Rhodopsin Reconstitution in Vesicles Formed from Simple, Fully Synthetic Amphiphiles

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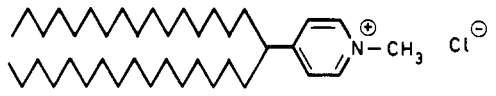
Abstract: The visual pigment bovine rhodopsin has been incorporated into closed bilayer vesicles formed from fully synthetic double-chained amphiphiles **1** and **2** carrying *N*-methylpyridinium head groups. The reconstituted rhodopsin exhibits the same absorption spectrum as in the intact biological membrane. This indicates that the chromophoric center of the pigment is not affected by reconstitution into the artificial, positively charged vesicles. Illumination of rhodopsin bound to vesicles of **1** at 25 or 16 °C results in spectrochemical changes indicative of the formation of metarhodopsin II and, subsequently, of metarhodopsin III and *all-trans*-retinal. These findings reveal that rhodopsin molecules are located in a membrane-like environment. Metarhodopsin I is the first detectable photoproduct when rhodopsin bound to vesicles formed from **1** is illuminated at 1.5 °C. This different behavior at lower temperature is primarily a kinetic effect and is not caused by an increased rigidity of the bilayer below the phase-transition temperature. Illumination of rhodopsin reconstituted in the presence of amphiphile **2** at 25 °C leads to less than 30% of metarhodopsin I. Electron micrographs obtained by both negative staining and freeze-fracture techniques provide strong additional evidence for the incorporation of rhodopsin into the hydrocarbon part of the vesicle membrane. It is concluded that this microenvironment allows the essential internal flexibility for the chromoprotein to retain its functional activity. Thus, vesicles derived from simple synthetic amphiphiles may well become powerful tools in mimicking membrane functions.

Introduction

Spherical or ellipsoidal closed bilayer structures formed from simple synthetic amphiphiles have been the subject of much recent interest.[2,3] It was observed that the chemical and physical properties of these vesicles are often quite similar to those of phospholipid vesicles. Therefore it may be anticipated that the "artificial" vesicles can also be employed to mimic cell membrane functions. In an approach in that direction, we describe here the first successful reconstitution of bovine rhodopsin employing bilayer membrane vesicles composed of the *fully synthetic amphiphiles* **1** and **2**. Rhodopsin plays a key role in the visual process whereby its location in uniquely organized membranes is essential for its function as photoreceptor.[4-7] Both studies of rhodopsin reconstituted in phospholipid membranes of well-defined composition and studies on native rod outer segment suspensions have provided useful insight into the microenvironmental effects on the process of photoreception.



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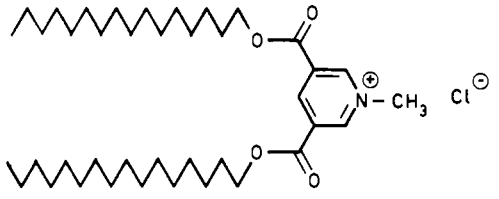

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In the present study, special attention has been focused on the photochemical behavior and the morphology of the reconstituted rhodopsin vesicles. In contrast to the previous reconstitutions using phospholipids,[8-18] the vesicle surface is not negatively charged or neutral[5] but bears a net *positive* charge.

Experimental Section

Materials. The synthetic amphiphiles 4-(17-tritriacontyl)-*N*-methylpyridinium chloride (**1**) and 3,5-bis[(*n*-hexadecyloxy)carbonyl]-*N*-methylpyridinium chloride (**2**) were prepared from the corresponding analytically pure iodides[3] using a standard procedure with silver chloride in 10% (v/v) ethanol–water. The precipitated silver iodide was filtered off and the solvent was removed under reduced pressure.

4-(17-Tritriacontyl)-*N*-methylpyridinium Chloride (1). The colorless crystals (mp 64 °C, from acetone) contained one molecule of water of crystallization. ^{1}H NMR ($CDCl_3/Me_4Si$): δ 0.93 (6 H, t, $J \sim 4$ Hz), 1.28 (60 H, m), 2.8 (1 H, q, $J < 2$ Hz, unresolved), 4.75 (3 H, s), 7.7–9.5 (4 H, m). A small concentration-dependent water signal was observed at about δ 4.4.

Anal. Calcd for $C_{39}H_{74}NCl\cdot H_2O$ (mol wt 610.50): C, 76.72; H, 12.55; Cl, 5.81. Found: C, 76.53; H, 12.42; Cl, 5.78.

(1) (a) University of Groningen. (b) University of Nijmegen.

(2) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860. Kunitake, T.; Okahata, Y.; Tanaki, K.; Kumamura, F.; Takayanagi, M. *Chem. Lett.* **1977**, 387. Kunitake, T.; Sakamoto, T. *J. Am. Chem. Soc.* **1978**, *100*, 4615. Okahata, Y.; Kunitake, T. *Ibid.* **1979**, *101*, 5231. Kunitake, T.; Okahata, Y. *Ibid.* **1980**, *102*, 549. Kunitake, T.; Nakashima, N.; Shimomura, M.; Okahata, Y. *Ibid.* **1980**, *102*, 6642. Deguchi, K.; Mino, J. *J. Colloid Interface Sci.* **1978**, *65*, 155. Tran, C. D.; Klahn, P. L.; Romero, A.; Fendler, J. H. *J. Am. Chem. Soc.* **1978**, *100*, 1622. Lim, Y. Y.; Fendler, J. H. *Ibid.* **1979**, *101*, 4023. Kano, K.; Romero, A.; Djermouni, B.; Ache, H. J.; Fendler, J. H. *Ibid.* **1979**, *101*, 4030. Mortara, R. A.; Quina, F. H.; Chaimovich, H. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 1080. Czarniecki, M. F.; Breslow, R. *J. Am. Chem. Soc.* **1979**, *101*, 3675. Murakami, Y.; Nakano, A.; Fukuya, K. *Ibid*, **1980**, *102*, 4253. Baumgartner, E.; Fuhrhop, J. H. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 550. Regen, S. L.; Czech, B.; Singh, A. *J. Am. Chem. Soc.* **1980**, *102*, 6638. Kunitake, T.; Okahata, Y.; Ando, R.; Shinkai, S.; Hirakawa, S.-i. *Ibid*, **1980**, *102*, 7877. Murakami, Y.; Nakano, A.; Yoshimatsu, A.; Fukuya, K. *Ibid.* **1981**, *103*, 728.

(3) Sudhölter, E. J. R.; Engberts, J. B. F. N.; Hoekstra, D. *J. Am. Chem. Soc.* **1980**, *102*, 2467.

(4) Ostray, S. E. *Biochim. Biophys. Acta* **1977**, *463*, 91.

(5) Montal, M. *Biochim. Biophys. Acta* **1979**, *559*, 231.

(6) Daemen, F. J. M.; de Grip, W. J. *Neurochemistry* **1980**, *1*, 539.

(7) van Breugel, P. J. G. M. Ph.D. Thesis, Nijmegen, 1977.

(8) Hong, K.; Hubbell, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2617.

(9) Hong, K.; Hubbell, W. L. *Biochemistry* **1973**, *12*, 4517.

(10) Chen, Y. S.; Hubbell, W. L. *Exp. Eye Res.* **1973**, *17*, 517.

(11) Hubbell, W. L. *Acc. Chem. Res.* **1975**, *8*, 85.

(12) O'Brien, D. F.; Costa, L. F.; Ott, R. A. *Biochemistry* **1977**, *16*, 1295.

(13) van Breugel, P. J. G. M.; Geurts, P. H. M.; Daemen, F. J. M.; Bonting, S. L. *Biochim. Biophys. Acta* **1978**, *509*, 136.

(14) de Grip, W. J.; Bovee-Geurts, P. H. M. *Chem. Phys. Lipids* **1979**, *23*, 321.

(15) Darzon, A.; Vandenberg, C. A.; Ellisman, M. H.; Montal, M. *J. Cell. Biol.* **1979**, *81*, 446.

(16) O'Brien, D. F. *Photochem. Photobiol.* **1979**, *29*, 679.

(17) Ikai, A.; Nishigai, M. *Arch. Biochem. Biophys.* **1979**, *192*, 352.

(18) Darzon, A.; Vandenberg, C. A.; Schönfeld, M.; Ellisman, M. H.; Spitzer, N. C.; Montal, M. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 239.

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3,5-Bis[(*n*-hexadecyloxy)carbonyl]-*N*-methylpyridinium Chloride (2). Mp 102.0–102.5 °C (from acetone). ^{1}H NMR ($CDCl_3/Me_4Si$): δ 0.85 (6 H, t, $J \sim 5$ Hz), 1.30 (56 H, m), 4.40 (4 H, t, $J < 7$ Hz), 5.03 (3 H, s), 9.20 (1 H, t, $J < 2$ Hz), 10.00 (2 H, d, $J < 2$ Hz).

Anal. Calcd for $C_{40}H_{72}NO_4Cl$ (mol wt 666.47): C, 72.10; H, 10.88; Cl, 5.32. Found: C, 71.44; H, 10.84; Cl, 5.23.

Phase Transitions. Differential scanning calorimetry (DSC; Perkin-Elmer DSC-2 apparatus, using sealed aluminum pans; heating rate, 10 °C min^{-1}) was used to study phase transitions in aqueous gels of **1** (29.5% by weight) and **2** (16.3% by weight). In the case of **1** the phase transition temperature (PT) is at 22 °C ($\Delta H°$ = 12.2 kcal·mol^{-1}) and for **2** at 37 °C ($\Delta H°$ = 10.8 kcal·mol^{-1}). No pretransitions were observed. Thus, the PT's are associated with a chain melting process, presumably directly from a flat $L_{\beta'}$ phase to a liquid-like L_α phase.[19]

Rhodopsin Reconstitution. The reconstitution was performed essentially as described by de Grip et al.[20] Thus, 100 μL of a stock solution of **1** or **2** (10 mg·mL^{-1}) in $CHCl_3$ was treated with a stream of dry nitrogen in a rotating test tube. The resulting amphiphilic film was then dissolved in 750 μL of a 22 mM solution of *n*-nonylglucoside[14] in a Mops buffer[21] and combined at ca. 2 °C under dim red light with 1500 μL of a buffered rhodopsin–*n*-nonylglucoside solution.[22] The test tube was gently shaken under a stream of nitrogen until a clear solution was obtained. This solution was dialyzed against 100 mL of Mops buffer[21] in a dark and cold (4–6 °C) room during 16–42 h. The buffer was refreshed twice. No precipitation was observed. Variation of the dialysis time had no significant effect on the results of the photolysis. After solubilization of the reconstituted sample in an Ammonyx-LO detergent solution (final concentration, 1% by weight), the recovery of detergent-free, reconstituted, and photosensitive rhodopsin was over 90%. Concentrations were determined from the optical density at 500 nm. However, when the concentrations were calculated from the optical density at 500 nm before solubilization, lower recoveries (22–55%) were obtained. This phenomenon probably originates from the presence of aggregated rhodopsin in the vesicle membranes, resulting in enhanced light scattering (vide infra) and deviation from the Lambert–Beer law. The rhodopsin/amphiphile molar concentrations were determined by absorption spectroscopy to be 1:54 and 1:90 for **1** and 1:106 for **2**. No correction for scattering was applied. The initital rhodopsin:amphiphile molar ratios were 1:82 for **1** and 1:75 for **2**.

The spectral measurements on the rhodopsin reconstitutions were carried out with a Rapid T3 double-beam spectrophotometer (Howaldtswerke, Deutsche Werft, Kiel, GFR) equipped with thermostated cuvettes or on a Pye-Unicam SP-1750 double-beam spectrophotometer for the spectra recorded at room temperature (ca. 25 °C). The spectra were recorded from long to short wavelength at a scanning speed of 4 nm·s^{-1}. No corrections for residual scattering were applied. Photolysis was initiated by illumination with a 100-W tungsten source through OG-530 and KG-1 infrared filters (Schott Jena, Mainz, GFR). Chemical regeneration was attempted by adding a fivefold molar excess of 11-*cis*-retinal in ethanol (final concentration, <0.5% by weight) to a fully bleached sample of reconstituted rhodopsin.[20] After incubation for 3 h at 25 °C an excess of NH_2OH in 1 M $NaHCO_3$ (pH 6.5) was added in order to convert unreacted 11-*cis*-retinal into the oxime.

Electron micrographs were obtained by using negative staining as well as freeze-fracture techniques. The negative-stained samples were prepared by employing the two-step droplet method on Formvar and carbon-coated grids. A 1% (by weight) solution of uranyl acetate was used as negative stain.

For freeze fractioning, small quantities of the concentrated suspension were micropipetted on phosphorbronze specimen carriers, prepared by the method of Mueller and Scheid,[23] and frozen in melting nitrogen. The freeze fracture and replication process were carried out in a freeze-etch apparatus described elsewhere.[24] The replicas were released in distilled water and thoroughly cleaned in bleach. Negative-staining preparations and freeze-fracture replicas were examined in a Philips 201 or 300 electron microscope at 80 kV and photographed at initial magnifications ranging from 10 000 to 50 000×.

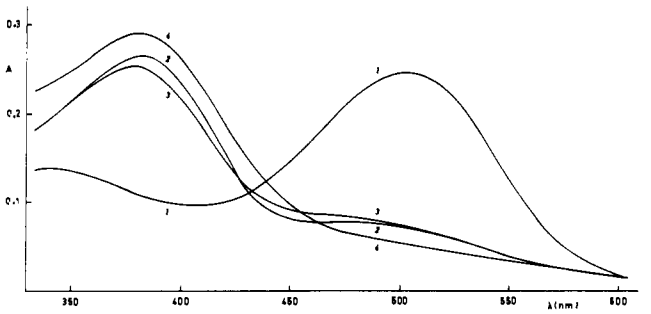

Figure 1. Photolysis of reconstituted rhodopsin. Absorption spectra (1) before illumination, (2) 2 min after illumination for 30 s, (3) 6 min after illumination for 30 s, and (4) after continuous illumination for 30 min. Rhodopsin:**1** molar ratio = 1:90; 25 °C.

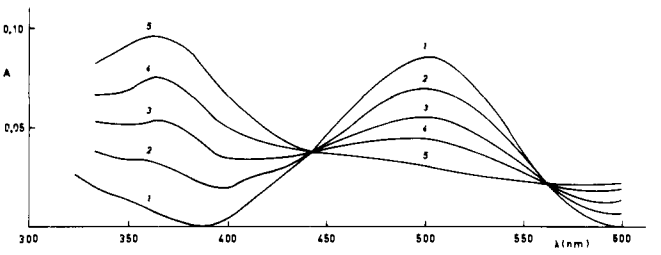

Figure 2. Photolysis of reconstituted rhodopsin. Absorption spectra (1) before illumination, (2) after illumination for 5 s, (3) after illumination for 10 s, (4) after illumination for 20 s, and (5) after illumination for 50 s. Rhodopsin:**1** molar ratio = 1:54; 25 °C.

Results and Discussion

The sequence of spectral changes in rhodopsin following illumination is a very specific property of the visual pigment. The late and slower part of these photochemical transitions at temperatures between 0 and 37 °C is accessible by conventional spectrophotometry on a time scale of minutes. We find that all vesicle preparations from **1** and **2** with rhodopsin exhibit a maximal absorbance around 500 nm, similar to that found in rod outer segment suspensions. Since the presence of this absorption band[25] indicates[4-7] that rhodopsin remained native and photochemically excitable, *our observation provides strong evidence for the conclusion that the chromophoric center of rhodopsin is not affected by reconstitution into these artificial, positively charged vesicles.*

Upon illumination of a sample of reconstituted rhodopsin in vesicles of **1** (rhodopsin:amphiphile **1** molar ratio 1:90) for 30 s at 25 °C the first detectable photoproduct is metarhodopsin II (λ_m = 384 nm; see Figure 1). The subsequent decrease in absorbance at 384 nm and gain in absorbance at 465 nm are interpreted as a conversion of metarhodopsin II into metarhodopsin III. An isosbestic point is observed at 427 nm. These findings are similar to those observed in native rod outer segment membrane suspensions and in phospholipid–rhodopsin reconstituted membranes.[26] The observation of the metarhodopsin III photoproduct provides a strong indication that the rhodopsin molecules are located in a membrane-like environment since in a micellar environment this photoproduct is not formed[26,27] but instead retinal (λ_m = 380 nm) is rapidly released from the protein in a hydrolytic process. Surprising was the increase in scattering with increasing illumination time. Aggregation of the apoprotein opsin could account for this phenomenon.[17,28] As depicted in Figure 2, the decrease in photochemically active rhodopsin (λ_m = 500 mm) parallels the formation of *all-trans*-retinal (λ_m = 380 nm) and, consequently, of opsin. The increase in turbidity is seen as an

(19) Janiak, M. J.; Small, D. M.; Shipley, G. G. *Biochemistry* **1976**, *15*, 4575.

(20) de Grip, W. J.; Daemen, F. J. M.; Bonting, S. L. In *Methods Enzymol.* **1980**, *67*, 301.

(21) 20 mM 4-morpholinepropanesulfonic acid (Mops), 130 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.1 mM EDTA; pH 7.2.

(22) 13.3 μM bovine rhodopsin, 20 mM *n*-nonylglucoside, 20 mM 1,4-piperazinediethanesulfonic acid (Pipes), 130 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.1 mM EDTA; pH 6.5. The isolation and purification of rhodopsin (spectral ratio A_{278}/A_{498} = 1.9) have been described.[20]

(23) Mueller, W.; Scheid. P. *J. Microsc. (Oxford)* **1979**, *115*, 113.

(24) Vos, J.; Kors, G.; Schuurhuis, G. J.; Stokroos, I. *Proc. 7th Eur. Cong. Electron Microsc.* **1980** *2*, 732.

(25) The bandwidth of the absorption band (Figure 1) is also similar to that observed for bovine rhodopsin: Ebrey, Th. G.; Honig, B. *Vision Res.* **1977**, *17*, 147.

(26) van Breugel, P. J. G. M.; Bovee-Geurts, P. H. M.; Bonting, S. L.; Daemen, F. J. M. *Biochim Biophys. Acta* **1979**, *557*, 188.

(27) de Grip, W. J., unpublished results.

(28) Osborne, H. B.; Sardet, C.; Hellenius, A. *Eur. J. Biochem.* **1974**, *44*, 383.

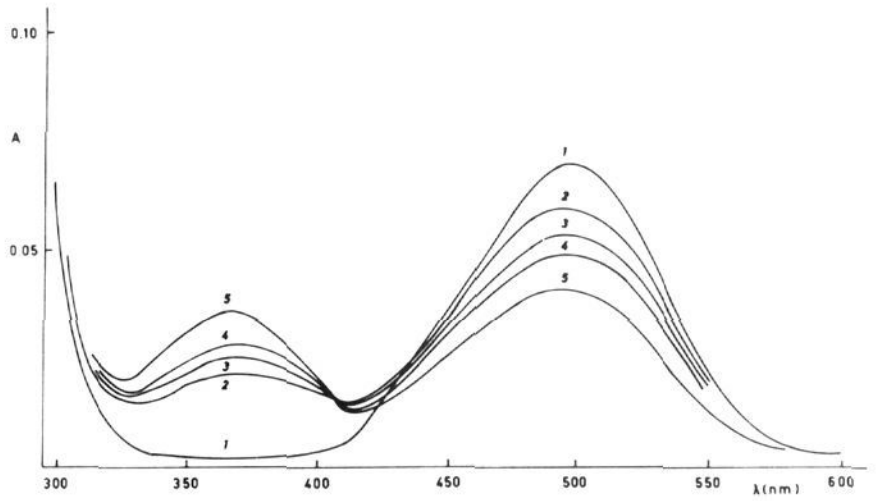

Figure 3. Photolysis of reconstituted rhodopsin. Absorption spectra (1) before illumination, (2) 1 min after illumination for 30 s, (3) 3 min after illumination for 30 s, (4) 5 min after illumination for 30 s, and (5) 14 min after illumination for 30 s. Rhodopsin:**1** molar ratio = 1:54; 1.5 °C.

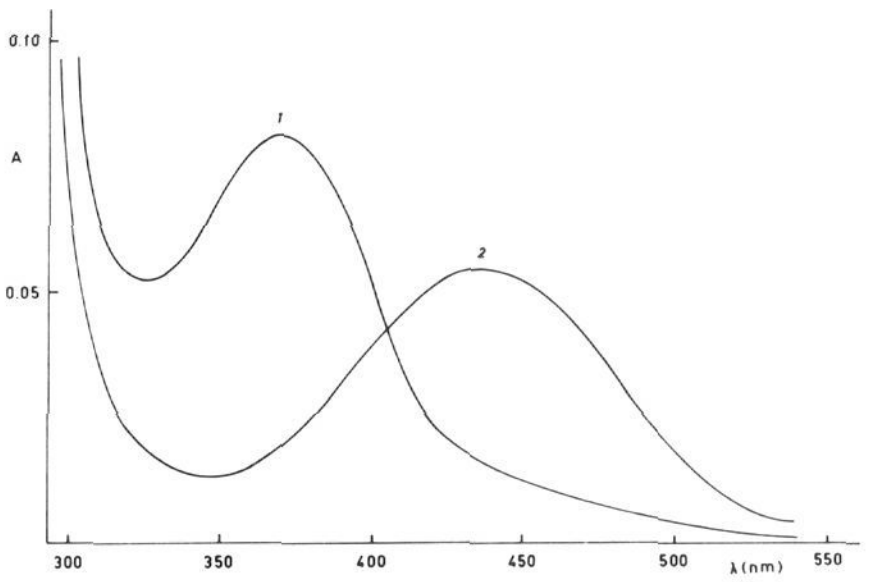

Figure 4. Photolysis of reconstituted rhodopsin at 1.5 °C. Absorption spectra (1) after continuous illumination for 30 min and (2) after acidification with excess HCl. Rhodopsin:**1** molar ratio ≈ 1:54.

increase in absorbance at $\lambda = 600$ nm. Isosbestic points are located at 440 and 560 nm. The increase in scattering strongly hampered a more detailed study of the conversion of metarhodopsin II into metarhodopsin III. When the photolysis of rhodopsin bound to vesicles of **1** (rhodopsin:**1** molar ratio = 1:54) is followed at 1.5 °C, metarhodopsin I ($\lambda_m = 478$ nm) is the first detectable intermediate. The isomerization of metarhodopsin I into metarhodopsin II as a function of time is shown in Figure 3. An isosbestic point is observed at 405 nm. This conversion is also retarded in the photoreceptor membrane by lowering the temperature (at pH 7.2 metarhodopsin I is then almost stable at 2 °C) or by reconstitution of rhodopsin in saturated phospholipids.[7,12,13] *In lipid and detergent-free rhodopsin preparations*, metarhodopsin I only slowly decays at 25 °C to products differing from metarhodopsin II.[3,12,29]

In order to check whether the 370-nm absorption (Figure 3) represents metarhodopsin II and/or free *all-trans*-retinal, samples were acidified with an excess of 3 M HCl. Acidification of the fully bleached sample at 1.5 °C shifts the 370-nm band completely to 437 nm. This band is characteristic for a protonated Schiff base (Figure 4). In a control experiment, the acid was added after the sample was warmed to room temperature. In this case, no band shift was observed, consistent with the formation of *all-trans*-retinal. Thus, in the present system metarhodopsin II is less stable than in phospholipid bilayers ($t_{1/2} = 7$–10 min) and decays rapidly at room temperature ($t_{1/2} < 5$ min) to yield predominantly *all-trans*-retinal.

Rhodopsin reconstitution with vesicles formed from amphiphile **2** could only be studied in less detail because of the strong absorption of **2** at wavelengths below 435 nm. Illumination of these

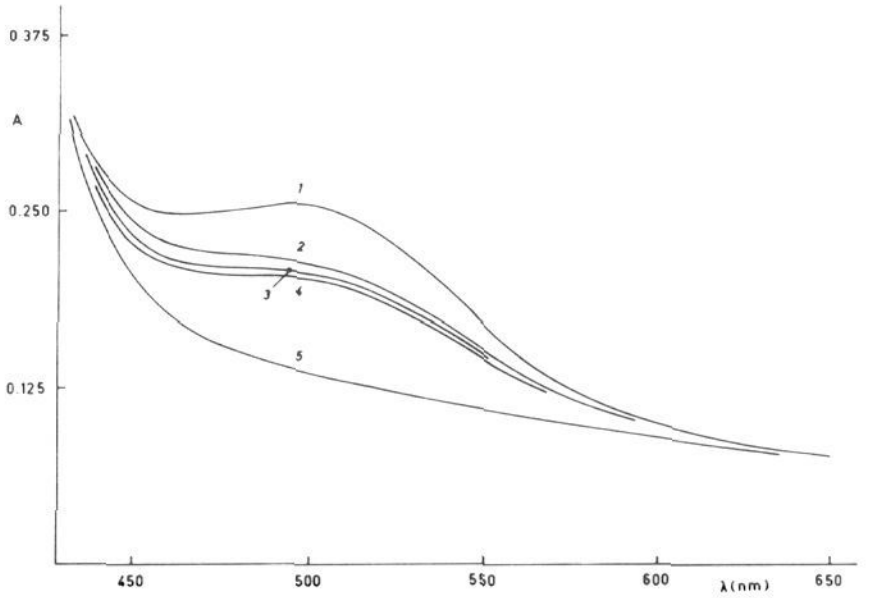

Figure 5. Photolysis of reconstituted rhodopsin. Absorption spectra (1) before illumination, (2) 1 min after illumination for 30 s, (3) 19 min after illumination for 30 s (4) 41 min after illumination for 30 s, and (5) after illumination for 5 min. Rhodopsin:**2** molar ratio = 1:106; 25 °C.

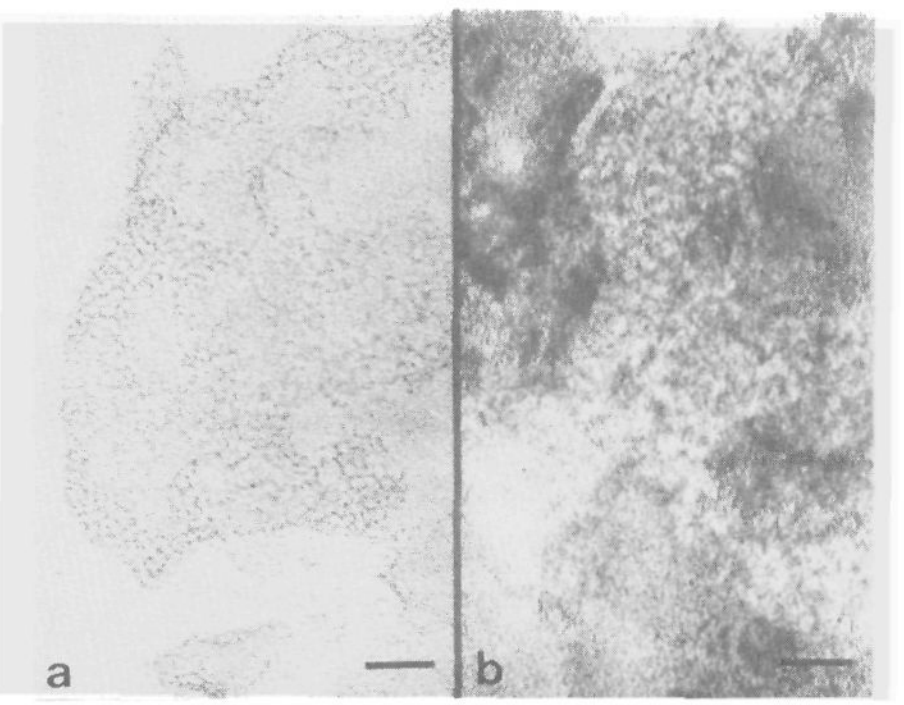

Figure 6. (a) Electron micrograph (negative staining) of reconstituted rhodopsin in vesicles formed from **1**. Initital magnifications, 25 000×. The marker line represents 250 Å. (b) Same as (a) for vesicles formed from **2**.

rhodopsin vesicles (rhodopsin:amphiphile molar ratio = 1:106) for 30 s at 25 °C (Figure 5) leads to only partial bleaching of rhodopsin (difference between spectra 4 and 1 shown in Figure 5). From this part only a fraction (<30%, difference between spectra 4 and 2 in Figure 5) can be attributed to metarhodopsin I; this pathway might be induced by the presence of some type of aggregated rhodopsin.[6,13] The remaining fraction is most likely metarhodopsin II, with its absorption hidden under the strong absorption of **2**.

The decrease in the rate of decay of metarhodopsin I observed *in vesicles of* **1** *upon lowering the temperature from ca. 25 to 1.5* °C could be due either to the phase transition (the PT is 22 °C; see Experimental Section), which renders the bilayer more rigid[30–32] (similar to phospholipid vesicles), or to the effect of temperature on the kinetics, as observed in the intact photoreceptor membranes. A photolysis experiment carried out in a reconstitution of rhodopsin and amphiphile **1** at 16 °C (i.e., below its PT) yields metarhodopsin II instantaneously. This provides strong evidence for the interpretation in terms of the kinetic effect.

Bilayers in vesicles formed from **1** apparently allow more freedom of motion below their PT than those composed of satu-

(29) Applebury, M. L.; Zuckerman, D. M.; Lamola, A. A.; Jovin, T. M. *Biochemistry* **1974**, *13*, 3448.

(30) Deguchi, K.; Mino, J. *J. Colloid Interface Sci.* **1978**, *65*, 155.
(31) Shinitzky, M.; Barenholz, Y. *J. Biol. Chem.* **1974**, *249*, 2652.
(32) Cogan, U.; Shinitzky, M.; Weber, G.; Nishida, T. *Biochemistry* **1973**, *12*, 521.

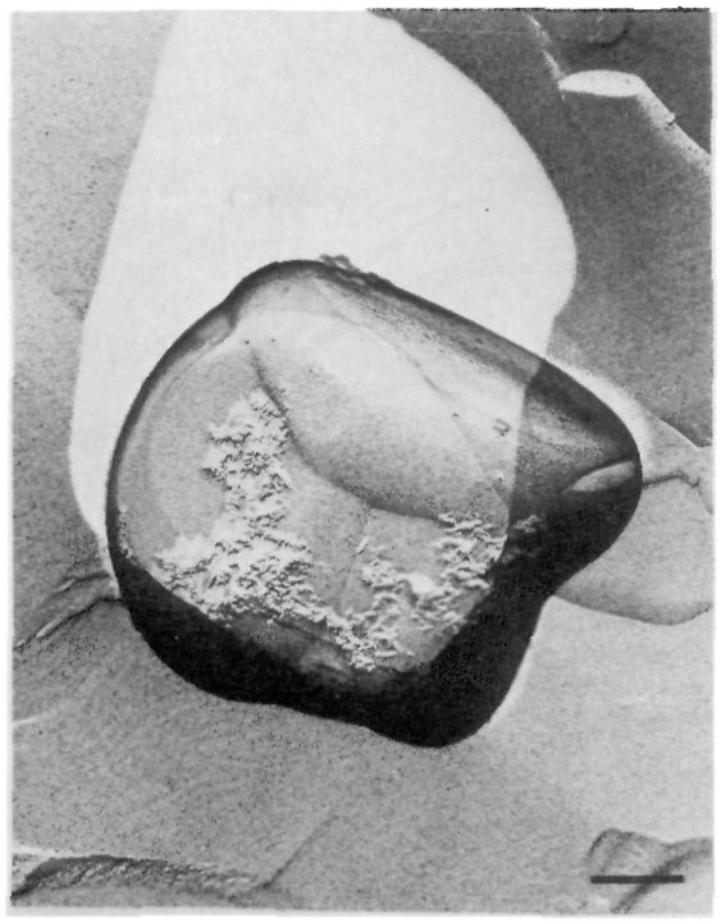

Figure 7. Electron micrograph of a replica of freeze-fractured reconstituted rhodopsin in vesicles formed from **2**. Initital magnification, 44 000×.

rated phospholipids,[7,12,13] since in reconstitutions with phospholipids below the PT metarhodopsin I is observed as the principal intermediate. This conclusion is in agreement with the observation that metarhodopsin I is only a minor photoproduct after illumination of rhodopsin in vesicles of **2** for 1 min at 25 °C (the PT of **2** is 37 °C, see Experimental Section). Differences in microviscosity within the double layer may account for the different behavior of both types of vesicles.

Attempts to regenerate completely bleached rhodopsin in vesicles formed from **1** and **2** by the addition of 11-*cis*-retinal were unsuccessful. However, if the regeneration of rhodopsin in vesicles formed from **1** is performed after solubilization of the fully bleached sample in 0.5% dodecylmaltose detergent solution,[14] 30% regeneration capacity was observed. The regeneration capacity could be increased to even 70% by solubilization of the reconstituted sample before bleaching. The observations can be rationalized by invoking aggregation of opsin and a concomitant shielding of the active site of the apoprotein. However, lipid and detergent-free rhodospin, which is heavily aggregated,[27] can be regenerated for 40–60%.[6] It cannot be excluded, therefore, that opsin, which is more sensitive than rhodopsin to changes in microenvironment,[33] is not well accommodated in the artificial vesicle system. This may result in conformational changes, loss of regeneration capacity, and aggregation.

Electron micrographs are shown in Figures 6 and 7. Both the negatively stained samples (Figure 6) and the freeze-fractured sample (Figure 7) provide strong evidence for a heterogeneous distribution of the unbleached rhodopsin molecules over the membrane. The negatively stained preparations clearly indicate the presence of lamellae-like structures while the freeze-fractured samples show spherical vesicles with the rhodopsin located in the bilayer of the vesicle. These samples were frozen from above as well as from below their PT. Since no homogeneous distribution of rhodopsin over the vesicle membrane was found, it appears that the difference in bilayer rigidity above and below the PT does not greatly influence the distribution. This contrasts with rhodopsin–phospholipid reconstitutions, in which the distribution of the protein is affected by the hydrocarbon state.[10]

Conclusion

Rhodopsin reconstitution can be successfully carried out in the presence of vesicles formed from simple, fully synthetic amphiphiles **1** and **2**. Apparently, the internal flexibility of the pigment molecule is retained in the microenvironment provided by the interior of the artificial bilayer membrane. This observation may encourage further studies of synthetic amphiphile vesicles as membrane models.

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Registry No. 1, 80243-88-5; **2**, 80243-89-6.

(33) de Grip, W. J. In *Methods Enzymol.*, in press.