L. M. (1972), *J. Bacteriol. 112,* 1090.
Op den Kamp, J. A. F., Kauerz, M. Th., and van Deenen, L. L. M. (1975), *Biochim. Biophys. Acta 406,* 169.
Pollack, J. P., Razin, S., and Cleverdon, R. C. (1965), *J. Bacteriol. 90,* 617.
Razin, S. (1969), in The Mycoplasmatales and the L-phase of Bacteria, Hayflick, L., Ed., Amsterdam, North-Holland Publishing Co., p 317.
Renooy, W., van Golde, L. M. G., Zwaal, R. F. A., and van Deenen, L. L. M. (1976), *Eur. J. Biochem. 61,* 53.
Rottem, S., Hasin, M., and Razin, S. (1973), *Biochim. Biophys. Acta 323,* 520.
Rottem, S., Hubbell, W. L., Hayflick, L., and McConnell, H. M. (1970), *Biochim. Biophys. Acta 219,* 1021.
Rottem, S., and Samuni, A. (1973), *Biochim. Biophys. Acta 298,* 32.
Träuble, H., and Overath, P. (1973), *Biochim. Biophys. Acta 307,* 491.
Tsai, K. H., and Lenard, J. (1975), *Nature (London) 253,* 554.
van Golde, L. M. G., McElhaney, R. N., and van Deenen, L. L. M. (1971), *Biochim. Biophys. Acta 231,* 245.
Verger, R., Mieras, M. C. E., and de Haas, G. H. (1973), *J. Biol. Chem. 248,* 4023.
Warren, G. B., Toon, P. A., Birdsall, N. J. H., Lee, A. G., and Metcalfe, J. C. (1974), *Biochemistry 13,* 5501.
Zwaal, R. F. A., Roelofsen, B., and Colley, C. M. (1973), *Biochim. Biophys. Acta 300,* 159.

Photochemical Functionality of Rhodopsin–Phospholipid Recombinant Membranes†

David F. O'Brien,* Larry F. Costa, and Rebecca A. Ott

ABSTRACT: Purified rhodopsin was incorporated into phospholipid bilayers to give recombinant membranes. The photochemical functionality in these systems was examined by low-temperature spectroscopy and by kinetic spectrophotometry. Changes in the absorption spectra of glycerol–water mixtures of rhodopsin–egg phosphatidylcholine and rhodopsin–asolectin recombinants were monitored after the sample was cooled to −196 °C, presented with light of wavelength greater than 440 nm, and then warmed gradually to room temperature. Absorption characteristics indicative of the spectral intermediates prelumirhodopsin, lumirhodopsin, metarhodopsin I, and metarhodopsin II were observed. The kinetics of the metarhodopsin I to metarhodopsin II transition in these recombinants was studied by flash photolytic observation of the decay of meta I and the formation of meta II. Recombinants prepared from unsaturated phospholipids, e.g., asolectin, egg phosphatidylcholine, egg phosphatidylethanolamine, and dioleoylphosphatidylcholine, showed first-order kinetics for the transition with rates comparable to that of rod outer segment membranes. Recombinants prepared from saturated phosphatidylcholines have a retarded rate of conversion from meta I to meta II and are considered to be nonfunctional. The photochemical functionality of rhodopsin–phospholipid recombinants is dependent upon the presence of phospholipid unsaturation and the fluidity of the phospholipid hydrocarbon chains, and is independent of the polar head group of the phospholipid.

The vertebrate rod outer segment is the site of light reception and energy transduction to electrical activity in the visual system. Rhodopsin is the major protein component of ROS[1] membranes (Daemen, 1973) and its chromophore, 11-*cis*-retinal, serves as the light receptor (Wald, 1968a,b). The subsequent role of rhodopsin in excitation remains to be clearly defined, although it has been proposed that rhodopsin mediates the permeability of disk membranes in ROS to transmitter substances (Yoshikami and Hagins, 1971; Hagins, 1972).

Rhodopsin is an integral protein in the ROS membrane. Data from x-ray diffraction (Blaise, 1972; Blaurock and Wilkins, 1972; Chabre, 1975) and freeze fracture electron microscopy experiments (Chen and Hubbell, 1973) suggest part of the protein molecule is in contact with the hydrophobic interior of the membrane. Thus, the properties of rhodopsin in the membrane should be partly dependent upon the physical and chemical nature of the lipids. Phospholipids comprise the major portion (80–85%) of the lipid content of ROS membranes (Daemen, 1973). The kind and composition of the phospholipids are known, as well as the fatty acid composition of the acyl groups. The chief phospholipids in cattle ROS are PC (41% of lipid phosphorus), PE (39%), and PS (13%) (Anderson and Maude, 1970). Nearly half of the fatty acids in ROS are polyunsaturated, which is high for natural membranes, although many membranes are composed of unsaturated phospholipids. The phospholipids in many native membranes exhibit rapid translational and rotational motion at room and physiological temperatures (Hubbell and McConnell, 1971; Devaux and McConnell, 1972). The dynamic properties of the polyunsaturated ROS membranes are demonstrated by the observed rotational (Brown, 1972; Cone,

† From the Research Laboratories, Eastman Kodak Company, Rochester, New York 14650. *Received July 26, 1976.*

[1] Abbreviations used are: ROS, rod outer segment; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; di-12:0-PC, 1,2-didodecanoylphosphatidylcholine; di-14:0-PC, 1,2-ditetradecanoylphosphatidylcholine; di-(18:1^c)-PC, 1,2-di(*cis*-9-octadecanoyl)-phosphatidylcholine; DTAB, dodecyltrimethylammonium bromide; meta I, metarhodopsin I; meta II, metarhodopsin II; DDAO, dodecyldimethylamine oxide; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; TrTAB, tridecyltrimethylammonium bromide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance.

1972) and translational (Poo and Cone, 1973) motions of rhodopsin in frog retinae.

Reconstitution of membranes from purified specific components is a convenient means to study protein–phospholipid interactions (Razin, 1972). It has been shown that purified, delipidated rhodopsin can be prepared in DTAB and recombined with a variety of selected phospholipids (Hong and Hubbell, 1973; Chen and Hubbell, 1973). The success of membrane reconstitution depends upon whether the system is functional. In the case of rhodopsin membranes, the in vivo functionality of the protein is not established so other criteria have been utilized. Hong and Hubbell (1973) tested the functionality of the recombinants by chemical regeneration with 11-*cis*-retinal after optical bleaching of the preparations. High regenerability was found for rhodopsin in phospholipid bilayers, in digalactosyl diglyceride bilayers, and in digitonin micelles, but not in other detergents such as DTAB or Triton X-100. They concluded that structural requirements rather than specific chemical interactions were important in maintaining a regenerable molecular configuration of the rhodopsin. The phospholipid bilayers appear to hold rhodopsin in a more native configuration than do strong detergents.

The present study of rhodopsin in recombinant membranes utilizes low-temperature spectroscopy and kinetic spectrophotometry to assess the existence of known intermediates with kinetics of relaxation comparable to that of rhodopsin in ROS membranes and in the retinae. These necessary conditions for the functionality of rhodospin–phospholipid recombinants will be termed photochemical functionality. Rhodopsin in ROS membranes and in detergent passes through a series of defined spectral intermediates (Yoshizawa, 1972; Tokunaga et al., 1976) which have known kinetic parameters for the relaxation processes (Abrahamson and Wiesenfeld, 1972). The interconversion from intermediates meta I (475 nm) and meta II (380 nm) is convenient to measure because of the large spectral shift, and the fact that the lifetimes are of the order of milliseconds at room temperature. In addition, it is known that the meta I to meta II relaxation is sensitive to the rhodopsin environment. The interconversion for rhodopsin in detergents (DDAO) is two orders of magnitude faster than that of rhodopsin in ROS membranes (Abrahamson and Wiesenfeld, 1972; Rapp, 1970; Applebury et al., 1974). The one report of the kinetics of interconversion from meta I to meta II in a rhodopsin recombinant indicates that the rate is comparable for ROS membranes and rhodopsin–egg PC bilayers (Applebury et al., 1974). In the present study, the effects of phospholipid composition in particular as well as pH and ionic strength on the photochemical functionality of rhodopsin–phospholipid recombinants are reported and discussed.

Experimental Section

Materials. Frozen, dark adapted bovine retinae were obtained from George A. Hormel Co., Austin, Minn. Egg PC, egg PE, 1,2-didodecanoyl-PC (di-12:0-PC), and 1,2-ditetradecanoyl-PC (di-14:0-PC) were obtained from Sigma Chemicals, St. Louis, Mo. Egg lysolecithin and 1,2-di(*cis*-9-octadecenoyl)-PC [di-(18:1^c)-PC] were obtained from P-L Biochemicals, Milwaukee, Wis. The phospholipid purity was evaluated by thin-layer chromatography (TLC), and where necessary the phospholipids were chromatographed on Unisil silicic acid (Clarkson Chemical Co., Williamsport, Pa.). Asolectin, a preparation of soybean phospholipids, was obtained from Associated Concentrates, Inc., Woodside, N.Y. It was purified by the procedure of Seufert (1970).

Spin-labeled phosphatidylcholines were prepared by acylation of egg lysolecithin with the anhydride of the appropriate spin-labeled fatty acid (Syva Chemical, Palo Alto, Calif.) by the method of Hubbell and McConnell (1971).

The tetraalkylammonium bromides were prepared from anhydrous trimethylamine and the appropriate alkyl bromide (Eastman Organic Chemicals) by the procedure of Hong and Hubbell (1973). The starting alkyl bromides were >98% pure as determined by gas–liquid partition chromatography (GLPC).

Buffers utilized were Hepes (Calbiochem, San Diego, Calif.) and Tris (Sigma Chemicals).

Isolation of Rod Outer Segments (ROS). All procedures which utilized rhodopsin were carried out under dim red light Kodak Safelight Filter, No. 1 (red), at 4 °C, unless otherwise specified. The buffer was 0.1 M sodium phosphate (pH 6.8). The procedure followed for isolation of ROS is essentially that of Hong and Hubbell (1973). After the sucrose flotations the final pellet was washed twice with water to remove the sucrose. The membranes were stored at −20 °C after lyophilization, or dissolved in selected detergents, or utilized directly for other experiments.

Rhodopsin in Detergent. The ROS membranes were solubilized with a tetraalkylammonium bromide, usually 100 mM TrTAB, in 15 mM sodium phosphate buffer (pH 6.6) and were chromatographed on hydroxylapatite (DNA grade, Bio-Gel HTP, Bio-Rad Laboratories, Richmond, Calif.) by the procedure of Hong and Hubbell (1973).

Rhodopsin–Phospholipid Recombinants. Purified, delipidated rhodopsin in detergent was combined with the selected purified phospholipid in 0.5 to 1 mL of the detergent. The phospholipid stock solution in chloroform had been stored under inert atmosphere at 4 °C, was evaporated under a dry nitrogen stream, and then pumped to dryness under vacuum for 2 h. The solution of rhodopsin and lipid in detergent was mixed thoroughly but gently and then allowed to equilibrate for a few hours at 4 °C. The detergent was removed by dialysis at 4 °C against a 10 mM Hepes buffer which had been deoxygenated with nitrogen with the desired concentration of dithiothreitol (Calbiochem), EDTA, and/or salts. The dialysis medium was changed every 10 to 14 h, for 2 to 4 days. The recombinants were removed from the dialysis bag and sometimes stored at 4 °C at that concentration; in other instances the membranes were collected by centrifugation at 80 000 to 100 000*g* or concentrated by ultrafiltration with a Diaflo filter and PM-30 or PM-10 membrane filters (Amicon Corporation, Lexington, Mass.). Usually, a five- to tenfold concentration of a typical recombinant was utilized to give optical densities of 0.2 to 0.6 (1 mm) at 500 nm for the low-temperature spectroscopy and flash photolysis experiments.

The rhodopsin recovery yield after recombinant membrane formation was estimated by:

$$(A_{498}/A_{278})(A_{278}'/A_{498}') \times 100$$

where A_{498} and A_{278} are the absorbances of the protein extracted from the recombinants and A_{498}' and A_{278}' are the corresponding absorbances before recombination as described by Hong and Hubbell (1973). A similar calculation was performed after concentration of the recombinant and is designated percent concentration recovery. All absorption spectra were taken with a Cary 14 or Cary 17 spectrophotometer except for analysis of column chromatography fractions where a Beckman DB-G spectrophotometer was utilized. Absorption spectra of ROS or recombinant membranes were obtained after solubilization with 100 mM TrTAB or 50 mM tetradecyltrimethylammonium bromide (TeTAB) in 10 mM Hepes

buffer. The molar extinction coefficient at 498 nm for rhodopsin in these detergents is 42 700 (Hong and Hubbell, 1972).

Low-Temperature Spectroscopy. Low-temperature spectra of rhodopsin in TrTAB or in recombinant membranes were obtained on a Cary 14 spectrophotometer which was fitted with a quartz liquid nitrogen Dewar flask equipped with a heater and a copper vs. constantan thermocouple. The samples were held in a 1-mm demountable cell in the Dewar flask. The rhodopsin solution or membrane suspension was diluted and mixed with an equal volume of spectrograde glycerol (Eastman Organic Chemicals). The measured quantity was spectral transmission density. The test sample and a control blank (lipid vesicles minus rhodopsin) were measured in a regular transmission geometry and the density of the blank was subtracted from the sample density. The density of the blank and density of the sample were normalized at zero at 700 nm. The spectrum thus obtained is corrected for front and back scattering of the blank. Some errors may remain after corrections due to differences in the scattering properties of the sample and blank.

Electron Microscopy. Samples of the recombinant membrane suspensions were observed after negative staining. The suspensions were diluted with the buffer to 0.1 to 0.2% (w/v) phospholipid and treated with ammonium molybdate on the grid. The grids were viewed in a Philips Model 201 electron microscope.

Kinetic Spectrophotometry. A single flash kinetic photometer was constructed to observe the relaxation of the intermediates of rhodopsin bleaching. In order to conserve rhodopsin recombinant preparations, a trinocular microscope (Olympus Optical Co., Tokyo, Japan) was utilized for the sample holder as well as focusing and collecting optics in a manner similar to that described by Porter and Strauss (1966). The photometer uses a xenon flash lamp (EG & G Model FX-42C-3, 400 J electrical energy at 10 kV) for the bleaching flash. The flash has a rise time of 20 μs and a decay of 100 μs. The monitoring light is a 12-V, 100-W quartz iodine lamp powered by a Kepco, Inc., stabilized power supply. Interference filters, Baird Atomic, Inc., were used to select the bleaching and monitoring wavelengths. The monitoring light was divided by a pellicle beam splitter mounted at a 45° angle below the condensing lens of the microscope. One part of the beam interrogated the sample on the microscope stage and passed through the optics to a photomultiplier tube (RCA 4526), while the other half was focused through a neutral density wheel (0–4.0 N.D.) to a second matched photomultiplier. The photomultiplier tubes were protected from the bleaching flash by combined cutoff and interference filters. The electrical output was connected to a differential amplifier of a Model 1090 digital oscilloscope (Nicolet Instrument Corp., Madison, Wis.) for display of the kinetic record. Small absorbance charges, usually in the range 0.003 to 0.05, could be readily measured. The information could be photographed and transferred to an X-Y recorder or to magnetic tape for permanent storage.

The data shown in this report were obtained from least-squares plots of ln $(A - A_\infty)$ as a function of time. The rate constants were derived from these plots. Oscilloscope traces are shown in Figure 5.

Results

ROS Membranes and Rhodopsin. Fresh ROS membranes isolated by the experimental procedure were suspended in phosphate buffer (pH 7.0) and used the same day for kinetic studies and for comparison to rhodopsin–phospholipid recombinants. A typical absorption spectrum of solubilized ROS in 100 mM TrTAB is shown in Figure 1. For comparison, a spectrum of rhodopsin in 100 mM TrTAB after hydroxylapatite chromatography is also shown. The major differences are a decrease in the A_{278}/A_{498} ratio, presumably due to removal of bleached rhodopsin, and a decrease in the absorption minimum between the β band at 350 nm and the protein peak at 278 nm. The spectral ratio A_{278}/A_{498} for purified rhodopsin was 1.70 ± 0.1, while that of A_{400}/A_{498} was 0.19 ± 0.01. Hong and Hubbell (1973) determined the phosphate content for similar rhodopsin preparations and found 0.2 to 0.8 mol of phosphate/mol of rhodopsin. The rhodopsin yields were 60–75% based on the ROS added to the column.

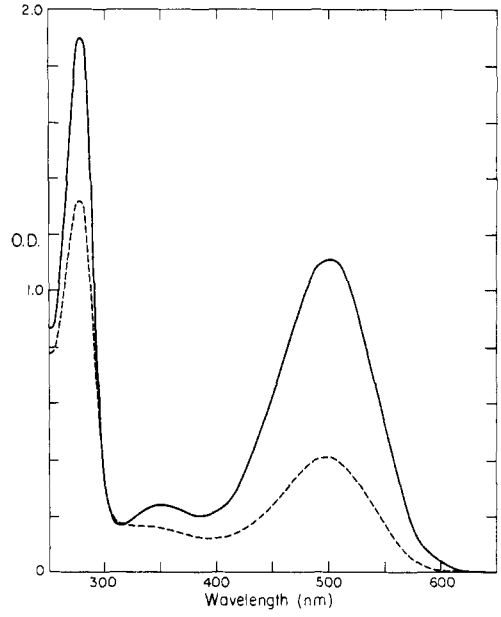

FIGURE 1: The absorption spectrum of purified rod outer segments (- - -) and purified rhodopsin (—) in 100 mM TrTAB–5 mM Hepes buffer (pH 6.6).

Recombinant Membranes. For these studies, the molar ratio of rhodopsin to phospholipid was 1:100, based on the initial rhodopsin concentration and the weight of phospholipid. In some experiments, the phospholipid content of the recombinant was evaluated by phosphate analysis and found to be consistent with the above ratio.

Recombinant formation was evaluated by electron paramagnetic resonance and electron microscopy. Usually 1% of the phospholipid had an attached nitroxide spin label, (12,3)-β-PC. It has been previously shown by Hubbell and O'Brien (1974) that the paramagnetic resonance spectrum of nitroxide-labeled phosphatidylcholines in phospholipid bilayers changes with the detergent concentration. When the rhodopsin–phospholipid is in the presence of high detergent concentrations the EPR spectrum (Figure 2) is consistent with a nearly isotropic, unrestricted motion of the spin-label. When the detergent concentration is low, the EPR spectrum (Figure 2) suggests a more rigid environment for the spin-label. The change in paramagnetic resonance line shape occurs over a narrow detergent concentration range, which closely coincides with the critical micelle concentration of the tetraalkylammonium bromides. These changes in EPR signal are believed indicative of membrane formation as the detergent is removed because of the observed correlation with increased turbidity of the material and the fact that electron micrographs show vesicle formation.

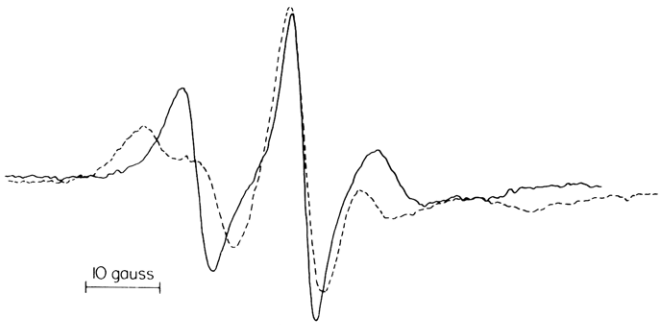

FIGURE 2: The paramagnetic resonance spectra of (12,3)-β-PC spin-label (1%) in a rhodopsin–egg PC (1:100) recombinant membrane before (- - -) and after (—) solubilization by 20 mM TrTAB–5 mM Hepes buffer (pH 6.6). Recorded at 23 °C with a Varian Associates Model E-4 spectrometer.

TABLE I: Recovery Yields of Rhodopsin during Recombinant Formation and Concentration.

Recombinant Lipid	% Recovery of Rhodopsin	
	Recomb. Formation	Concn
Asolectin	77–95	95–108
Asolectin with DTT	80	110
Egg PC	89–95	95–97
Egg PC with DTT	83	108
Egg PC–egg PE	90	106
Di-14:0-PC	95	100
Di-12:0-PC	81	106
Di-12:0-PC with DTT	83	99
Di-(18:1^c)-PC	86	103

Electron micrographs from negative stained recombinant membranes are shown in Figure 3 for rhodopsin–egg PC (1:100) and rhodopsin–asolectin (1:00). The vesicles generally appear bounded by a single wall which has a thickness appropriate for a phospholipid bilayer. A high proportion of the vesicles appear to have their interior volume isolated from the negative stain. The diameter of the vesicles ranges from 300 to 4000 Å. The population of small recombinant vesicles may be increased and the turbidity decreased by sonication at 0–5 °C under nitrogen with a sonic probe equipped with a microtip.

Recovery yields were generally 75–90% in these recombinants (Table I). The rhodopsin concentration in these preparations after dialysis was normally $1–2 \times 10^{-5}$ M. In order to obtain optical densities of 0.2 to 0.6 at 498 nm for a 1-mm path, the recombinants were concentrated by ultrafiltration with Amicon PM-30 or PM-10 membranes. The procedure was conducted in the dark in an ice bath to minimize thermal and optical bleaching over the filtration period (1–2 h). The filtrate was devoid of rhodopsin absorption, although some rhodopsin was lost on the membrane filter (2–3%). The desired increase in rhodopsin recombinant concentration was achieved with very high recovery yields (Table I). Recovery values in excess of 100% may be due to loss of some phospholipid through the PM-30 filter. The ultrafiltration technique provided a simple method of concentrating the recombinant preparation without significant loss of rhodopsin or thermal bleaching.

Low-Temperature Spectroscopy. The rhodopsin–phospholipid recombinants were examined at low temperature for evidence of spectral intermediates by the technique of Yoshizawa and Wald (1963). A sample of rhodopsin–asolectin (1:100) in 10 mM Hepes buffer–1 mM EDTA at pH 6.6 was diluted 1:1 with spectrograde glycerol to give a preparation ~4

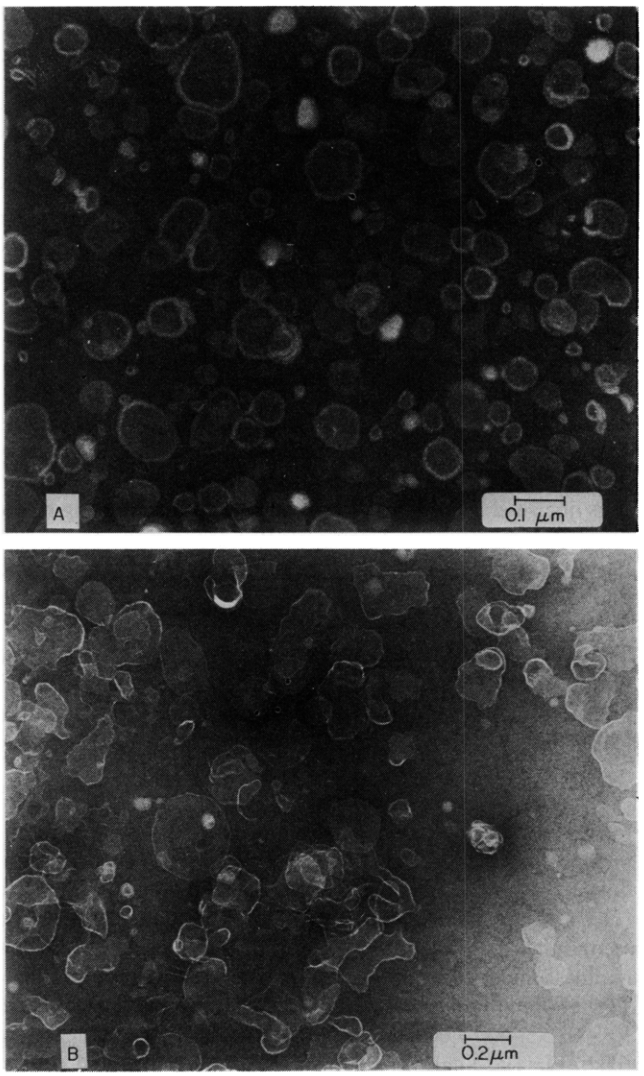

FIGURE 3: Electron micrographs of negatively stained rhodopsin–phospholipid recombinant membranes: (A) egg PC; (B) asolectin.

$\times 10^{-5}$ M in rhodopsin. The recombinant had been sonicated to reduce the vesicle size and improve the optical transmission. The sample was examined at low temperatures in a thermostated liquid nitrogen optical Dewar flask which was suitable for transmission spectra at defined temperatures; however, the spectra showed considerable scatter at shorter wavelengths due to the vesicles and cracks in the water–glycerol glass. These spectra were corrected by determination of the spectra of water–glycerol solutions of phospholipid vesicles at the appropriate temperatures.

On cooling to −196 °C, the absorption maximum shifts from 497 to 502 nm with a corresponding increase in the optical extinction by 25% (Figure 4, curve 2). This is accompanied by a sharpening of the chromophore band as expected at these temperatures. The sample was exposed with several flashes of a Honeywell Strobonar Model 782 through a 440-nm cutoff filter and an infrared filter to prevent heating the sample. The flashing at −196 °C causes part of the main band to shift to the red with a new maximum at 512 nm, with a higher optical density all along the red limb on the curve (Figure 4, curve 3). This new spectrum is stable at −196 °C in the dark and may be photoreversed to the original by flash excitation through a broad band interference filter (550–650 nm). Upon warming the red-shifted sample without additional illumination, new

TABLE II: Kinetic Data for Meta I Decay of Rhodopsin Membranes.

Temp (°C)	ROS Membranes	k_1 (s^{-1}) at pH 7.0 for Rhodopsin–Phospholipid Recombinant: Asolectin	Egg PC	Egg PC–Egg PE	Di-14:0-PC	Di-12:0-PC	Di-(18:1)-PC
13	19[a]	15					
16	33						
18	41[a]	30	<0.3	<0.3	<0.3	<0.3	35
22	75	67	77				69
24	111[a]	96	106	127			96
28	310[a]	184	173	180	<2	<2	177
31		290	260				300

[a] Rapp (1970).

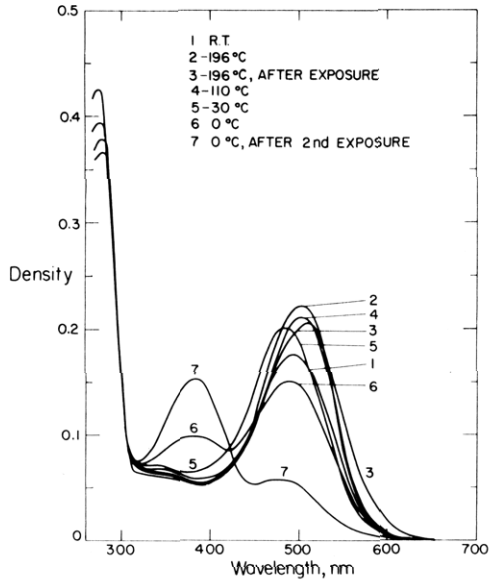

FIGURE 4: Absorption spectra of rhodopsin–asolectin recombinant membranes in glycerol–water (1:1) in an optical Dewar flask, 1-mm cell, at selected temperatures. The tracings are corrected for light scatter (see Experimental Section).

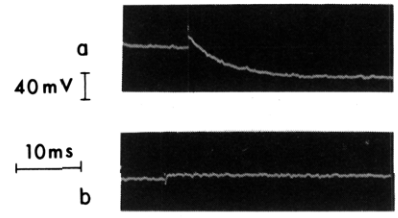

FIGURE 5: Oscilloscope record of 470-nm absorption of recombinant membranes upon flash excitation at 28 °C, pH 7.0: (a) rhodopsin–asolectin recombinant; (b) rhodopsin–di-12:0-PC recombinant; amplifier band pass, 0.5 MHz.

spectral shifts were observed. The first of these is recorded at −110 °C and shows a shift of the maximum to 500 nm (Figure 4, curve 4). This corresponds to a change from batho- to lumirhodopsin. A similar absorption is seen at −90 °C. No spectra were measured between −90 and −30 °C because of the opalescence of the glycerol–water glass at these temperatures (Yoshizawa and Wald, 1966). At −30 °C, the absorption maximum is at 480 nm (Figure 4, curve 5), which has been assigned to metarhodopsin I for rhodopsin in digitonin (Yoshizawa and Wald, 1963). Continued warming yields a decrease in the 480-nm band and an increase in absorption at 380 nm, meta II. The spectrum at 0 °C (Figure 4, curve 6) reflects this partial change and also shows some residual rhodopsin absorption as a long-wavelength shoulder of the 480-nm absorption band. This rhodopsin remains from the initial exposure, which produced a mixture of rhodopsin and bathorhodopsin. Subsequent warming from −196 °C allows bathorhodopsin to decay, but leaves rhodopsin unbleached. A second exposure of the sample at 0 °C (Figure 4, curve 7) bleaches the residual rhodopsin and yields a spectrum that has a band at 380 nm, meta II, and at 480 nm, meta I. There may be an isosbestic point near 420 nm for these samples, but the correction technique is not sufficiently precise to say with certainty. Matthews et al. (1963) report an isosbestic point at 417 nm for meta I–meta II in digitonin at 3.2 °C.

A similar series of spectral changes which are associated with the same previously identified rhodopsin intermediates was observed for water–glycerol solutions of rhodopsin–egg PC (1:100) recombinant and for rhodopsin in 100 mM TrTAB.

Kinetics. Typical data for a rhodopsin–asolectin recombinant are shown in Figure 5a. Excitation by xenon flash initiates a transient increase in 470-nm absorbance followed by an apparent exponential decrease. These changes are associated with the formation and decay of metarhodopsin I. The decay at 470 nm is paralleled by an increase in absorbance at 380 nm, which is ascribed to metarhodopsin II formation. The experimental data have been plotted as ln $(A - A_\infty)$ vs. time, where A_∞ is taken directly at 8 to 10 lifetimes. A least-squares slope of the data was calculated and the rate constant obtained. The plot is linear for at least three lifetimes. The correlation coefficients varied from 0.994 to 0.999 for these data, and inspection of the graphs showed only random variations from the least-squares slope. The error in each derived rate constant is less than ±2%. The linearity of the data plot indicates that the rhodopsin–asolectin recombinant has a single first-order rate for the disappearance of meta I. Similar kinetic data were observed with the other recombinants that showed a natural relaxation from meta I (Table II).

Reproducibility of the kinetic data is good. Repeated experiments with the same preparation of rhodopsin–asolectin (pH 7.0) over several days gave values for k_1 at 28 °C that varied from 178 to 187 s^{-1}. A second preparation of the same recombinant had a k_1 (28 °C) of 192 s^{-1}.

Freshly prepared ROS membranes in phosphate buffer (pH 7.0) were examined by flash photolysis for comparison with the recombinant preparations. The data were consistent with a single first-order process for meta I decay and are given in Table II. The rate constants are similar to those of Rapp (1970)

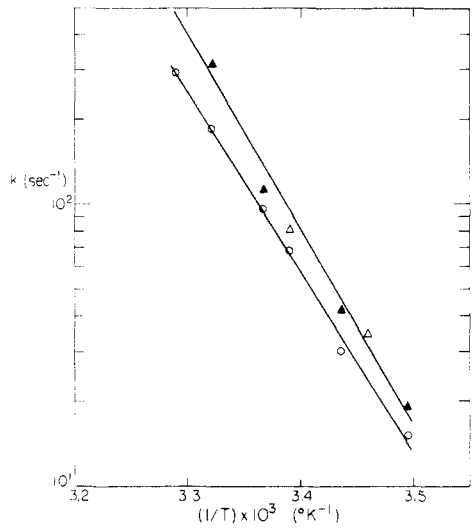

FIGURE 6: Arrhenius plot of kinetic data for the decay of metarhodopsin I (pH 7.0): (△) ROS membranes; (▲) data of Rapp (1970); (◎) rhodopsin–asolectin recombinant.

and Applebury et al. (1974). An Arrhenius plot of kinetic data at several temperatures is shown in Figure 6. The inverse temperature dependence observed is in good agreement with the results Applebury et al. (1974) obtained for meta II formation in ROS and with the results Rapp (1970) obtained for meta I decay. The enthalpy of activation is 29 kcal/mol, and the entropy of activation is 50 eu for rhodopsin–asolectin recombinant.

Several rhodopsin–phospholipid recombinants were examined to evaluate the effect of phospholipid on the meta I to meta II relaxation (Table II). The recombinants fall roughly into two categories: (1) those which show the meta I to meta II transition at a rate comparable to that of ROS membranes; and (2) those which show evidence of rapid meta I formation, but where further decay is retarded compared to ROS membranes.

Two rhodopsin recombinants with defined saturated phospholipids, 1,2-didodecanoyl-PC (di-12:0-PC) and 1,2-ditetradecanoyl-PC (di-14:0-PC), were examined. The phase transitions, T_m, were previously determined for fully hydrated bilayers of saturated phospholipids by Chen and Hubbell (1973). The transition for di-12:0-PC is 3 °C and that of di-14:0-PC is 22 °C. The inclusion of rhodopsin (1:100) in these phospholipid bilayers increases the characteristic temperature, T_c, a few degrees and broadens the transition. These data imply that the hydrocarbon interiors of the recombinant bilayers are in a fluid state for the temperature range examined for rhodopsin–di-12:0-PC and at 28 °C for rhodopsin–di-14:0-PC. Surprisingly, the kinetic behavior of these recombinants was not affected by temperature from 18 to 40 °C (Table II). Both preparations show an increase in 470-nm absorbance upon excitation; however, the meta I intermediate does not appreciably decay over 100 ms. (See Figure 5b for the rhodopsin–di-12:0-PC recombinant.) Examination of these recombinants over several minutes in a spectrophotometer shows a decrease in 470-nm absorbance with concurrent increase in 380-nm absorbance. Although it is not surprising that rhodopsin would be frozen at meta I in phospholipid bilayers below the bilayer characteristic temperature, it is unpredicted that the meta I decay would be hindered above the characteristic temperature.

Similar results were obtained with rhodopsin–egg PC recombinants at 18 °C. The meta I intermediate does not decay over several milliseconds. At temperatures above 20 °C, the increase in 470-nm absorbance was followed by a decay over a few milliseconds. The kinetics are first order and the rate constants are comparable to those of ROS. The limiting transition temperature for hydrated egg PC is −15 to −7 °C (Chapman, 1975).

Recombinants prepared from rhodopsin with asolectin, a natural mixture of phospholipids from soybean (40% PC, 29% PE, 14% monophosphoinositide, 4% PS, with small amounts of lysophospholipids[2]), showed immediate meta I formation upon excitation followed by an exponential decrease in 470-nm absorbance at temperatures from 13 to 31 °C (Table II). The magnitude of $(A - A_\infty)$ was generally greater for the asolectin recombinants than the egg PC recombinants under similar experimental conditions. The change in absorbance at 470 nm (28 °C) was typically 0.035 to 0.04 for ROS and asolectin preparations and 0.015 to 0.02 for egg PC recombinants, which corresponds to 5 to 15% rhodopsin bleaching. These conditions were utilized to minimize photogeneration effects (Williams and Breil, 1968). Although these data are only semiquantitative, it does suggest that more of the rhodopsin is photochemically active in asolectin recombinants than in egg PC at 28 °C.

Both egg PC and asolectin recombinants were examined for the effect of pH. Recombinants were prepared in 10 mM Hepes buffer at pH 6.6 as well as 7.0, and the kinetics of meta I decay determined. The rate constant was approximately twice as great at pH 6.6 as at 7.0. At 28 °C the k_1 values for rhodopsin–asolectin recombinants were 184 and 432 s^{-1} at pH 7.0 and 6.6, respectively. Similar values were obtained with the respective egg PC recombinants. At 18 °C the k_1 values for the asolectin preparations were 30 and 70 s^{-1} at pH 7.0 and 6.6, respectively, whereas this pH change did not affect the very slow meta I decay observed for rhodopsin–egg PC recombinants at 18 °C. This is consistent with the observation of Matthews et al. (1963) that the meta I–meta II equilibrium is shifted in favor of meta II at lower pH. Stieve et al. (1973) reported a similar effect of pH on the rate of the meta I–meta II transition in sonicated ROS. These comparisons lend support to the hypothesis that rhodopsin in these phospholipid bilayers is functioning photochemically in a manner comparable to rhodopsin in ROS membranes.

Some recombinants were prepared in the presence of 1 mM dithiothreitol, as well as 10 mM Hepes and 1 mM EDTA. In the cases examined, asolectin (pH 7.0), egg PC (pH 6.6), and di-12:0-PC (pH 7.0), the presence of dithiothreitol did not alter the rate of decrease of 470-nm absorbance. It did appear to decrease the magnitude of the signal by 15–25%. This may be due to an optical effect or perhaps some of the rhodopsin is rendered photochemically inactive by the presence of the sulfhydryl reagent.

Matthews et al. (1963) report that neutral salts favor meta II in the equilibrium between the metarhodopsins. An initial experiment on the effect of salts, which we hope to report on more fully at a later time, shows that added salts do increase the rate of meta I decay. A rhodopsin–egg PC recombinant was examined in 10 mM Hepes–1 mM EDTA (pH 6.6), 28 °C, with and without 115 mM NaCl and 2.5 mM KCl. In the presence of salts, the k_1 is 630 s^{-1}, half again as large as the value observed in their absence, 460 s^{-1}.

The positive results with asolectin suggest that either the

[2] Analysis was kindly provided by Professor G. V. Marinetti, University of Rochester, Rochester, N.Y.

TABLE III: Fatty Acid Composition of Phospholipids Used in Recombinants.

Material	T_c (°C)	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:4	22:5	22:6
ROS, PC, bovine[a]				32.3	2.3	17.7	10.7	1.8	4.3	1.1	2.0	20.6
Didodecanoyl-PC	0	100										
Ditetradecanoyl-PC	22		100									
Dioleoyl-PC	−11						100					
Egg PC[b]	−15/7			32.0	1.0	16.0	30.0	17.0	3.0			
Asolectin phospholipids[c]				22.0		5.4	7.0	56.0			2.9	
Soy PE[d]				50.0		4.0	5.0	38.0				
ROS, PE, bovine[a]				10.3	0.7	21.9	6.1	2.2	5.0	3.4	5.7	31.7

[a] Anderson and Maude (1970). [b] Ansell and Hawthorne (1964). [c] Values for total soy phospholipids were kindly provided by Professor G. V. Marinetti, University of Rochester. [d] Stearns and Morton (1973).

presence of PE and/or phospholipid unsaturation is important for the functional photochemical behavior of rhodopsin–phospholipid recombinants. Two experiments favor the importance of unsaturation and do not suggest any special requirement for PE. In one, a recombinant was prepared from 1 part of rhodopsin and equal parts (50) of egg PC and egg PE. Its kinetic behavior was similar to that of egg PC recombinants. Decay of meta I was blocked at 18 °C, but at 28 °C a decay of the 470-nm absorbance was observed with a rate of 180 s^{-1}. The addition of a natural PE does not appear to alter the rhodopsin behavior.

A recombinant prepared from rhodopsin and dioleoyl-PC [di-(18:1[c])-PC] showed decay of meta I at rates comparable to that observed for rhodopsin with asolectin (Table II). Thus, functional photochemical behavior can be realized in rhodopsin recombinant membranes prepared from pure defined phosphatidylcholines with sufficient fatty acid unsaturation.

Discussion

The technique of membrane reconstitution from purified, characterized components is a powerful method of studying structure and function of biological systems (Razin, 1972). It is especially attractive in the rhodopsin membrane system, since 80–90% of the protein content in ROS is rhodopsin, and phospholipids comprise 85–90% of the ROS lipids (Daemen, 1973). Recombinant membranes prepared from the major components of ROS allow examination of pertinent protein–phospholipid interactions. Prime considerations in reconstitution experiments are the purity of the components, the nature of the detergent, and the functionality of the recombinant.

It has been previously demonstrated that rhodopsin may be purified and delipidated in DTAB or its C_{13} and C_{14} homologues (Hong and Hubbell, 1973), in DDAO (Applebury et al., 1974), and in octyl β-D-glucoside (Stubbs et al., 1976). Successful reconstitution requires that the detergent not only be effective at solubilizing rhodopsin and lipids, but that it must be readily removed by dialysis, it must not irreversibly denature rhodopsin, and it should be transparent in the ultraviolet region of the spectrum to allow optical characterization of the rhodopsin. DTAB, TrTAB, DDAO, and octyl β-D-glucoside satisfy these requirements.

Rhodopsin has been successfully incorporated into phospholipid bilayers by dialysis to remove DTAB (Hong and Hubbell, 1972) or DDAO (Applebury et al., 1974). Since the in vivo functionality of rhodopsin is not established, other standards for native behavior were utilized. The functionality of their preparations was examined by chemical regenerability and by the kinetics of metarhodopsin II formation, respectively.

The information available from spectrophotometric and kinetic methods comprises a generous portion of our knowledge about rhodopsin in detergents and membranes. The rhodopsin spectral intermediates and the rates of their formation and decay have been established in vitro and in vivo. Upon absorption of light, rhodopsin is excited with the production of bathorhodopsin (545 nm), which decays thermally to lumirhodopsin (497 nm), metarhodopsin I (478 nm), and metarhodopsin II (380 nm) (Yoshizawa, 1972). The first intermediate may be observed spectrophotometrically after photolysis at −196 °C, and the others appear upon warming (Yoshizawa and Wald, 1963). In our experiments, we found evidence for this sequence of intermediates for rhodopsin recombinant preparations with asolectin or egg PC as well as rhodopsin in TrTAB. Since the rhodopsin was not completely bleached by the flash lamp, the post-flash absorption spectra represent a mixture of rhodopsin and the intermediate(s) stable at the particular temperature. The presence of unbleached rhodopsin is reflected in the bathochromic shift of only 10 nm for the partially bleached sample at −196 °C, whereas total rhodopsin to bathorhodopsin conversion should yield a 40-nm shift (Yoshizawa, 1972). The residual rhodopsin is also apparent in the long-wavelength shoulder of the 480-nm band at 0 °C. In spite of this, the spectra clearly demonstrate that the known spectral intermediates occur in the rhodopsin recombinants and that bathorhodopsin may be photoreversed to rhodopsin in the recombinant membranes examined.

These recombinants were examined in a 1:1 water–glycerol mixture. To demonstrate that the phospholipid bilayer was not significantly altered by the presence of the glycerol, the paramagnetic signal from an incorporated spin-labeled PC was determined. Large order parameters, 0.7, both for rhodopsin–asolectin in water and water–glycerol (1:1), show that the part of the phospholipid bilayer that is sampled by the spin-label is unperturbed by the presence of the glycerol. The glycerol also did not appear to significantly affect the rhodopsin since kinetics of meta I decay for rhodopsin–asolectin recombinant at 22 °C, buffered at pH 6.6 in water and in water–glycerol (1:1), were both first order with rate constants of 128 and 96 s^{-1}, respectively.

Previous kinetic studies on rhodopsin in digitonin have found evidence of multiple first-order rates for the meta I to meta II transition (Abrahamson and Wiesenfeld, 1972). First-order conversions of meta I to meta II were found for in vivo systems (Hagins, 1957, 1972) as well as for fresh, sonicated ROS membranes (Rapp, 1970; Applebury et al., 1974). Applebury et al. (1974) have suggested that the serial first-order kinetics in the presence of digitonin may be due to a nonuniform solubilization of the ROS membrane. Dissolution of ROS with

stronger detergents such as hexadecyltrimethylammonium bromide yields a preparation with a rate of meta II formation that is ten times greater than ROS in digitonin (Williams and Breil, 1968). Purified, delipidated rhodopsin in DDAO shows a first-order meta II formation rate that is 100 times that of sonicated ROS (Applebury et al., 1974). The presence of lipids and detergents with rhodopsin results in multiple first-order kinetics (Williams et al., 1974), whereas delipidated rhodopsin in detergent yields first-order kinetics that are significantly greater than those of sonicated ROS membranes. Fresh ROS membranes display kinetics for the transition for meta I to meta II that are comparable (1.5×10^{-3} s^{-1} at 37 °C; Rapp, 1970) to the in vivo values reported for rat, 3×10^{3} s^{-1}, and rabbit, 0.9×10^{3} s^{-1}, at 37 °C (Hagins, 1972). For our studies, we utilized fresh ROS membranes as a standard of comparison for the rhodopsin recombinants.

Rhodopsin recombinants prepared from saturated phosphatidylcholines have a retarded rate of conversion from meta I to meta II, even above the characteristic temperature of the recombinant where the phospholipid hydrocarbon chains are fluid. In contrast, rhodopsin–dioleoyl-PC recombinant showed meta I decay comparable to ROS, which is indicative of photochemical functionality. Recombinants prepared from egg PC and egg PC–egg PE, as well as asolectin and a mixture of soya PC and PE, showed first-order decay of metarhodopsin I. However, at temperatures below 20 °C, meta I decay was inhibited in the rhodopsin recombinants containing egg PC. The kinetic results with different lipids indicate that there is not a specific requirement for phosphatidylethanolamine for photochemical functionality. Hong and Hubbell (1973) concluded that specific phospholipid polar head groups were not required for chemical regeneration.

In our experiments conducted to date, photochemical functionality was only observed above the characteristic temperature of the phospholipids. It is not unexpected that fluidity in the hydrocarbon interior of the bilayer is necessary for rhodopsin functionality. As Daemen (1973) points out in his review, ROS membranes have a high phospholipid concentration compared to other characterized membranes. A high lipid-to-protein content appears to favor a lamellar arrangement in biomembranes. The cholesterol content is low in ROS, and there is a large amount of unsaturation in the fatty acids of the phospholipids (Table III). These factors permit high fluidity at physiological temperatures as demonstrated by the rotational (Brown, 1972; Cone, 1972) and translational (Poo and Cone, 1973) motions of rhodopsin in ROS. ROS membranes are highly organized yet very dynamic membranes.

Fluidity of the phospholipid bilayer is necessary for recombinant functionality, but it may not be a sufficient condition. In contrast to the chemical regeneration experiments (Hong and Hubbell, 1973), where no particular fatty acid chain was required for successful regeneration (which we have also observed), unsaturation in the hydrocarbon chains appears to be necessary for a meta I to meta II transition with kinetics comparable to ROS. The results suggest that functional behavior of the rhodopsin–phospholipid recombinant is related to the degree of unsaturation of the phospholipid. Recombinants from saturated phospholipids were nonfunctional over the entire temperature range. Egg PC recombinants had a smaller temperature range for functional kinetics than soya PC recombinants. Soya phospholipids have a higher percentage of unsaturated hydrocarbon chains (Table III) and a greater fraction of polyunsaturated residues than phospholipids derived from egg.

The requirement for unsaturated lipids for functional recombinant behavior has been observed for preparations of the calcium ATPase of sarcoplasmic reticulum (Warren et al., 1974a,b, 1975). Reconstituted ATPase with dioleoyl-PC was functional, yet with increasing amounts of the saturated phospholipids (di-14:0-PC or di-16:0-PC) the recombinant was rendered progressively inactive. Functional activity of the ATPase requires a first shell or annulus of unsaturated phospholipid to surround the protein. Penetration of the annulus by saturated phospholipids or cholesterol decreases the ATPase activity (Warren et al., 1975).

The requirement for phospholipid unsaturation in rhodopsin–phospholipid recombinants may indicate a minimum phospholipid bilayer fluidity is necessary to allow the protein to undergo the conformational changes associated with the meta I–meta II transition. Alternatively, the lack of photochemical functionality in the rhodopsin–saturated phospholipid recombinants could be due to rhodopsin aggregation in the bilayers. The effect of unsaturation may relate to the general requirements of solvation of the protein. Chen and Hubbell (1973) observed evidence for protein aggregation in their freeze fractive studies of rhodopsin recombinants with di-12:0-PC and di-14:0-PC among others.

In conclusion, photochemical behavior has been observed in selected rhodopsin–phospholipid recombinant bilayers that is comparable to that of rhodopsin in ROS membranes. A specific phospholipid head group is not required, whereas unsaturation in the phospholipid hydrocarbon chains in addition to membrane fluidity is a necessary condition for photochemical functionality of the recombinants.

Acknowledgment

The authors wish to thank Lee Brady and Peter Rawleigh, Eastman Kodak Research Laboratories, for the electron microscopy. We also wish to thank Professors Wayne L. Hubbell and Richard A. Cone for their advice, and for their generous hospitality to D. F. O'Brien during his sabbatical.

References

Abrahamson, E. W., and Wiesenfeld, J. R. (1972), *Handb. Sens. Physiol. 7 (Part 1)*, 69.

Anderson, R. E., and Maude, M. B. (1970), *Biochemistry 9*, 3624.

Ansell, G. B., and Hawthorne, J. N. (1964), in Phospholipids, Amsterdam, Elsevier, p 422.

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

Blaurock, A. E., and Wilkins, M. H. F. (1972), *Nature (London) 236*, 313.

Blaise, J. K. (1972), *Biophys. J. 12*, 191.

Brown, P. K. (1972), *Nature (London), New Biol. 236*, 35.

Chabre, M. (1975), *Biochim. Biophys. Acta 382*, 322.

Chapman, D. (1975), *Q. Rev. Biophys. 8*, 2.

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

Cone, R. A. (1972), *Nature (London), New Biol. 236*, 39.

Cone, R. A., and Cobbs, W. H. (1969), *Nature (London) 221*, 820.

Daemen, F. J. M. (1973), *Biochim. Biophys. Acta 300*, 255.

Devaux, P., and McConnell, H. M. (1972), *J. Am. Chem. Soc. 94*, 4475.

Hagins, W. A. (1957), Thesis, University of Cambridge, Cambridge, G.B.

Hagins, W. A. (1972), *Annu. Rev. Biophys. Bioeng. 1*, 131.

Hong, K., and Hubbell, W. L. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2617.
Hong, K., and Hubbell, W. L. (1973), *Biochemistry 12,* 4517.
Hubbell, W. L. (1975), *Acc. Chem. Res. 8,* 85.
Hubbell, W. L., and McConnell, H. M. (1971), *J. Am. Chem. Soc. 93,* 314.
Hubbell, W. L., and O'Brien, D. F. (1974), unpublished results.
Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G. (1963), *J. Gen. Physiol. 47,* 215.
Poo, M. M., and Cone, R. A. (1973), *Exp. Eye Res. 17,* 503.
Porter, G., and Strauss, G. (1966), *Proc. R. Soc. London, Ser. A 295,* 1.
Rapp, J. (1970), Ph.D. Thesis, Case Western Reserve University, Cleveland, Ohio.
Razin, S. (1972), *Biochim. Biophys. Acta 265,* 241.
Seufert, W. D. (1970), *Biophysik 7,* 60.
Stearns, E. M., and Morton, W. T. (1973), *Lipids 8,* 668.
Stieve, H., Wilms, M., and Nöll, G. (1973), *Z. Naturforsch. C 28,* 600.
Stubbs, G. W., Smith, H. G., and Litman, B. J. (1976), *Biochim. Biophys. Acta 425,* 46.
Tokunaga, F., Kawamura, S., and Yoshizawa, T. (1976), *Vision Res. 16,* 633.
Wald, G. (1968a), *Nature (London) 219,* 800.
Wald, G. (1968b), *Science 162,* 230.
Warren, G. B., Housley, M. D., Metcalfe, J. C., and Birdsall, N. J. M. (1975), *Nature (London) 225,* 684.
Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974a), *Proc. Natl. Acad. Sci. U.S.A. 71,* 622.
Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974b), *Biochemistry 13,* 52.
Williams, T. P., Baker, B. N., and McDowell, J. H. (1974), *Exp. Eye Res. 18,* 69.
Williams, T. P., and Breil, S. J. (1968), *Vision Res. 8,* 777.
Yoshikami, S., and Hagins, W. A. (1971), *Biophys. J. 11,* 47.
Yoshizawa, T. (1972), *Handb. Sens. Physiol. 7 (Part 1),* 146.
Yoshizawa, T., and Wald G. (1963), *Nature (London) 197,* 1279.
Yoshizawa, T., and Wald G. (1966), *Nature (London) 212,* 483.

Effect of Lysolecithin on the Structure and Permeability of Lecithin Bilayer Vesicles†

Yunko Lee and Sunney I. Chan*

ABSTRACT: In order to elucidate the role of lysolecithin in membranes, we have examined the effect of lysolecithin on the structure and permeability of lecithin bilayer membranes. Small L-α-dimyristoyllecithin (DML) vesicles with myristoyllysolecithin (MLL) incorporated as well as small L-α-dipalmitoyllecithin (DPL) vesicles with palmitoyllysolecithin (PLL) were studied by nuclear magnetic resonance (NMR) methods at temperatures both above and below the α-gel ⇌ liquid crystalline phase transition temperature (T_c) and as a function of the concentration of the incorporated lysolecithin. Europium(III) ion was used as a probe to measure the permeability of the vesicular bilayer membrane. At temperatures below T_c, these vesicles were found to be extremely permeable to europium(III) ions. The ion translocation was found to be too fast to be measured by the NMR method under these conditions. However, above the phase transition temperature the ionic permeability decreases to a rate which could be conveniently monitored, and the permeability was shown to increase with temperature and lysolecithin concentration. Analysis of the lysolecithin concentration dependence suggests the formation of ion channels within the lipid bilayer involving four lysolecithin molecules. The data below T_c suggest a phase separation below the phase transition temperature of the host lipid, leading to the formation of patches of lysolecithin molecules within the lecithin matrix. These lysolecithin clusters are presumably long-lived under these conditions and are sufficiently structurally perturbed or disordered to serve as channels for rapid ion permeation.

Lysolecithin is an important metabolite, produced by many cells and widely distributed in a variety of tissues. For example, chromaffin granules from the adrenal medulla contain a fairly high concentration of lysolecithin in their membranes (Blaschko et al., 1967; Kirshner, 1974). The functional role of lysolecithin in these membranes is not fully understood. Since it has been proposed that lysolecithin is capable of promoting fusion between cells (Guttler and Clausen, 1969; Poole et al., 1970; Croce et al., 1971), the function of lysolecithin in these membranes may be related to its fusion inducing property. It has been shown that chromaffin granules from the adrenal medulla undergo fusion among themselves (Edwards et al., 1974), and it has been proposed that these granules fuse with the chromaffin plasma membrane during the process of catecholamine release (Douglas, 1966; Diner, 1967; Winkler, 1971; Smith and Van Orden, 1973).

In order to elucidate the role of lysolecithin in these membranes, we have investigated the effect of lysolecithin on the structure and ionic permeability of lecithin bilayer membranes.

† Contribution No. 5402 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125. *Received September 14, 1976.* This work was supported by U.S. Public Health Service Grant No. GM-22432 from the National Institute of General Medical Sciences.