data. The reaction between cytochrome b_5 and cytochrome c is uniquely suited to test this theory, and the excellent fit to the ionic strength dependence of the reaction rate, the reasonable value obtained for R, and the good agreement between the calculated number of complementary charge interactions and the results of the chemical modification studies all combine to justify the validity of the general approach. In extending this technique to reactions between other electron transport proteins, it will be important to use small monovalent electrolytes such as NaCl at ionic strengths above 0.1 M and where possible corroborate the results with chemical modification studies.

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Proton and Carbon-13 Nuclear Magnetic Resonance Studies of Rhodopsin–Phospholipid Interactions†

Nicholas Zumbulyadis and David F. O'Brien*

ABSTRACT: Proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra of rhodopsin–phospholipid membrane vesicles and sonicated disk membranes are presented and discussed. The presence of rhodopsin in egg phosphatidylcholine vesicles results in homogeneous broadening of the methylene and methyl resonances. This effect is enhanced with increasing rhodopsin content and decreased by increasing temperature. The proton NMR data indicate the phospholipid molecules exchange rapidly ($<10^{-3}$ s) between the bulk membrane lipid and the lipid in the immediate proximity of the rhodopsin. These interactions result in a reduction in either or both the frequency and amplitude of the tilting motion of the acyl chains. The ^{13}C NMR spectra identify the acyl chains and the glycerol backbone as the major sites of protein lipid interaction. In the disk membranes the saturated *sn*-1 acyl chain is significantly more strongly immobilized than the polyunsaturated *sn*-2 acyl chain. This suggests a membrane model in which the lipid molecules preferentially solvate the protein with the *sn*-1 chain, which we term an edge-on orientation. The NMR data on rhodopsin–asolectin membrane vesicles demonstrate that the lipid composition is not altered during reconstitution of the membranes from purified rhodopsin and lipids in detergent.

The light-sensitive protein rhodopsin comprises at least 85% of the protein content of the outer segment membranes of visual receptor cells (Daeman, 1973). Rhodopsin is considered to be an integral membrane protein. Data from X-ray diffraction (Blaise, 1972; Blaurock & Wilkins, 1972; Chabre, 1975), neutron diffraction (Yaeger, 1975; Saibil et al., 1976), freeze-fracture electron microscopy (Chen & Hubbell, 1973), and electron paramagnetic resonance spectroscopy (Hong & Hubbell, 1972) indicate that at least part of the protein is in

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contact with the membrane interior. Studies on rhodopsin in BLMs[1] (Montal et al., 1977) and in rhodopsin–egg phosphatidylcholine (PC) membrane vesicles (O'Brien et al., 1977b) suggest that rhodopsin spans the membrane lipid bilayer. This has been confirmed by a recent chemical-labeling study (Fung & Hubbell, 1978). In natural membranes rhodopsin moves freely, as demonstrated by the observed rotational (Brown, 1972; Cone, 1972) and translational (Poo & Cone, 1974; Liebman & Entine, 1974) motions of rhodopsin in retinas. The photochemical behavior of rhodopsin–phospholipid membrane vesicles is affected by the fluidity of the phospholipid bilayer (O'Brien et al., 1977a).

Understanding the interaction of the transmembrane protein with the phospholipid of the membrane bilayer is of prime importance for the elucidation of the functionality of rhodopsin. Nuclear magnetic resonance (NMR) is a useful tool in probing the dynamic properties of membranes. ^{13}C NMR (Millett et al., 1973) and ^{1}H NMR (Brown et al., 1977a,b) of bovine rod outer segment membranes have been reported. We have demonstrated by the use of ^{1}H and ^{31}P NMR that the permeability of rhodopsin–phospholipid membrane vesicles to transition-metal cations is significantly increased upon exposure of rhodopsin to light (O'Brien et al., 1977b). In the present work we report the results of ^{1}H and ^{13}C experiments designed to probe the interaction of rhodopsin with various regions of the phospholipid molecules.

The interaction between proteins and lipids manifests itself primarily in the modification of the fluidity of the host membrane matrix. Two different, and to a certain extent independent, parameters contribute to the notion of membrane fluidity: (1) the average degree of ordering of the hydrocarbon chains, (2) the rates of the different types of motion such as segmental trans–gauche isomerizations, lateral diffusion, and chain reorientations. When a transmembrane protein is incorporated in the bilayer, a number of phospholipid molecules solvate the hydrophobic portion of the protein, forming what can be thought of as a two-dimensional solution. Hence, the rate of exchange of a phospholipid molecule between the lipids in proximity to the protein and the bulk lipid is a third necessary parameter to characterize the dynamic structure of membranes and lipid–protein interactions.

Experimental Section

Materials. Frozen, dark-adapted bovine retinas were obtained from George A. Hormel Co., Austin, MN. Egg PC was obtained from Sigma Chemicals, St. Louis, MO, and was purified by chromatography on Unisil silicic acid. The purity was evaluated by TLC. Asolectin, a preparation of soybean phospholipids, was obtained from Associated Concentrates, Inc., Woodside, NY, and purified as described previously (O'Brien et al., 1977a).

All procedures with rhodopsin were done under dim red light (Kodak safelight filter no. 1) at 4 °C, unless otherwise specified. The procedure for isolation of ROS and preparation of purified rhodopsin in detergent was essentially that of Hong & Hubbell (1973).

Purified, delipidated rhodopsin in 100 mM detergent, usually tridecyltrimethylammonium bromide (O'Brien et al., 1977a) with an A_{278}/A_{498} spectral ratio of 1.7 ± 0.1, was combined with purified egg PC which was dissolved in 0.5–1 mL of 100 mM detergent, and the detergent was removed by dialysis against a buffer of 10 mM Hepes and 1 mM EDTA, which had been deoxygenated with argon. The recombinants were removed from the dialysis bag and concentrated by ultrafiltration to a phospholipid concentration of 18–36 mM. The recombinant membranes were sonicated in an ice bath under nitrogen with a Branson sonic probe, yielding clear suspensions of recombinant vesicles which were used in the NMR experiments.

The samples for ^{1}H NMR spectroscopy were exchanged into deuterium oxide containing 1 mM Hepes and 1 mM EDTA via repeated dilutions with D_2O and ultrafiltration concentrations.

The rhodopsin recovery yields after recombinant membrane formation and ultrafiltration concentration were estimated as described by Hong & Hubbell (1973). The recovery yields for both of these steps were 90–95%. All absorption spectra were taken with a Cary 118 spectrophotometer, after solubilization with 100 mM TrTAB in 10 mM Hepes buffer, pH 7.0.

ROS disk membranes were isolated by the procedure of Smith et al. (1975); argon-saturated solutions were used to avoid oxidation of the fatty acids of the lipids (Farnsworth & Dratz, 1976). The ROS phospholipids were extracted with freshly distilled argon-saturated solvents which contained butylated hydroxytoluene by the technique of Folch et al. (1957) and purified on silicic acid to remove retinal and retinol (Brown et al., 1976). The disk membranes and ROS phospholipid membranes were sonicated in an ice bath under nitrogen as described above.

Electron Microscopy. Samples of the membrane vesicle suspensions were observed after negative staining. The suspensions were diluted with buffer to 0.1–0.2% (w/v) phospholipid and treated with ammonium molybdate on the grid. The grids were viewed in a Philips Model 201 electron microscope. The image for egg PC vesicles ranged from 300 to 320 Å in diameter, and the images of the rhodospin–egg PC membrane vesicles ranged in size from 350 to 500 Å in diameter, the majority having a diameter of 420–440 Å.

Nuclear Magnetic Resonance. ^{1}H Fourier transform spectra were obtained by using a Varian HA-100 spectrometer. Details have been published (O'Brien et al., 1977b). Some measurements of the line width of egg PC vesicles were made at 60 and 270 MHz by using the JEOL FX-60Q and Bruker WH-270 NMR instruments. Spectral simulations and line width calculations were made by using a HP67 programable calculator. The ^{13}C spectra were obtained at 22.6 and 67.8 MHz by using Bruker HX-90 and Bruker WH-270 instruments. The chemical shifts were referred to external Me_4Si in benzene solution.

Results and Discussion

Proton Line Width Studies of Rhodopsin–Egg PC Recombinants. We have previously shown that the inclusion of rhodopsin in egg PC vesicles leads to a broadening of the methyl, methylene, and phosphate lipid resonances as the rhodopsin concentration in the vesicles is increased (O'Brien et al., 1977b). The chemical shifts for these resonances are independent of the rhodopsin concentration. The spectrum of the methyl and methylene regions for the rhodopsin–egg PC (1:200) membrane vesicle at 36 °C is shown in Figure 1, together with the simulated spectrum from which the line widths were determined. Two Lorentzian curves were sufficient to simulate the observed spectrum.

Examination of the sonicated unilamellar membrane vesicles by electron microscopy indicates that the average vesicle di-

[1] Abbreviations used: NMR, nuclear magnetic resonance; BLM, bilayer lipid membrane; PC, phosphatidylcholine; PE, phosphatidylethanolamine; ROS, rod outer segments; TrTAB, tridecyltrimethylammonium bromide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; Me_4Si, tetramethylsilane; Pipes, 1,4-piperazinediethanesulfonic acid.

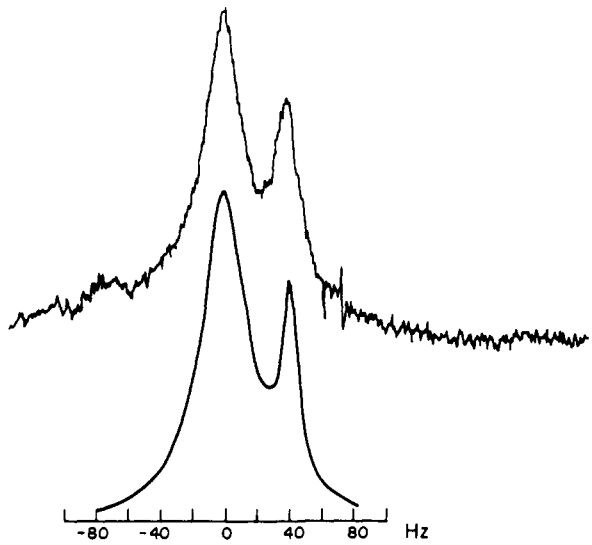

FIGURE 1: 100-MHz ^1H NMR spectrum of the methyl and methylene region of the 1:200 rhodopsin–PC membrane vesicle. The simulated spectrum composed of two Lorentzians is shown below.

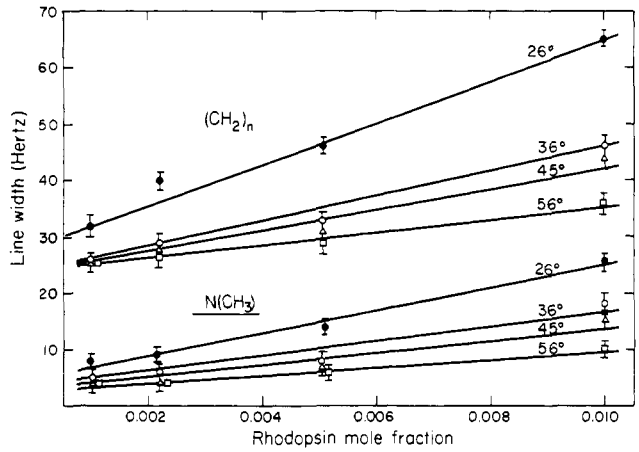

FIGURE 2: Dependence of methylene and choline methyl proton line widths on rhodopsin concentration at various temperatures.

ameter (~400 Å) or the degree of dispersity is independent of the rhodopsin concentration from 1:500 to 1:100 (rhodopsin/lipid mole ratio). Size variation can thus be discounted as the source of the observed line broadening.

The time scale of the protein–lipid interactions was probed by studying the temperature dependence of methylene and choline line widths. The effects of temperature and rhodopsin concentration on the line widths of the methylene and choline resonances are shown in Figure 2. The line width dependence on rhodopsin concentration (for the concentration range examined) is linear within experimental error and displays a steeper slope at lower temperature. The data converge at vanishing rhodopsin concentration.

The relative intensities (peak areas) of the choline methyl and chain methylene resonances have been determined for the spectra used to construct Figure 2, and their ratio (1:1.6 ± 0.1) is independent of rhodopsin concentration at the temperature and concentration range studied.

Since the choline methyl proton signal is least affected by lipid immobilization or changes in vesicle size (Sheetz & Chan, 1972; Prestegard & Fellmeth, 1974), the constancy of the relative intensity of choline and methylene resonances in these membrane vesicles argues against the existence of a large lipid fraction that is permanently associated with rhodopsin. The data show a homogeneous average broadening of the methylene protons. The simplest explanation of the observed line broadening is a two-site lipid-exchange process between the lipid molecules in the proximity of the protein (referred to henceforth as the proximate lipid) and the bulk lipid. The methylene protons of the proximate lipids experience a shorter transverse relaxation time (T_2) than those of the bulk lipids. In order to evaluate whether the exchange of lipids between the two environments is in the slow- or fast-exchange region, consider the relationship (eq 1) (Dwek, 1973; Lindman & Forsen, 1976)

$$1/T_2(\text{obsd}) = (1 - x_L)/T_2^0 + x_L/(T_{2p} + \tau) \quad (1)$$

where $1/T_2(\text{obsd})$ is the observed transverse relaxation rate (related to the line width at half-height by $\pi\Delta\nu = 1/T_2$), $1/T_2^0$ is the relaxation rate of the protons in the absence of protein, x_L is the mole fraction of the proximate lipid, T_{2p} is the transverse relaxation time of the proximate lipid, and τ is the mean lifetime for a given phospholipid molecule in the proximate lipid region. At the fast-exchange limit, $T_{2p} \gg \tau$ and eq 1 becomes

$$1/T_2(\text{obsd}) = (1 - x_L)/T_2^0 + x_L/T_{2p} \quad (2)$$

$$\Delta\nu(\text{obsd}) = (1 - x_L)\Delta\nu^0 + x_L\Delta\nu_p \quad (2a)$$

Since the relaxation time T_{2p} generally increases with temperature, a decrease in the slope of the plot of line width vs. rhodopsin mole fraction with increasing temperature is expected for the fast-exchange case.

The data in Figure 2 clearly demonstrate a decrease in the slope as the temperature is increased; therefore, the exchange of the lipid molecules between the two sites is *fast* on the NMR time scale. Slow exchange ($\tau \gg T_{2p}$) would yield an increase in the slope of the plot with increasing temperature. The observation of fast lipid exchange between the proximate and bulk lipid regions will be examined in more detail in the last section.

The large change in slope between 26 and 36 °C shown in Figure 2 can be explained in terms of an abrupt change in T_{2p} at 26 °C with relatively little change in T_{2p} above 36 °C. This is analogous to the NMR behavior of pure phospholipid bilayer membranes around their phase-transition temperature. Thus, the data seem consistent with the onset of a discontinuous change in fluidity of the proximate lipid near 26 °C.

We have reported (O'Brien et al., 1977a) that the rate of interconversion of the photochemical intermediates meta-rhodopsin I to meta II in rhodopsin–egg PC vesicles is significantly reduced below ~20 °C. Since this photochemical change is associated with a conformational change in the protein, it is reasonable that the reduced fluidity of the proximate egg PC molecules would inhibit the transition.

^{13}C NMR Studies of Pure Asolectin and Rhodopsin–Asolectin Membrane Vesicles. A more detailed picture of phospholipid–rhodopsin interactions can be obtained by the use of ^{13}C NMR spectroscopy since the various carbon sites can be studied individually.

A diversity of motional states is apparent in the ^{13}C T_1 studies of egg PC vesicles by Godici & Landsberger (1974). They have shown that the NCH_2 and OCH_2 groups of the choline moiety and the first methylene along the acyl chain display the least mobility (relaxation times 0.30, 0.41, and 0.26 s, respectively). The mobility increases toward the choline $N(CH_3)_3$ group and the termini of the fatty acid chains.

Figure 3 shows the ^{13}C NMR spectra of purified asolectin vesicles and rhodopsin–asolectin membrane vesicles. The carbonyl, olefinic, and aliphatic regions of the spectrum display

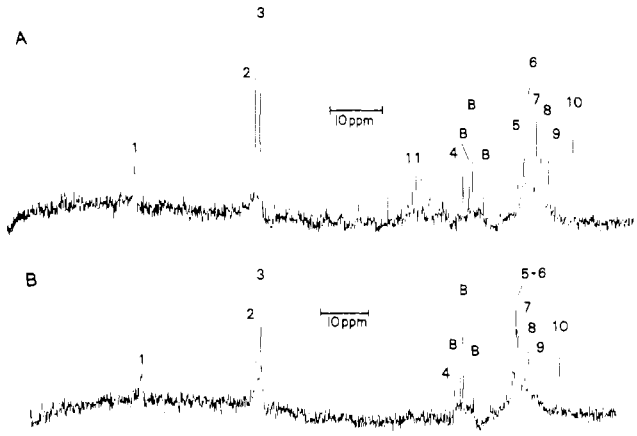

FIGURE 3: 22.6-MHz ^{13}C NMR spectra of (A) asolectin vesicles and (B) rhodopsin–asolectin (1:500) membrane vesicles in D_2O. 3×10^4 transients were collected, with 8K data points at ambient probe temperature (in parts per million from external Me_4Si): (1) C=O, 174.3; (2) C=C, 130.9; (3) conjugated –C=C–, 129.1; (4) $N(CH_3)_3^+$, 54.8; (5) $CH_2CH_2CH_3$, 32.8; (6) $(CH_2)_n$, 30.7; (7) $CH_2CH{=}CH$, 28.4; (8) $=CCH_2C=$, 26.8; (9) CH_2CH_3, 23.9; (10) CH_3, 15.1; (11) backbone, 72.3–67.3; (B) buffer.

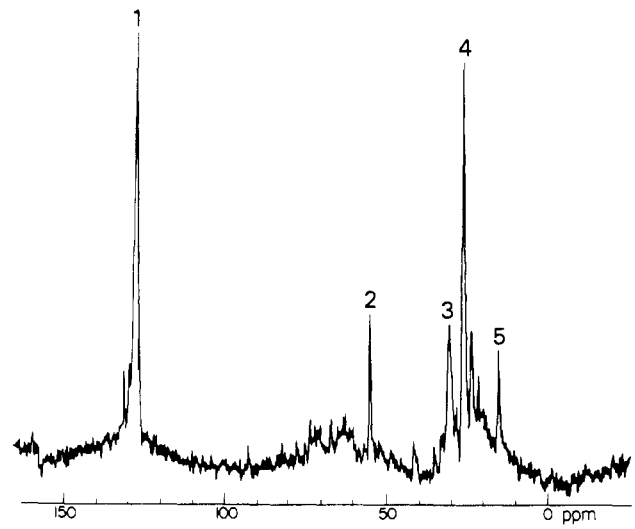

FIGURE 4: 67.8-MHz ^{13}C NMR spectrum of sonicated ROS membranes in D_2O. 4.9×10^4 transients were collected, with 16K data points at ambient probe temperature using quadrature detection (in parts per million from external Me_4Si): (1) C=C, 128.7; (2) $N(CH_3)_3^+$, 54.8; (3) $(CH_2)_n$, 29.7; (4) $C{=}CCH_2C{=}C$, 25.8; (5) CH_3, 14.5.

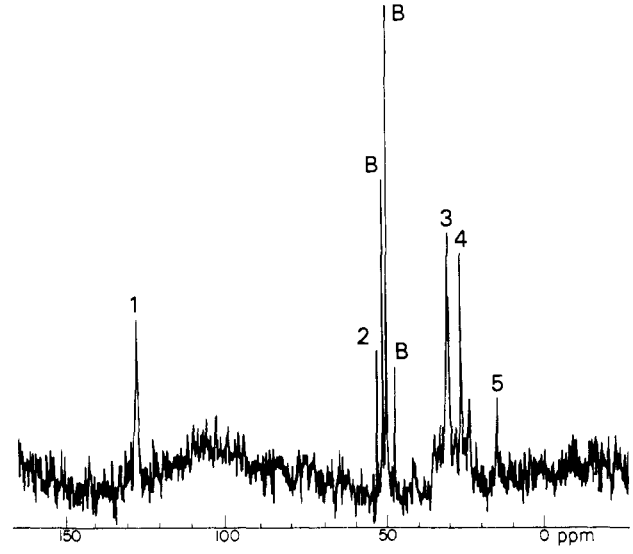

FIGURE 5: 67.8-MHz ^{13}C NMR spectrum of sonicated ROS lipid vesicles in D_2O. 5×10^4 transients were collected, with 16K data points at ambient probe temperature. Assignments are the same as those in Figure 4. Peaks labeled with B belong to the buffer.

well resolved resonances. The major peak assignments given in Figure 3 are referred to external Me_4Si and are essentially identical with previous assignments (Godici & Landsberger, 1974; Shapiro et al., 1975). The instrumental error is ±0.06 ppm (1.35 Hz). A comparison of parts A and B of Figure 3 reveals that the incorporation of rhodopsin into the bilayer membrane modifies the line widths of various carbon resonances.

The peaks at 67.3, 70.6, and 72.3 ppm are assigned to the choline methylene and glycerol methylene carbons of the backbone. These carbons exhibit the most dramatic line broadening. The backbone is known from T_1 measurements (Godici & Landsberger, 1974) to be the least mobile portion of the phospholipid molecule in the bilayer membrane, and from the present work it appears to be the region whose mobility is modified the most by the presence of rhodopsin.

The choline CH_3 groups (54.8 ppm) and the terminal methyl groups (15.1 ppm) show no apparent change either in line width or in relative intensity and can be used to estimate the PC content of asolectin. We find PC to constitute 43% of the lipids (mole/mole), in reasonable agreement with analytical data published elsewhere (O'Brien et al., 1977a). The constancy of the relative peak intensity in the lipid and recombinant vesicles demonstrates that the lipid composition of asolectin was not modified upon recombinant preparation.[2]

The three peaks labeled by B (53.2, 52.0, and 47.9 ppm) stem from the buffer (Pipes).

^{13}C NMR Spectra of Sonicated Disk Membranes and Derived Lipid Vesicles. The ^{13}C NMR spectra of sonicated disk membranes and extracted disk lipids (derived lipids) have been obtained at 22.6 and 67.8 MHz. The high-field spectra are shown in Figures 4 and 5. The ROS disks were ~15% optically bleached. The ^{13}C NMR spectrum of the ROS is insensitive to the degree of light exposure of the membrane. Absorption spectra of the ROS disks taken before and after data accumulation were very similar, which indicates that the degree of optical or thermal bleaching as well as lipid oxidation was minimal during the experiment.

The peaks at 128.7 ppm belong to the unsaturated carbons. The methylene region displays two clearly separated signals. The resonance centered at 29.7 ppm stems from the CH_2 groups, whereas those around 25.8 ppm originate mainly from unsaturated chains and correspond to doubly allylic methylene groups.

A comparison of the spectra of sonicated disks and derived lipids (Figures 4 and 5) reveals some striking differences. Comparison of the line widths and the peak intensities for the saturated methylene and doubly allylic methylene resonances in the two spectra shows the following. In Figure 4, the ROS disks, the envelope line width of the $(CH_2)_n$ is ~100 Hz, which is several times the width for this resonance in the lipid vesicles where a doublet is observed, whose individual components are ~15 Hz (Figure 5). In contrast, the peak at 25.8 ppm, the

[2] The published composition of asolectin is 40% PC and 29% PE. ^{31}P NMR spectra of asolectin lipid vesicles and rhodopsin–asolectin membrane vesicles show two prominent peaks corresponding to PC and PE. The molar ratio PE/PC in the lipid vesicles was 0.75, and in the rhodopsin membrane vesicles it was 0.69. These values are in reasonable agreement with the analytical value of 0.72 and agree with the ^{13}C NMR data.

doubly allylic methylene, is sharp in both spectra. The line width for this resonance in the ROS disks is 20 Hz, and in the lipid vesicles it is 12 Hz. The relative area ratios of the choline and allylic carbon resonances are the same in both samples, whereas the relative area of the $(CH_2)_n$ signal in the ROS disk spectrum is about one-half that observed in the lipid vesicle spectrum.

Since 80–90% of the lipids in ROS are phospholipids, the ^{13}C data are representative of phospholipid molecules in the membranes. The major phospholipids in ROS and ROS disks are phosphatidylcholine and phosphatidylethanolamine (each ~40%) and some phosphatidylserine (10–13%) (Anderson & Maude, 1970; Crain et al., 1978). There is a large amount of polyunsaturation in the fatty acids of these phospholipids, and the unsaturated fatty acids are generally associated with the *sn*-2 position of the glycerol backbone (Anderson & Sperling, 1971). Approximately 70% of the *sn*-2 chain fatty acids are composed of docosahexenoic acid (22:6). The *sn*-1 position fatty acids are 95% saturated fatty acids in bovine ROS and are primarily palmitic (16:0) and stearic (18:0) acids (Anderson & Sperling, 1971). Therefore, the observed broadening of the saturated CH_2 resonance in the ROS disk spectrum is due primarily to an effect on the *sn*-1 saturated chain of the phospholipids.

The most immediate explanation of this observation is the assumption that the *sn*-1 chain is the primary site of interaction between the hydrophobic portion of the protein and the lipid, whereas the unsaturated *sn*-2 chains retain their mobility. This conclusion finds further support in the ^{13}C spin–lattice relaxation measurements reported earlier for unbleached ROS disks (Millett et al., 1973). These authors have observed a short relaxation time for the saturated CH_2 carbons (0.3 ± 0.1 s) and a much longer T_1 for the doubly allylic carbons (1.0 ± 0.1 s), an additional indication of the higher mobility of the *sn*-2 over the *sn*-1 chain in the presence of rhodopsin.

These data suggest a model in which the lipid molecules solvate the protein with one hydrocarbon chain, which we term an edge-on orientation, rather than with both hydrocarbon chains, termed side-on orientation, or random interactions. The preference for *sn*-1 acyl chain solvation of the hydrophobic region of the protein may be due to the ability of the saturated chain to conform to the geometrical requirements of the protein surface. The observed rhodopsin interaction with the glycerol backbone and *sn*-1 acyl chain is consistent with the current view of the preferred lipid conformation in which these portions of the molecule are aligned.

General Discussion

Previous authors have demonstrated that rhodopsin undergoes rapid rotational (Cone, 1972) and lateral (Poo & Cone, 1974; Liebman & Entine, 1974) diffusion in the membranes of photoreceptor cell outer segments. The extent to which rhodopsin is associated with particular lipids during diffusion has been a matter of conjecture. The present results indicate that for membrane vesicles of rhodopsin–egg phosphatidylcholine the lipids exchange rapidly on the NMR time scale between the bulk membrane lipids and the lipids which directly solvate the rhodopsin.

Although it is unclear whether the fast lipid exchange observed for rhodopsin–egg PC membranes also occurs in the more complex ROS membranes, it is known that the ROS membrane lipids may be readily removed from rhodopsin by detergent action and chromatography using detergents such as dodecyltrimethylammonium bromide (Hong & Hubbell, 1973), dodecyldimethylamine oxide (Applebury et al., 1974), and octyl glucoside (Stubbs & Litman, 1978). Chemical cross-linking studies of aminophospholipids to rhodopsin in ROS disk membranes (Crain et al., 1978) show a random array of the lipids with respect to their fatty acid content. These previous observations on natural membranes taken together with the NMR data reported here on rhodopsin–egg PC membrane vesicles are consistent with a membrane in which both protein and lipids are in rapid motion and few, if any, of the lipids are strongly associated with the rhodopsin.

The fast exchange between proximate and bulk lipids documented by an analysis of the ^1H NMR results implies that $T_{2p} \gg \tau$. This is to be contrasted with the results of Dratz and co-workers (Brown et al., 1977a,b), who report that $T_1 \ll \tau$ for sonicated ROS disk vesicles. The apparent discrepancy might lie with the fact that the recombinant vesicles studied in the present work are smaller in diameter and have a greater curvature than the sonicated disk membranes investigated by Brown et al. (1977a,b). The small recombinant vesicles studied in this work display rather sharp methylene signals (25–65 Hz, cf. Figure 2), and the protein-induced broadening is a significant fraction of the total line width. The study of small vesicles, therefore, presents definite advantages in elucidating protein–lipid interactions, particularly at low protein concentrations. As the vesicle diameter approaches 1000 Å, as, for example, in sonicated ROS studies reported previously (Brown et al., 1977a,b), vesicle tumbling quickly becomes the dominant spin–spin relaxation mechanism leading to line widths in the neighborhood of 100 Hz. The increase in the methylene proton line width due to the presence of rhodopsin in the bilayer then represents only a small fraction of the total width, rendering the study of protein–lipid interactions in these larger vesicle systems by T_2 measurements more difficult than in the membrane vesicles used in this study.

The ^1H NMR results can be used to develop a detailed picture of the motional state of acyl chains of the lipids in proximity to the rhodopsin. The line width data shown in Figure 2 allow the calculation of the spin–spin relaxation time of methylene protons of the proximate lipids. The value for $1/T_2^0$ in eq 2 can be obtained by extrapolation to vanishing rhodopsin concentration. For egg PC recombinants and assuming ~25 lipid molecules/protein, we calculate the methylene T_{2p} in the proximate lipid as ~2 ms (corresponding to a line width of 140–150 Hz) and 3–3.5 ms (90–100 Hz) at 26 and 36 °C, respectively. Therefore, the time scale of exchange between bulk and proximate lipid is $<10^{-3}$ s.

An analysis of the proton line widths in phospholipid vesicles was recently given by Petersen & Chan (1977). These authors treat the effects of both vesicle tumbling and chain reorientations in the methylene line widths and derive a mathematical expression for T_2 as a function of various dynamic parameters. It follows qualitatively from their treatment that the presence of rhodopsin dampens the amplitude and/or correlation time of the chain tilting motion of lipids in contact with the protein, leading thus to an enhancement of the methylene T_2 values. A quantitative description of the influence of rhodopsin on the cooperative processes within the bilayer can also be given, if one obtains best estimates for the various adjustable parameters of the Petersen–Chan equation, for both recombinant and protein-free vesicles.

Frequency-dependent line width measurements of protein-free egg PC vesicles, 300–320 Å in diameter, indicate a homogeneous line width of 19–20 Hz at 36 °C. The rhodopsin–egg PC membrane vesicles are on the average 420–440 Å in diameter regardless of their rhodopsin content. Extrapolation of the line width to vanishing rhodopsin concentration yields for protein-free vesicles the size of the recombinants a

line width of ~25 Hz (cf. Figure 2). Both measurements are in agreement with the values of 19 and 27 Hz, respectively, predicted by the Petersen–Chan theory using an average angle of tilt of ~85° and a correlation time for chain reorientation of $\sim 3 \times 10^{-8}$ s and taking into consideration the differences in vesicle size. The observed methylene line width in freshly sonicated limiting size egg PC vesicles (220 Å in diameter) is 16–17 Hz. The predicted value employing the same parameters is 18 Hz.

The estimated line width of the proximate lipids (~100 Hz at 36 °C) can be explained in terms of either a reduction in the average angle of tilt by 10°, to a value of 75°, or an increase in the chain tilt correlation time to 2×10^{-7} s. A distinction between these two possibilities is not possible from the present data. Both conclusions agree with theoretical considerations. Statistical mechanical calculations on thin nematogen layers have shown, for instance (Schröder, 1977), that rigid boundaries (as a protein might appear to a lipid) have the effect of damping order-parameter fluctuations, which is reflected in an increased degree of order within the boundary region. Petersen & Chan (1977), on the other hand, consider the acyl chain tilting as a cooperative process and conclude that the cooperative unit is larger within regions of the membrane where protein–lipid interactions are strong, predicting thus an increase in the correlation time.

The latter view is supported by the extensive deuterium NMR experiments of Oldfield and co-workers (Oldfield et al., 1978; Kang et al., private communication; Rice and Oldfield, private communication). These authors observe a decrease in the residual deuterium quadrupole splittings for several protein–lipid systems, an observation which might actually imply an *increase* in the amplitude of the chain tilt. The decrease in the quadrupolar splitting is accompanied, however, by an increase in the intrinsic line width of the individual isochromats comprising the powder pattern spectra. Based on the broader intrinsic line widths, the above authors conclude that lipid chains in the proximity of a protein are slowed down in their motions as they move to fill spaces between amino acid side chains. Our estimate of the reduction of the rate of conformational transition to values in the range of 10^7 s^{-1} generally agrees with the estimates of Rice and Oldfield (private communication) for lipid–gramicidin A systems (10^5–10^7 s^{-1}).

Greater refinement of the conclusions drawn in this paper must await progress in the theory of membrane NMR and the results of further experiments such as ^{31}P and deuterium spin–lattice relaxation times in appropriately labeled recombinant systems.

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