Hubbell, W. L., & McConnell, H. M. (1971) *J. Am. Chem. Soc. 93*, 314–326.
Ito, T., Ohnishi, S., Ishinaga, M., & Kito, M. (1975) *Biochemistry 14*, 3064–3069.
Jost, P. C., & Griffith, O. H. (1980) *Ann. N.Y. Acad. Sci. 348*, 391–407.
Mabrey, S., & Sturtevant, J. M. (1978) *Methods Membr. Biol. 9*, 237–274.
Mason, J. T., Broccoli, A. V., & Huang, C. (1981a) *Anal. Biochem. 113*, 96–101.
Mason, J. T., Huang, C., & Biltonen, R. L. (1981b) *Biochemistry 20*, 6086–6092.
Privalov, P. L., Plotnikov, V. V., & Filimonov, V. V. (1975) *J. Chem. Thermodyn. 7*, 41–47.
Radda, G. K. (1975) *Methods Membr. Biol. 4*, 97–188.
Scandella, C. J., Devaux, P., & McConnell, H. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 2056–2060.
Seelig, J. (1970) *J. Am. Chem. Soc. 92*, 3881–3887.
Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353–418.
Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1649–1653.
Sturtevant, J. M., Ho, C., & Reimann, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2239–2243.

Effect of Hydrogen Ion Concentration on Rhodopsin–Lipid Interactions†

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ABSTRACT: This study concerns the role titratable chemical groups play in rhodopsin–lipid interactions. Tempo partitioning techniques are used to construct DMPC–rhodopsin partial binary phase diagrams. Such diagrams are constructed at pH 7.0, where rhodopsin has a net negative charge, and at pH 5.0, where rhodopsin is virtually isoelectric. It is found that at pH 5.0, increases in rhodopsin content induce a depression in temperature of the solidus curve. At pH 7.0, the solidus curve is not a strong function of temperature. Additional studies, involving nitroxide-labeled palmitic acid, methyl palmitate, and lecithins, indicate that the pH-dependent changes in the phase behavior of DMPC–rhodopsin systems are due to the titration of rhodopsin's carboxylic acid groups.

A great deal of knowledge has emerged over the last decade concerning the motional state of membrane components in rhodopsin–phospholipid systems. Model systems, involving lecithin–rhodopsin recombinant membranes, have been particularly useful in this regard and the results from studies of such systems provided the background for this work.

Hong et al. (1975) have found that rhodopsin incorporation into lecithin bilayers resulted in an increase in the average viscosity of the bilayer as sensed by several nitroxide-labeled lecithin probes.

Fischer & Levy (1981) reported experiments involving DMPC[1]–rhodopsin recombinants that demonstrated several new features concerning the effect of rhodopsin on the motional state of lipids. First, proton spin–lattice (T_1) relaxation measurements involving DMPC–rhodopsin recombinants demonstrated that each rhodopsin molecule immobilized approximately 50 choline quaternary amine groups. Second, experiments involving proton T_1 studies of phospholipid chain terminal methyl groups as well as spin-labeled fatty acids with the nitroxide group placed in the center of the DMPC bilayer indicated that rhodopsin had a smaller effect on molecular motion in the center of the bilayer than on that in the outer half of the hydrophobic region of the bilayer.

Experiments utilizing rhodopsin–lecithin systems in which the rhodopsin has been covalently labeled with various ESR probes have also been performed (Davoust et al., 1980). These experiments demonstrated both a restriction of probe mobility in fluid membranes and the existence of a highly immobilized spectral component that was induced by decreases in temperature and/or lipid content.

Kusumi et al. (1980) have also performed experiments involving spin-labels convalently bound to rhodopsin in DMPC membranes. Utilizing saturation transfer techniques, they found that rhodopsin experienced relatively free rotational motion in the fluid phase. This rotational motion became more restricted as the temperature was lowered below the gel-to-liquid-crystalline phase transition temperature.

The above-mentioned experiments involving rhodopsin have measured the effect of a protein on various types of molecular motion within the membrane. In contrast, relatively few studies have addressed questions concerning the specific nature of protein–lipid interactions. For example, what chemical groups are involved in the rhodopsin–phospholipid interactions that produce immobilization? The ESR studies reported in this article address such questions. We have systematically examined the effect of pH on the phase behavior of rhodopsin–DMPC systems. This was accomplished by using Tempo partitioning techniques (Shimshick & McConnell, 1973) to identify fluidus and solidus curves. We have also examined the effects of pH on the behavior of several spin-labeled amphiphiles in DMPC and DMPC–rhodopsin membranes. The results of these two kinds of studies begin to define the role that titratable groups play in rhodopsin–lipid interactions.

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[1] Abbreviations: DMPC, L-α-dimyristylphosphatidylcholine; Tempo, 2,2,6,6-tetramethylpiperidinyl-1-oxy; PC, phosphatidylcholine; FA, fatty acid; DTAB, dodecyltrimethylammonium bromide; ME, methyl ester.

0006-2960/82/0421-5101$01.25/0

Materials and Methods

The following procedures, and those for the analytical characterization of the resulting recombinants, have been discussed in more detail elsewhere (Fischer & Levy, 1981). Rod outer segment (ROS) isolation from cattle retinas (Hormel Co.) utilized the procedure of Papermaster & Dreyer (1974). The procedure of Van Breugel & Daemen (1977), involving conconavalin A affinity chromatography, was used to prepare delipidated rhodopsin. This procedure typically yielded delipidated rhodopsin suspensions with A_{280}:A_{500} ratios between 2.1 and 2.5. The rhodopsin concentration of the delipidated rhodopsin suspension was obtained by measuring the absorbance at 500 nm, a molar extinction coefficient of 40 000, and a molecular weight of 36 500. Recombination of rhodopsin with DMPC utilized the procedure of Hong & Hubbell (1973). This involved adding a defined amount of finely powdered DMPC (Sigma) to a aliquot of the delipidated rhodopsin suspension. The DMPC:rhodopsin ratios reported under Results were based on the ratios of DMPC and rhodopsin that were mixed before dialysis. Recombination of rhodopsin with DMPC utilized the procedure of Hong & Hubbell (1973). Sample pH was adjusted during the dialysis of the rhodopsin–DTAB–DMPC suspensions. Over a 4-day period, DTAB-solubilized samples were dialyzed first against one change of 100 mM buffer and then against three changes of 10 mM buffer. Citrate buffer was used for pH 5.0 samples, while phosphate buffer was used for pH 7.0 samples. Recombinant samples were harvested by centrifugation followed by lyophilization to obtain a freeze-dried sample that contained buffer salts. Recombinant samples maintained a "native" unbleached absorption spectrum. Pure DMPC samples were treated with DTAB and dialyzed in exactly the same manner as recombinant samples. Protein (Lowry & Rosebrough, 1951) and phosphate (Bartlett, 1959) analysis of recombinant samples yielded DMPC:rhodopsin ratios that were within experimental error of the ratios expected on the basis of the predialysis mixing of DMPC and rhodopsin.

DMPC and DMPC–rhodopsin samples were examined with light and electron microscopes. Negative staining techniques were used in the electron microscopic examination (Sheetz & Chan, 1972). The samples contained mainly large unilamellar vesicles ranging from 700 to 5000 Å in diameter, as well as a small amount of multilamellar structures. The heterogeneity of the rhodopsin content of the individual particles was analyzed by performing ultracentrifugation experiments on a linear density gradient. The density of the membranes was found to be an increasing function of the rhodopsin content. Samples gave a single, sharp band with a thickness that indicated no greater than a 5% spread of rhodopsin content about the mean rhodopsin concentration. The "sidedness" of the recombinant membranes utilized in this study was not examined.

For Tempo measurements, freeze-dried samples were vortexed with a 1 mM Tempo solution prepared from distilled, deionized water or with a 1 mM Tempo solution that contained the appropriate concentrations of NaCl. Samples containing (10,3)ME, (10,3)FA, or (10,3)PC (cf. Figure 1) were prepared in a similar manner by vortexing freeze-dried samples with deionized, distilled water. The resulting suspensions were then transferred to a second vial onto which the spin-labeled amphiphiles had been evaporated from chloroform stock solutions with a stream of dry N_2, followed by placement under a high vacuum. After being vortexed, samples picked up about half of the spin-label, and the final spin-label concentration was 0.2 mM. This spin-label concentration was well below the concentration necessary for spin-exchange effects, as determined by titrating DMPC with increasing amounts of each spin-labeled amphiphile used in this experiment. The total DMPC concentration in all samples prepared in this experiment was 100 mg/mL. The final pH values were measured with a pH probe (Microelectrode, Inc.) and were found to agree consistently with the pH of the dialysis solution.

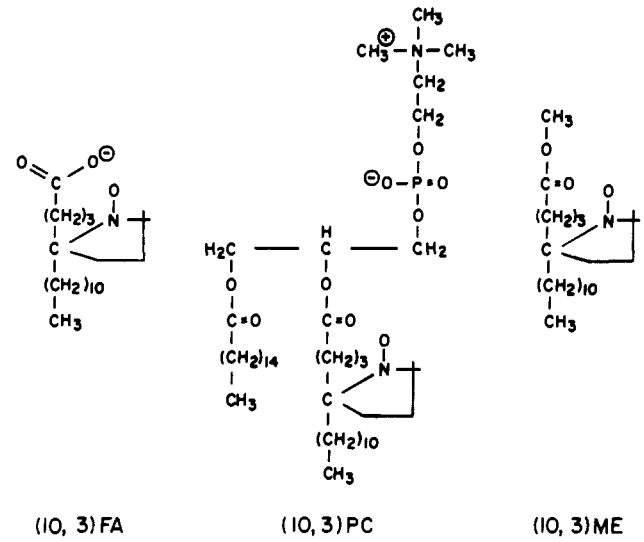

FIGURE 1: Molecular structure of the spin-labeled probes utilized in this experiment.

Electron spin resonance spectra were recorded on a Varian E-12 spectrometer at 9.12 GHz. Samples were positioned so that the long axis of the sample cell was horizontal. A Varian variable-temperature controller was used for temperature regulation, the temperature being monitored continuously with a YSI Model 42sc Tele thermometer. The temperature could be controlled to within 0.2 °C for a series of measurements performed in any one ESR session. Measurements obtained in different ESR sessions could be calibrated to within 0.5 °C and were made in ascending temperature order with a heating rate of approximately 10 °C/h. Some samples were examined in less detail in descending temperature order.

The (10,3)ME, (10,3)FA, and (10,3)PC utilized in this experiment were synthesized according to the methods of Hubbell & McConnell (1971). DMPC was obtained from Sigma Chemical Co. TLC analysis on silica HF plates utilizing $CHCl_3$–CH_3OH–7 M NH_4OH (80:30:4) as an elutant indicated that the DMPC was free from lysolecithin and fatty acid contamination.

Results

Two types of ESR experiments were conducted to answer questions concerning the role of titrable chemical groups in rhodopsin–lipid interactions. First, the Tempo spectral parameter, f (Shimshick & McConnell, 1973), which was approximately proportional to the fraction of Tempo dissolved in the membrane, was measured as a function of temperature for DMPC and DMPC–rhodopsin recombinants that contained varying amounts of rhodopsin. This approach allowed analysis of the effect of rhodopsin incorporation on the DMPC gel-to-liquid-crystalline phase transition. Tempo measurements were conducted at pH 7.0, where rhodopsin had a net negative charge, and at pH 5.0, which was near rhodopsin's isoelectric points (Planter & Kean, 1976). The results of these measurements are depicted in Figures 2–4. Several features of the curves in these figures should be noted. First, at both pH 7.0 and pH 5.0, added rhodopsin progressively broadened the gel-to-liquid-crystalline phase transition at a DMPC:rhodopsin

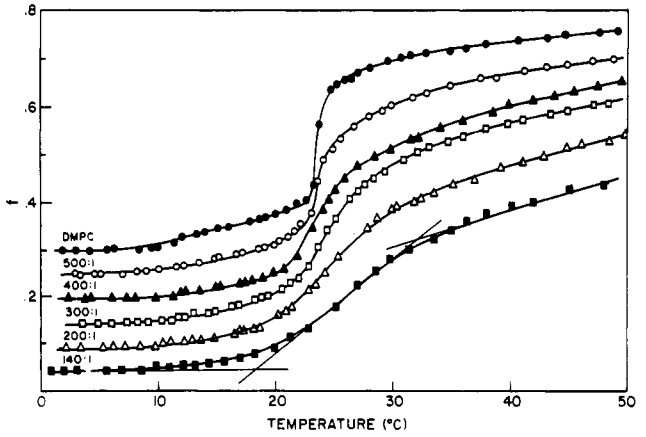

FIGURE 2: pH 7.0 Tempo spectral parameter (f) vs. temperature curves for DMPC:rhodopsin = 140:1 (■), 200:1 (△), 300:1 (□), 400:1 (▲), and 500:1 (○) and DMPC control (●). Each curve above the 140:1 curve is displaced upward 0.05 f unit for reasons of clarity. The method used for determining breakpoints is demonstrated for the DMPC:rhodopsin = 140:1 sample.

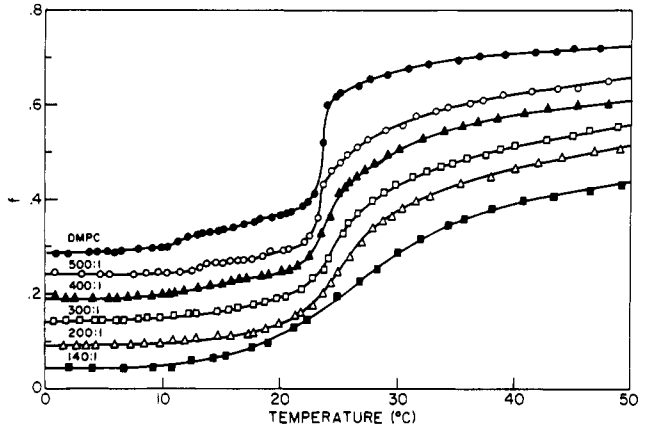

FIGURE 3: pH 5.0 Tempo spectral parameter (f) vs. temperature curves for DMPC:rhodopsin = 140:1 (■), 200:1 (△), 300:1 (□), 400:1 (▲), and 500:1 (○) and DMPC control (●). Each curve above the 140:1 curve is displaced upward 0.05 f unit for reasons of clarity.

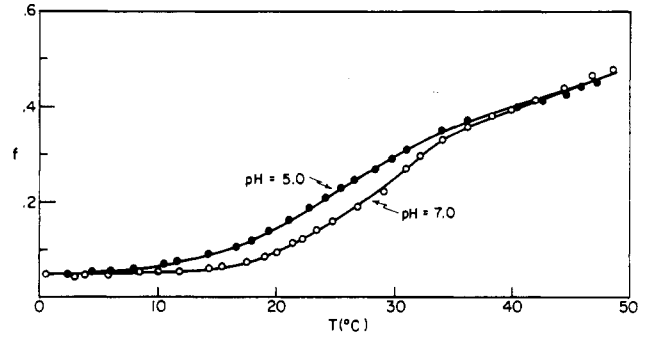

FIGURE 4: Tempo spectral parameter (f) vs. temperature curves for DMPC:rhodopsin = 100:1 at pH 5.0 (●) and pH 7.0 (○).

molar ratio of 400:1 or less. At DMPC:rhodopsin ratios greater than 400:1, only slight "rounding" of the f vs. temperature curves at temperatures above the midpoint of the phase transition (T_c) was observed. Important differences were noted between pH 5.0 and pH 7.0 samples at a DMPC:rhodopsin ratio of 100:1. This effect was demonstrated in Figure 4. At pH 5.0, the low-temperature breakpoint in the f vs. temperature curves occurred at a progressively lower temperature as the rhodopsin content was increased. On the other hand, at pH 7.0, the low-temperature breakpoint remained constant at about 18 °C as the rhodopsin content was increased. The effect of rhodopsin bleaching of Tempo binding was not examined, because bleaching caused rapid loss of the Tempo signal.

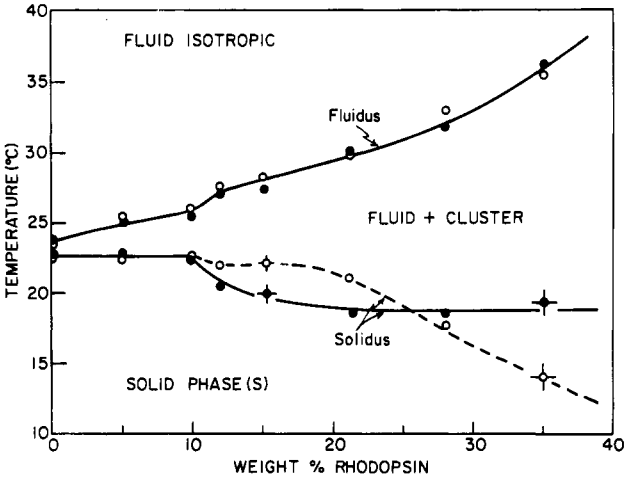

FIGURE 5: Partial binary phase diagram for the rhodopsin–DMPC system at pH 5.0 (○) and pH 7.0 (●). Error bars are included on only four points for clarity.

The Tempo binding curves in Figures 2 and 3 were constructed in ascending temperature order. Several samples were examined in less detail in descending temperature order. At pH 5.0 and pH 7.0, the pure DMPC samples displayed a small hysteresis effect involving an approximately 0.8 °C displacement of the main phase transition to a lower temperature when analyzed in descending temperature order. Rhodopsin incorporation reduced the magnitude of this effect, eliminating it altogether at a DMPC:rhodopsin ratio of 200:1.

It was noted that in the fluid-isotropic phase at pH 7.0 and pH 5.0, the amount of Tempo dissolved in the membrane continued to increase as the temperature was elevated. TLC analysis of the DMPC utilized in this study indicated that this effect was not due to contamination. The fluid-phase increase in Tempo binding has been noted in other studies (Luna & McConnell, 1977a,b) and might involve continued changes in the physical state of membranes as the temperature is increased.

The Tempo data depicted in Figures 2–4 was used to construct the partial phase diagrams in Figure 5. This involved the measurement of fluidus and solidus curves by identifying the breakpoints in f vs. temperature curves (Shimshick & McConnell, 1973). Breakpoints were identified by drawing lines that were tangential to the Tempo binding curves at 0 °C, 50 °C, and the transition midpoints. As demonstrated for the DMPC:rhodopsin = 140:1 sample in Figure 2, the intersection of the tangential lines gave the breakpoints. The vertical error bars in Figure 5 represented uncertainty in the positioning of the tangential lines. This uncertainty increased as the rhodopsin content of the membrane increased. The horizontal error bars in Figure 5 reflected preparatory difficulties in obtaining a defined DMPC:rhodopsin ratio. The freeze–fracture studies of Chen & Hubbell (1973), as well as the theoretical discussions concerning protein–lipid phase diagrams by Kleemann & McConnell (1976), were important aids in labeling the phase domains in Figure 5. The major difference between the pH 5.0 and the pH 7.0 phase diagrams was the decrease in temperature of the solidus curve as the rhodopsin content was increased at pH 5.0.

The effect of NaCl on the phase behavior of the DMPC–rhodopsin system was also analyzed. Samples with

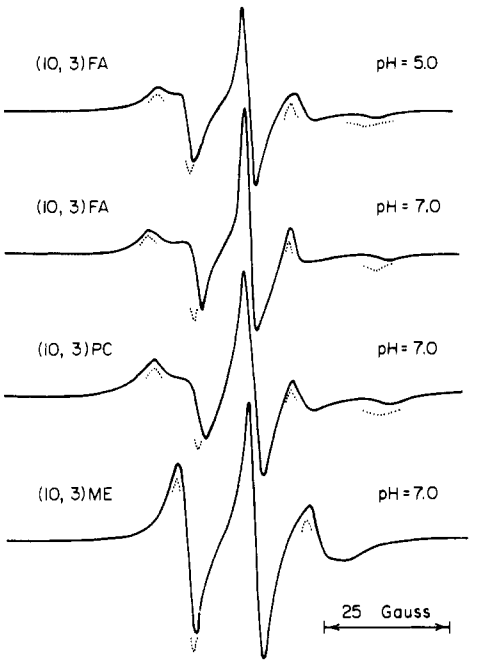

FIGURE 6: ESR spectra of (10,3)FA, (10,3)PC, and (10,3)ME at 37.0 °C in DMPC:rhodopsin = 200:1 (—) and DMPC control (···) samples.

Table I: pH Dependence of ESR Spectral Splittings at 37.0 °C

probe	sample	pH	$T_{\parallel}$ (G)	$T_{\perp}$ (G)
(10,3)FA	DMPC	7.0	24.25	9.25
(10,3)FA	DMPC	5.0	21.05	10.15
(10,3)FA	200:1	7.0	24.55	9.10
(10,3)FA	200:1	5.0	21.65	10.05
(10,3)PC	DMPC	7.0	23.55	9.50
(10,3)PC	DMPC	5.0	23.50	9.45
(10,3)PC	200:1	7.0	24.05	9.35
(10,3)PC	200:1	5.0	24.07	9.30
(10,3)ME	DMPC	7.0		11.65
(10,3)ME	DMPC	5.0		11.65
(10,3)ME	200:1	7.0		11.45
(10,3)ME	200:1	5.0		11.50

DMPC:rhodopsin = 200:1 that contained 150 mM NaCl were prepared. These samples gave f vs. temperature curves that were virtually identical with the 200:1 curve in Figure 2.

The second type of ESR experiment performed in this study utilized spin-labeled amphiphiles in an attempt to understand what might have caused the pH-induced differences in phase behavior. ESR spectra of (10,3)ME, (10,3)FA, and (10,3)PC in DMPC and DMPC:rhodopsin = 200:1 samples were obtained at pH 5.0 and at pH 7.0. Measurements were conducted at 37.0 °C (in the fluid-isotropic phase) to allow free diffusion of membrane components and avoid phase separation effects. The results of this series of measurements are summarized in Figure 6 and Table I. It was found that the ESR line shape of (10,3)ME and (10,3)PC were virtually independent of pH. In contrast, the ESR lineshape of (10,3)FA changed between pH 5.0 and pH 7.0. The changes in $T_{\parallel}$ and $T_{\perp}$ that accompanied this change are presented in Table I. These line-shape changes were thought to involve the titration of the (10,3)FA carboxyl group, because (10,3)ME and (10,3)PC, which contained no chemical functions that titrate between pH 5.0 and pH 7.0, had line shapes that were pH independent. The lecithin phosphate function titrates at pH values less than 3.0 (Trauble & Eibl, 1974), while many carboxylic acid containing compounds have pK_a values between 5.0 and 7.0. Our findings concerning the titration of (10,3)FA were similar in many respects to the findings of Barratt & Laggner (1974), who titrated spin-labeled fatty acids in egg lecithin bilayers.

Discussion

The results of these experiments demonstrate several new points about the phase behavior of DMPC–rhodopsin systems and, in more general terms, about protein–lipid interactions.

Perhaps the most interesting finding of this study is that at a pH value near or more acidic than the isoelectric point(s) of rhodopsin the temperature of the solidus curve is significantly lowered. While the titration properties of rhodopsin in DMPC have not been determined, the isoelectric point measured in the detergent Emulophogene by Planter & Kean (1976) shows three values for unbleached rhodopsin, namely, 4.99, 5.33, and 5.91. Bleached rhodopsin gave a single pI value of 5.22. Higher values for both bleached (pI = 6.0) and unbleached (pI = 5.7) nonphosphorylated rhodopsins in Triton X-100 have been reported by Kuhn & McDowell (1977). Similar values were found by Huang et al. (1973) for Emulophogene-solubilized rhodopsin. In addition, the presence of lipids associated with the Triton X-100–rhodopsin system was shown to have no effect on the measured isoelectric point (Kuhn & McDowell, 1977). While the actual state of phosphorylation of rhodopsin in the DMPC recombinants is not known, the work of Shichi & Williams (1979) and Kuhn & McDowell (1977) would suggest that rhodopsin prepared from retinas obtained in the frozen state from meatpacking companies may contain as much as 50% of the rhodopsin in the phosphorylated state. The pI for phosphorylated rhodopsin was determined to range from pH 6.0 to pH 4.5, depending on the extent of phosphate group incorporation (Kuhn & McDowell, 1977). Thus, no matter what the extent of phosphorylation the pI for the rhodopsin in the DMPC recombinant is probably still very close to pH 5.0. It is certainly closer to pH 5.0 than to pH 7.0. It, therefore, seems reasonable to assume that at pH 7.0 the DMPC–rhodopsin system bears a net negative charge while at pH 5.0 it is close to electrical neutrality.

The large number of dicarboxylic acid residues in rhodopsin, i.e., some 20 aspartates and 25–30 glutamates, would suggest that the major ionization changes occurring between pH 5.0 and pH 7.0 are the titration of some of these carboxylic acid containing amino acid residues. At pH 7.0, where rhodopsin has a net negative charge, the solidus curve was not lowered in temperature. At pH 5.0 where many of the carboxylic acid side chains will no longer be ionized, the solidus curve is lowered in proportion to the amount of rhodopsin present in the recombinant once the weight percent of rhodopsin exceeds 10%. Rhodopsin is reported to contain eight arginine, ten lysine, and five histidine residues as well as the large number of dicarboxylic acid residues. However, in general, arginine, lysine, and histidine residues titrate under basic conditions at pH values greater than 8.0 and are probably not involved here. The hypothesis that the pH-induced change in phase behavior results from titration of dicarboxylic acid residues of the rhodopsin molecule is further supported by the finding that membrane-bound spin-labeled fatty acids titrate between pH 5.0 and pH 7.0. Because the carboxylic acid groups of both rhodopsin and the spin-labeled fatty acids are bound to the membrane, it is a reasonable inference that some of rhodopsin's carboxylic acid groups are titrated over the same range as the fatty acid carboxylic acid groups.

While the oligomeric state of rhodopsin in the fluid-isotropic phase is not precisely known, the freeze-fracture studies of Chen & Hubbell (1973) place an upper diameter size limit of 110 Å on any possible oligomeric structures. This suggests

that in the fluid-isotropic phase, rhodopsin is monomeric or associated with only a few molecules of its own kind. This question could be clarified if direct rotational and translational diffusion measurements of rhodopsin in DMPC membranes were available, but such measurements have not been reported as of this writing. It should be noted that rhodopsin is not fully photochemically functional in DMPC membranes (O'Brien et al., 1977; Calhoon et al., 1981), which suggests that while the configuration of the chromophoric part of the protein is not significantly altered, the protein as a whole might not be in a "native" configuration.

While the pH 7.0 phase diagram reported in this study is very similar to the previously reported isoionic phase diagram (Fischer & Levy, 1981), the measurement of more points on the present diagram demonstrates an interesting feature: Until the rhodopsin content is increased to a molar ratio of about 400:1, the DMPC gel-to-liquid-crystalline phase transition is not significantly broadened. This effect results in the discontinuity in the fluidus and solidus curves at about 10% rhodopsin content. Beyond this discontinuity, the fluid plus cluster domain is gradually expanded as more rhodopsin is added.

Experiments involving the effect of NaCl on the phase behavior of DMPC–rhodopsin systems are reported because inorganic salts have important effects on many membrane systems. For example, the photoinduced changes in the ROS membrane surface potential are virtually eliminated in the presence of 100 mM NaCl (Cafiso & Hubbell, 1980). This effect is thought to involve a screening of the surface potential by the NaCl. Another example involves the erythrocyte membrane. Reynolds & Trayer (1971) report that the removal of inorganic salts from erythrocyte ghost membranes causes the release of proteins from the membrane. This effect is postulated to involve a disruption of ion-mediated lipid–protein interactions. In contrast to the results of the above-mentioned studies, no ion-induced effects were observed in this study with NaCl. This indicates that the type of ionic protein–lipid interactions that are disrupted or mediated by salts do not play a major role in modulating the phase behavior of DMPC–rhodopsin systems. This conclusion is further supported by the similarity of the pH 7.0 phase diagram in Figure 5 of this paper and a previously reported DMPC–rhodopsin phase diagram that was constructed under isoionic conditions (Fischer & Levy, 1981). In those experiments, under isoionic conditions, the bulk sample pH varied from 7.0 to 8.0, depending on the rhodopsin content.

In conclusion, this study demonstrates how titratable chemical groups on a membrane-bound protein affect the phase behavior of a model system. This study assumes direct importance in view of the extensive study by many investigators of rhodopsin–lecithin membranes.

Acknowledgments

We are indebted to M. Cooper and J. Schrader for performing the ROS isolations, to Dr. B. Gaffney and Dr. A. Deese for much useful advice concerning probe synthesis, and to B. Tranchand and B. Williams for help in preparing the manuscript. We are also grateful to B. Baker for her invaluable guidance and technical assistance.

References

Barratt, M. D., & Laggner, P. (1974) *Biochim. Biophys. Acta 363*, 127.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466.

Cafiso, D. S., & Hubbell, W. L. (1980) *Biophys. J. 30*, 243.

Calhoon, R. D., Ebreb, T. G., & Tsuda, M. (1981) *Biophys. J. 33*, 290a.

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

Davoust, J., Bievenue, A., Fellmann, P., & Devaux, P. (1980) *Biochim. Biophys. Acta 596*, 28.

Fischer, T. F., & Levy, G. C. (1981) *Chem. Phys. Lipids 28*, 7.

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

Hong, K., Chen, Y. S., & Hubbell, W. L. (1973) *Ann. N.Y. Acad. Sci. 222*, 523.

Huang, H. V., Molday, R. S., & Dreyer, W. J. (1973) *FEBS Lett. 37*, 285.

Hubbell, W. L., & McConnell, H. (1971) *J. Am. Chem. Soc. 93*, 314.

Kleemann, W., & McConnell, H. M. (1976) *Biochim. Biophys. Acta 419*, 206.

Kuhn, H., & McDowell, J. H. (1977) *Biophys. Struct. Mech. 3*, 199.

Kusumi, A., Sakaki, T., Joshizawa, T., & Ohnishi, S. (1980) *J. Biochem. (Tokyo) 88*, 1103.

Lowry, O. H., & Rosebrough, N. J. (1951) *J. Biol. Chem. 193*, 265.

Luna, E. J., & McConnell, H. M. (1977a) *Biochim. Biophys. Acta 466*, 381.

Luna, E. J., & McConnell, H. M. (1977b) *Biochim. Biophys. Acta 470*, 303.

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

Papermaster, D. S., & Dreyer, W. (1974) *Biochemistry 13*, 2438.

Planter, J. J., & Kean, E. L. (1976) *Exp. Eye Res. 23*, 281.

Reynolds, J. A., & Trayer, H. (1971) *J. Biol. Chem. 246*, 7337.

Sheetz, M., & Chan, S. (1972) *Biochemistry 11*, 4573.

Shichi, H., & Williams, T. C. (1979) *J. Supramol. Struct. 12*, 419.

Shimshick, E. J., & McConnell, H. M. (1973) *Biochem. Biophys. Res. Commun. 53*, 446.

Trauble, H., & Eibl, H. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 214.

Van Breugel, P. J., & Daemen, F. (1977) *Exp. Eye Res. 24*, 581.