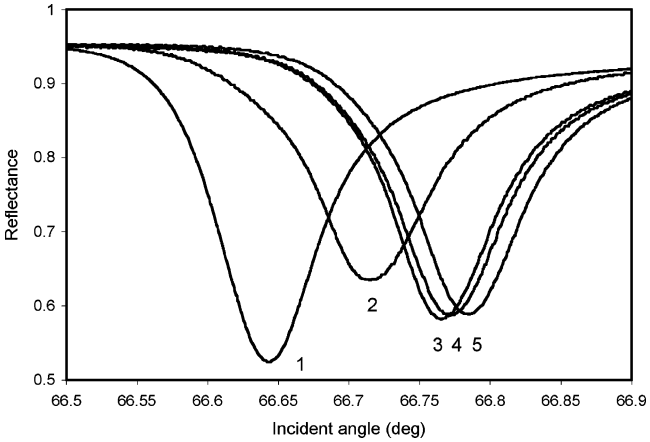

Figure 1. *s*-Polarized PWR spectra: obtained with only pH 5.5 buffer in the PWR cell (1), after bis-SorbPC PSLB formation (2), after Rho incorporation (3), after UV polymerization (4), and after YL activation of Rho (5). Spectra were excited at 632.8 nm.

Table 1. PWR Spectral Shifts (*p*, *s*; in +mdeg) Due to PSLB Formation, Rho Incorporation, PSLB Polymerization, and Yellow Light Activation at pH 5.5 for Different Types of Lipids

lipid membrane composition	+PSLB (*p*, *s*)	+Rho (*p*, *s*)	+UV (*p*, *s*)	+YL (*p*, *s*)
poly(bis-SorbPC)[a]	108, 73	70, 51	4, 3	13, 12
bis-SorbPC[a]	116, 80	70, 51	—[b]	11, 11
DOPC[a]	113, 79	70, 50	—[b]	13, 8
poly(bis-SorbPC)[c]	118, 81	100, 56	4, 3	18, 16
poly(bis-SorbPC)[c]	119, 84	64, 22	4, 3[d]	7, 6

[a] Rho concentration in PWR cell = 1 μM. [b] PSLB was not exposed to UV light. [c] Rho concentration in PWR cell = 1.5 μM. [d] PSLB was polymerized before the Rho reconstitution step.

irradiation induces much smaller shifts (~3 mdeg), indicating that cross-linking polymerization minimally alters the membrane optical properties.

YL activation of Rho in poly(bis-SorbPC) induces the MI−MII transition. The accompanying conformational change alters the mass distribution of the proteolipid membrane, which is detected as further shifts in the resonance angles.[7] The magnitude of the shifts (~12 mdeg in both polarizations) is equivalent to those measured for YL activation of Rho in an unpolymerized bis-SorbPC bilayer and in a fluid dioleoylphosphatidylcholine (DOPC) bilayer (Table 1). Thus, Rho photoactivity is unaffected by lipid polymerization. Increasing the Rho concentration in the PWR cell by 50% produces larger shifts in the resonance angles induced by Rho insertion into bis-SorbPC (Table 1), which is expected due to a greater Rho surface coverage. After polymerization, YL activation induces correspondingly larger shifts (~17 mdeg in both polarizations), verifying that the measured shifts indeed result from Rho photoactivity. In another control, a bis-SorbPC PSLB was polymerized before Rho was injected into the PWR cell. The reduced magnitude of the resonance angle shifts (Table 1) show that although Rho does interact with the poly(lipid) bilayer, ~50% less protein is incorporated relative to an unpolymerized bilayer (see SI). The shifts induced by YL activation are also smaller, consistent with a lower Rho surface coverage and possibly partial hindrance of the conformational change accompanying the MI−MII transition.

In summary, these data demonstrate for the first time that Rho photoactivity can be maintained in a highly cross-linked poly(PSLB). UV irradiation of SorbPC lipids produces an elastomeric bilayer composed of cross-linked oligomers, with a number-average degree of polymerization (X_n) of 3−10.[9] This relatively low X_n is sufficient to stabilize the membrane to conditions that would destroy a fluid bilayer[2,9] (see SI), yet the cross-linked poly(lipid) clearly retains sufficient flexibility to accommodate the conformational change(s) accompanying activation of a reconstituted GPCR. This finding has potentially important implications for development of TMP-based biosensors and biochips.

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Supporting Information Available: Absorbance spectra of Rho, bis-SorbPC synthesis, data verifying polymerization, and additional PWR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) After dilution in the buffer in the PWR cell, the nominal bulk concentrations of Rho and OG were 1 μM and 3 mM, respectively. Dilution of OG below its critical micelle concentration of 25 mM caused spontaneous insertion of Rho into the PSLB.[7,11]

(14) Uniaxial insertion with the long axis of Rho normal to the PSLB plane should produce a larger shift in the *p*-polarized resonance angle relative to the *s*-polarized resonance angle, as observed here (Table 1). However, the observation of optical thickness anisotropy does not unambiguously define the direction of Rho insertion. In addition, the degree of nonspecific adsorption of Rho to the PSLB cannot be quantitatively determined from the PWR data, although based on the observed optical thickness anisotropy, it appears to be minor.

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