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Structure and Motion of Phospholipids in the Chromatophore Membrane from *Rhodospirillum rubrum* G-9†

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ABSTRACT: The structure and motion of the phospholipids in chromatophore membranes from *Rhodospirillum rubrum* G-9 have been investigated by using differential scanning calorimetry (DSC), ^{31}P NMR, and electron spin resonance (ESR) spin-labeling. ^{31}P NMR shows that the phospholipids in chromatophore membranes are present essentially as a bilayer between the growth temperature (25–30 °C) and −20 °C; furthermore, the total lipids extracted from chromatophore membranes form a smectic lamellar phase over the same temperature range when dispersed in aqueous solvents. DSC indicates that the lipids in chromatophore membranes undergo a broad, reversible, endothermic phase transition of low enthalpy between about −10 and 10 °C. There are several irreversible, endothermic transitions between about 40 and 90 °C which have been tentatively assigned to membrane proteins. In protein-free lipid bilayers made from the lipid extract, the phase transition is shifted to lower temperatures by about 20 °C. At the growth temperature, the chromatophore membrane is therefore functioning well above the lipid phase transition, possibly in the liquid-crystalline state. ^{31}P NMR indicates that the motionally averaged conformation of the phosphodiester group in chromatophore membranes is very similar to that in liquid-crystalline bilayers made from the lipid extract or from pure phosphatidylethanolamine or phosphatidylglycerol. Lipid–protein interactions in chromatophore membranes, therefore, have no detectable effect on this average conformation. ESR spin-labeling shows that at the growth temperature phospholipid bilayers made from the lipid extract are tightly packed and highly ordered. In chromatophore membranes, the phospholipids are even more highly ordered, and the value of the order parameter, $S_{33} = 0.75$, is among the highest reported so far and comparable to that measured in the purple membrane of *Halobacterium halobium*. The effect of membrane protein is to increase the packing order by ~20% and to raise the lipid phase transition temperature by about 20 °C.

In recent years, some components of the photosynthetic membrane of purple, non-sulfur bacteria such as *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* have been isolated and some of their properties studied in isolated systems (Clayton, 1978; Cogdell, 1982). However, little is known about the physical structure and thermodynamic state of the photosynthetic membrane. In this paper, some fundamental structural and motional properties of the phospholipids in the intact membrane and in their extracted form have been investigated by using differential scanning calorimetry, ^{31}P NMR, and electron spin resonance (ESR) spin-labeling.

Materials and Methods

Cultures of the carotenoidless mutant *Rhodospirillum rubrum* G-9 were grown at low light intensity, and chromatophores were prepared as described previously (Snozzi & Bachofen, 1979). Freeze–fracture electron microscopy revealed that the membrane preparation consists of vesicles bounded by a single membrane, the chromatophore membrane. The size ranged between 50 nm and 0.5 μm, with most vesicles being between 50 and 200 nm (data not shown). Unless otherwise stated, chromatophore membranes were dispersed in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, and 5 mM ethylenediaminetetraacetic acid (EDTA) and handled in the dark. Protein concentrations were determined by using the modified Lowry assay of Peterson (1977) with bovine serum albumin as the standard. The native state of the chromatophores was checked by the near-IR absorption spectrum. When stored at 4 °C, they were stable over several days.

Preparation of the Extracted Lipids. Chromatophores (0.4 g of total protein dispersed in 18 mL of buffer) were extracted twice under nitrogen with 65 mL of CH_2Cl_2/CH_3OH (1:2 v/v) until the protein pellet was free of bacteriochlorophyll (Ames, 1968). The blue organic phase obtained after centrifugation was dried under a stream of N_2 to a small volume (approximately 4 mL), applied to a Sephadex LH 20 column (30 × 2.5 cm) equilibrated with CH_2Cl_2/CH_3OH (3:1 v/v), and eluted with the same solvent. This separated the lipids from

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extracted proteins which were eluted in the void volume. The fractions containing the lipids were pooled, and after the volume was reduced in a N_2 stream to about 2 mL, the fractions were applied to a silica gel column (30 × 2.5 cm). Bacteriochlorophyll and a red pigment, probably rhodoquinone, were eluted with CH_2Cl_2/CH_3OH (9:1 v/v) followed by the phospholipids with $CH_2Cl_2/CH_3OH/H_2O$ (25:25:4) and with CH_3OH alone. Phospholipid-containing fractions were pooled, dried under high vacuum in a round-bottom flask, and weighed. The lipids were redissolved in a small volume of CH_2Cl_2 containing a few drops of CH_3OH and stored at −22 °C in a sealed tube. In this form, the lipids, which were protein free, were stable for several weeks. The chromatophore lipids were analyzed by thin-layer chromatography using $CHCl_3/CH_3OH/H_2O$ (85:30:3 v/v) as the solvent. Spots were detected by iodine vapor followed by detection with a molybdate or a ninhydrin spray. Dispersions of the lipid extract in buffer were made as described below.

Differential Scanning Calorimetry (DSC). DSC was performed by using a Perkin-Elmer DSC 2 instrument. Pelleted chromatophores were scraped into stainless-steel pans which were hermetically sealed by pressure and immediately transferred to the DSC instrument. Before the first heating run, the samples were cooled to 255 K and then heated at 2.5 K/min.

^{31}P NMR Measurements. Chromatophore membranes were pelleted and transferred to NMR tubes. Lipids extracted from chromatophore membranes were dried under high vacuum in an NMR tube overnight. Dispersions of the lipid extract were prepared by adding 50 wt % buffer to the dried lipid and vortexing. Alternatively, the lipids were dispersed in buffer, the liposomes formed were pelleted by centrifugation, and the pellet was filled into the NMR tube.

^{31}P NMR spectra were recorded at 121.5 MHz in the Fourier-transform mode using a Bruker CXP 300 spectrometer equipped with a high-power proton decoupler. The spectral width was about 31 kHz, and a line broadening of 80 Hz was used for all spectra. Spectra were accumulated by using the Hahn spin–echo sequence $[90(+x)-\tau-180(+x)-\tau-AQ-PD]_n$ with a 90° pulse of 3 μs, a refocusing time τ of 90 μs, an aquisition time (AQ) of 150 ms, and a pulse delay time (PD) of 8–10 s. The refocusing time was determined with a sample of 1-palmitoyl-2-oleoylphosphatidylcholine dispersed in the standard buffer. The observation frequency was set at approximately +69 ppm from the isotropic frequency, i.e., outside the powder spectrum at the $\sigma_\perp$ side to avoid effects of pulse distortion. Great care was taken to find the optimal decoupling conditions. Continuous decoupling with a power of about 20 W was used except that the decoupler was switched off during the pulse delay, thus avoiding heating of the sample. ^{31}P T_1 relaxation measurements were performed according to the inversion recovery method using a $[180(+x)-\tau-90(+x)-AQ-PD]_n$ pulse sequence where τ was varied from 1 ms to 12 s. The relaxation time (T_1) was found to be independent of chemical shift; hence, T_1 values were derived from the most intense peak in the powder spectrum (corresponding to $\sigma_\perp$) by using a linear least-squares routine.

ESR Spin-Labeling. The fatty acid spin-label 2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxy (5-doxylstearic acid) was incorporated into chromatophore membranes or into lipids extracted from this membrane. ESR spectra were recorded at 9.16 GHz and evaluated as described before (Hauser et al., 1982). Spectra were measured by using the following temperature sequence: measurements were started at 30 °C, and the sample was cooled in 2 °C steps to −30 °C. The sample was then warmed slowly to 30 °C and then heated in 2 °C steps to 60 °C. Measurements to 80 °C were made in 5 °C steps.

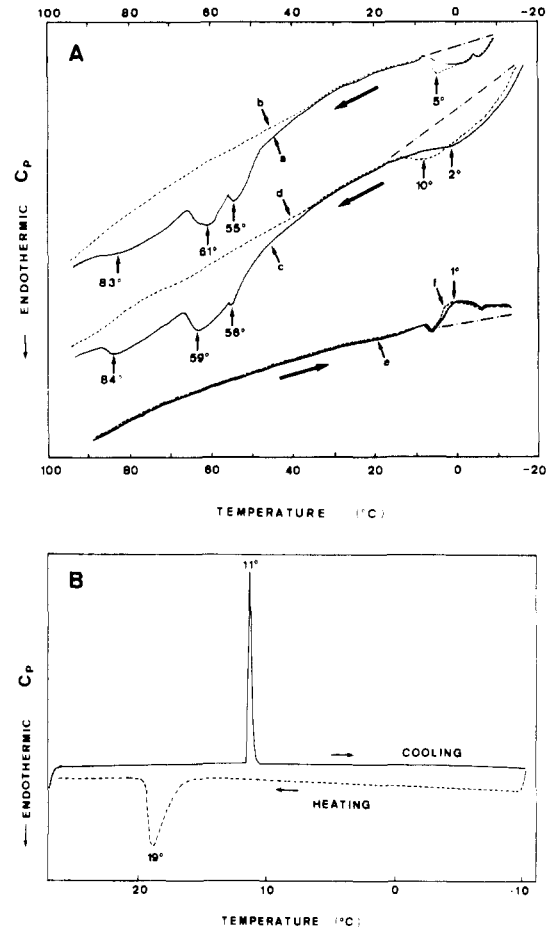

FIGURE 1: (A) Differential scanning calorimetry (DSC) of chromatophore membranes from *R. rubrum*. Curves a and b represent the first and second heating runs of the same membrane sample, and curves c and d are the analogous runs for membranes which had been suspended in 15% glycerol at −15 °C prior to measurement. Curves e and f show the first and second cooling curves, respectively, of the sample shown in curves a and b. The intermittent dashed line (–·–) is an arbitrary base line produced by extrapolation of the high-temperature base line of the lipid phase transition. (B) DSC curves of the extracted lipids dispersed in water (~1:2 w/w lipid:water ratio). The sample was equilibrated at 27 °C for 10 min prior to the first cooling run.

RESULTS

Differential Scanning Calorimetry (DSC). Figure 1 shows heating and cooling curves of native membranes and aqueous dispersions made from extracted lipids. Chromatophore membranes show two main transition regions, one between −10 and 8 °C and another extended region between about 40 and 90 °C with peak temperatures of about 55 and 60 °C and a broad hump at approximately 80 °C (Figure 1A). The low-temperature transition was reversible: cooling scans showed reproducibly an exothermic transition shifted by a few degrees toward lower temperatures (Figure 1A, curves e and f). For a second heating curve (Figure 1A, curve b, dashed line) obtained after heating chromatophore membranes to about 90 °C followed by cooling to about −15 °C, the enthalpy of the low-temperature transition region was slightly increased. The onset temperature of this region remained the same, whereas the high-temperature region was completely abolished. On the basis of these observations, the low- and high-temperature transitions were assigned to lipid and protein, re-

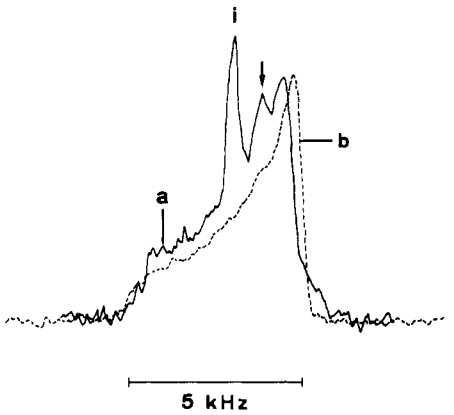

FIGURE 2: ^{31}P NMR spectra of chromatophore membranes from *R. rubrum* (solid line, a) and of an unsonicated lipid dispersion (dotted line, b) made from the total lipids extracted from chromatophore membranes. The chromatophore membranes dispersed in buffer (0.01 M Tris-HCl, pH 8.0, and 5 mM EDTA) were pelleted at 100000*g*. Liposomes dispersed in the same buffer were incubated at −30 °C for about 1 h prior to the NMR measurement. ^{31}P NMR spectra were recorded at 20 °C, and about 5000 and 1200 scans were required for chromatophore membranes (30–50 mg of lipid/mL) and liposomes (~30%), respectively. The arrow indicates the high-field edge of possibly a second bilayer component. The central, relatively narrow peak i resonates at the isotropic frequency.

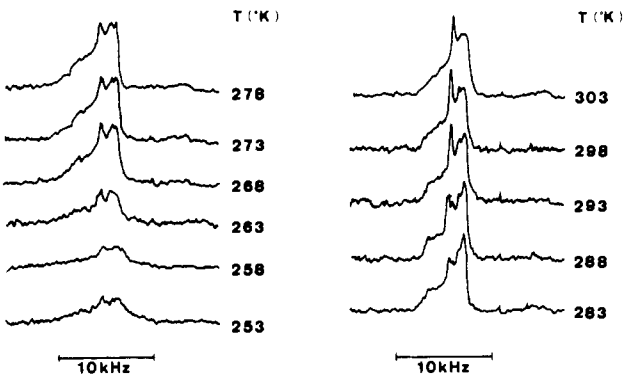

FIGURE 3: Temperature dependence of the ^{31}P NMR spectra of pelleted chromatophore membranes from *R. rubrum* dispersed in buffer (0.01 M Tris-HCl, pH 8.0, and 5 mM EDTA).

spectively. Unfortunately, the freezing of water, which in the membrane pellet occurred at about −15 °C, obscured the lipid phase transition at temperatures less than −10 °C. Addition of 15% glycerol to depress the freezing point of water broadened the lipid phase transition, and its onset temperature was reduced to about −15 °C (Figure 1A, curve c). Figure 1B shows the heating and cooling curves obtained with liposomes made from the extracted chromatophore lipids. The first cooling run gave a sharp exothermic transition at 11 °C, and the first heating run showed an endothermic peak at 19 °C, the area of which was about twice that of the exothermic peak. This behavior was observed reproducibly on subsequent cooling and heating cycles. However, after storage of the sample at 4 °C for 2 days, there was no detectable transition between −10 and −60 °C except for a very smmall peak at about 8 °C (data not shown).

^{31}P NMR. The ^{31}P NMR spectrum recorded from a pellet of chromatophore membranes at 20 °C is shown in Figure 2a. The spectrum of an unsonicated lipid dispersion made from the total lipid extract of chromatophore membranes is included for comparison (Figure 2b). Chromatophore membranes give a composite spectrum, its main component being characteristic of phospholipid molecules in liquid-crystalline bilayers undergoing rapid rotation about the bilayer normal. This interpretation is also true for the lipid extract (Figure 2b). At the growth temperature (25–30 °C), the chemical shielding anisotropy of the major component of the chromatophore spectrum is $\Delta\sigma \simeq -41$ ppm and thus very close to the value derived from the spectrum of the lipid extract (Figure 2b). This value is also in good agreement with that measured for aqueous dispersions of pure phosphatidylethanolamine or phosphatidylglycerol in the liquid-crystalline phase (data not shown). Besides the axially symmetric powder spectrum, there is an isotropic component in the ^{31}P NMR spectrum of chromatophore membranes (Figure 2a) that is observeed over the total temperature range shown in Figure 3. It is present even at −20 °C where the bulk water is frozen. Its intensity is fairly constant (6 ± 1%) over the temperature range of −20 to 30 °C. Between the isotropic peak and the high- field edge of the spectrum ($\sigma_\perp$), there is yet another peak (see arrow in Figure 2a) which is detectable over most of the temperature range shown (Figure 3, left panel). Assuming it is the high-field edge of a second axially symmetric powder spectrum, an estimate of its shielding anisotropy, $\Delta\sigma$, may be obtained from

$$|\Delta\sigma| = \frac{3|\nu_\perp - \nu_i|}{\nu_0} = 25 \text{ ppm}$$

where $\nu_\perp$ and ν_i are the frequencies of the high-field edge of this peak (arrow) and the isotropic peak, respectively, and ν_0 is the ^{31}P resonance frequency. The $\Delta\sigma$ value is smaller than that of an aqueous cardiolipin dispersions ($\Delta\sigma \simeq 30$ ppm; Cullis et al., 1978).

Figure 4A shows the temperature dependence of the ^{31}P NMR spectra recorded from lipid dispersions made from the extracted lipids. The ^{31}P NMR spectrum of the lipid extract consists of a predominant narrow peak at the isotropic frequency and a second minor component which is an axially symmetric powder spectrum. The latter is more noticeable at lower temperatures. The effect of freezing the lipid dispersion and holding it at −20 °C for 1 h is shown in Figure 4B. This treatment effected a marked change in the ^{31}P NMR spectrum in as much as the isotropic peak was replaced by an axially symmetric powder spectrum. Upon heating this dispersion from −20 °C, the isotropic peak appeared between 25 and 30 °C (Figure 4B), and its intensity increased with increasing temperature. These changes were reversible. Below about −10 °C, the spectra broadened gradually, indicating that the axial rotation of the phospholipid molecules is slowed down; however, even at −25 °C, this motion was apparently still sufficient to produce an axially symmetric shielding tensor. Only at −30 °C was the spectrum drastically broadened, probably reflecting the immobilization of the phosphate group. Figure 5 shows the temperature dependence of the chemical shielding anisotropy $\Delta\sigma$ for both chromatophore membranes and the lipid dispersion made from the extracted lipids. At the growth temperature (25–30 °C), very similar $\Delta\sigma$ values were measured for both systems. For chromatophore membranes, $\Delta\sigma$ remained fairly constant between 10 and 30 °C; below about 10 °C, $\Delta\sigma$ decreased from about −39 to −48 ppm at −5 °C. At even lower temperatures, significant line broadening precluded the determination of $\Delta\sigma$. In contrast, $\Delta\sigma$ of the lipid extract decreased steadily from 35 °C to about −10 °C below which temperature $\Delta\sigma$ dropped markedly, similar to the behavior observed for chromatophore membranes at temperatures below ~10 °C.

When the ^{31}P powder spectra of intact chromatophore membranes were plotted as a function of the delay time τ, there was no apparent dependence of the spectral phase on the chemical shift (data not shown). Hence, the most intense high-field peak ($\sigma_\perp$) was used to determine T_1. At the ac-

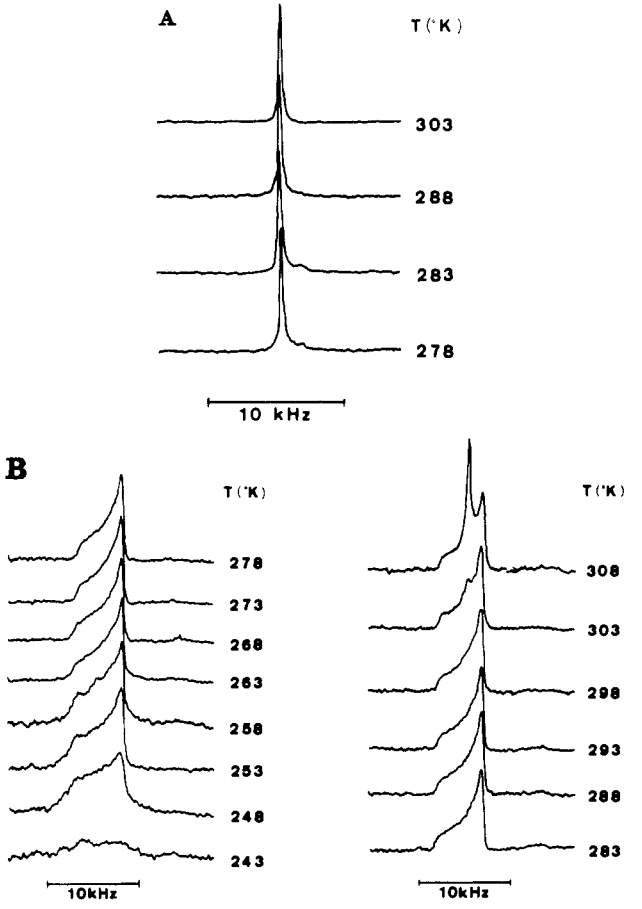

FIGURE 4: (A) Temperature dependence of the ^{31}P NMR spectra of freshly prepared lipid dispersions made from the lipid extract of chromatophore membranes. About 60% lipid dispersions in buffer (0.01 M Tris-HCl, pH 8.0, and 5 mM EDTA) were prepared as described under Materials and Methods, the dispersion was pelleted by centrifugation, and the pellet was transferred to the NMR tube. (B) The lipid dispersion in buffer as described under (A) was incubated at −20 °C for 1 h, and ^{31}P NMR spectra were recorded at the indicated temperatures.

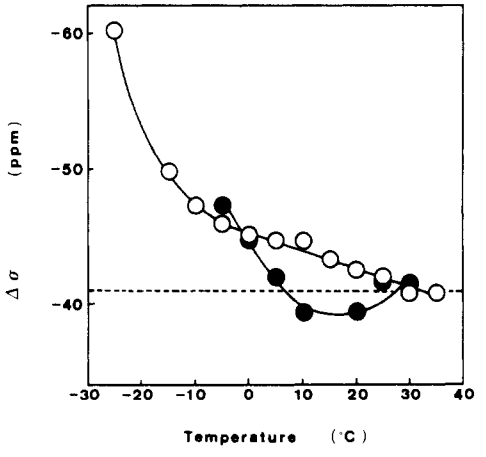

FIGURE 5: Temperature dependence of the ^{31}P chemical shielding anisotropy, $\Delta\sigma$, for chromatophore membranes (*R. rubrum*) in buffer (●) and for lipid dispersions made from the lipid extract (○) in the same buffer (0.01 M Tris-HCl, pH 8.0, and 5 mM EDTA). $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$ was derived from the low-filed ($\sigma_{\parallel}$) and high-field edges ($\sigma_{\perp}$) of the ^{31}P NMR spectrum.

curacy of our measurement, the relaxation process is apparently described by a single exponential (Figure 6A). The T_1 value thus obtained for bilayer phospholipids in chromatophore membranes was 1.2 ± 0.1 s. For the isotropic component, the T_1 relaxation time was about 10% longer, T_1 = 1.4 ± 0.1 s.

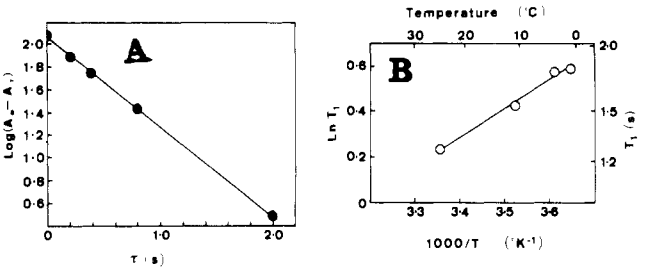

FIGURE 6: (A) Log $(A_\infty - A_\tau)$ as a function of τ where A_∞ and A_τ are the signal heights of the high-field peak (corresponding to $\sigma_\perp$) at $\tau \rightarrow \infty$ and τ (in seconds), respectively. (B) Arrhenius plot of T_1 values measured at four different temperatures.

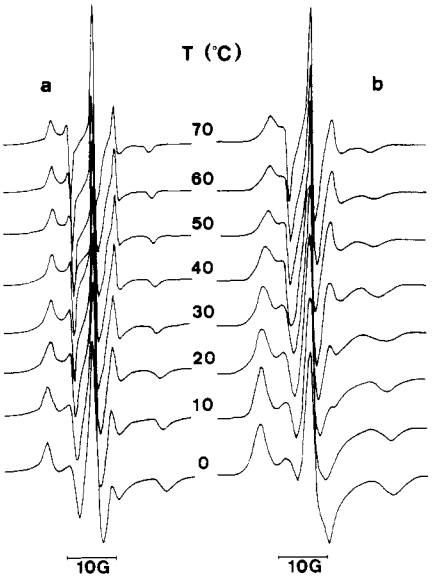

FIGURE 7: Temperature dependence of the ESR first-derivative spectra of 5-doxylstearic acid incorporated in liposomes made from the lipid extract (a) and in chromatophore membranes (*R. rubrum*) (b). Experimental spectra were digitized and normalized by using the intensity of the central peak. Temperatures (in degrees centrigrade) are indicated between the two sets of spectra.

The temperature dependence of T_1 for the bilayer phospholipid is shown in Figure 6B. The positive slope of the Arrhenius plot (Figure 6B) indicates that the rate of reorientation of the phosphate group is in the slow correlation time regime where $\nu_0\tau >> 1$ (ν_0 is the Lamor frequency in radians per second and τ is the correlation time for motion of the phosphate group).

ESR Spin-Labeling. The temperature dependence of the ESR spectra of 5-doxylstearic acid incorporated in chromatophore membranes and in lipids extracted from these membranes is shown in Figure 7. For both systems, the spectra are characteristic of rapid but anisotropic motion, i.e., rapid rotation about the long axis of the phospholipid molecule. Below about 20 °C, the spectra from chromatophore membranes indicate increasing immobilization (Figure 7b). The spectrum at 0 °C is characteristic of highly immobilized phospholipids with the observed maximum hyperfine splitting ($A_\parallel$) approaching the A_{zz} tensor component.

In contrast, the ESR spectrum from the lipid extract at 0 °C is more mobile; the maximum hyperfine splitting ($A_\parallel \simeq$ 25 G) is significantly smaller than the A_{zz} tensor component. The temperature dependence of the maximum hyperfine splitting $A_\parallel$ and of the order parameter S_{33} of both chromatophore membranes and lipid extract is shown in panels A and B, respectively, of Figure 8. At temperatures below ~50 °C, for both parameters higher values were measured in chromatophore membranes. The values are high compared to

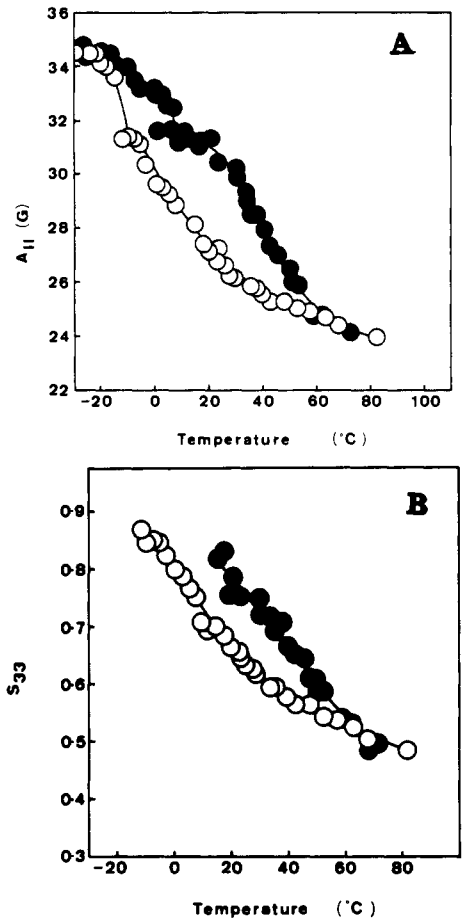

FIGURE 8: Temperature dependence of the maximum hyperfine splitting $A_{\parallel}$ (A) and the order parameter S_{33} (B) for chromatophore membranes (●) and for dispersions of lipids extracted from these membranes (○). Order parameters were derived from the ESR spectra (shown in Figure 7) at temperatures where the outer and inner hyperfine splittings, $2A_{\parallel}$ and $2A_{\perp}$, respectively, were well resolved and could be taken directly from the spectrum. The order parameter S_{33} was calculated from $S_{33} = (A_{\parallel} - A_{\perp})/[A_{zz} - {}^1/_2(A_{xx} + A_{yy})](a_0/a) = \langle 3\cos^2\theta - 1\rangle/2$ where A_{xx}, A_{yy}, and A_{zz} are the principal components of the hyperfine splitting tensor taken from Hubbell & McConnel (1971), a_0/a is the correction factor for the polarity dependence of the hyperfine splittings (Hauser et al., 1982), and θ is the time-dependent angle between the direction of the nitrogen $2p\pi$ orbital and the director axis (=bilayer normal). The broken brackets indicate that the time average of the expression is taken.

mammalian plasma membranes but comparable to those measured in other bacterial membranes (Hauser et al., 1982).

At the growth temperature (25–30 °C) of the cells, the values obtained were $A_{\parallel} \simeq 30$ G and $S_{33} \simeq 0.7$. The lowest temperature at which S_{33} could still be derived directly from the observed ESR spectrum was about 10 and −10 °C for chromatophore membranes and lipid dispersions, respectively. Below these temperatures, the molecular motion was restricted so that the hyperfine splitting $A_{\perp}$ could no longer be taken from the ESR spectrum. At these minimum temperatures, the molecular order is comparable in chromatophore membranes and in the lipid extract as is evident from the similar values of $S_{33} \simeq 0.85$ (Figure 8B). It is interesting to note that the minimum temperatures at which the order parameter could be measured are in good agreement with the onset temperatures at which the chemical shielding anisotropy $|\Delta\sigma|$ undergoes a significant increase (Figure 5).

Discussion

Phospholipids of the chromatophore membrane from *R. rubrum* consist of phosphatidylethanolamine (~65%), phosphatidylglycerol (~25%), and cardiolipin (~10%), and the phospholipid/protein ratio has been determined to be 0.62 μmol of phosphate/mg of protein (Snozzi & Bachofen, 1979). The ^{31}P NMR spectra of chromatophore membranes indicate that most of the phospholipid (~90%) is present as a bilayer, the remaining ~10% being contributed by an isotropic component. The same is true for aqueous lipid dispersions made from the lipid extract. This is consistent with ^{31}P NMR studies on *Escherichia coli* phospholipids of a similar composition (Gally et al., 1981).

The reversible, broad, low-temperature transition observed in chromatophore membranes may be assigned to membrane lipids, probably being due to the gel to liquid-crystal transition of bilayer lipids, whereas the irreversible high-temperature transitions may be assigned to membrane proteins. The latter transition was suggested to be due to changes in protein quaternary and possibly tertiary structure but was shown not to involve protein secondary structure (Ghosh et al., 1984).

The thermal behavior of the phospholipid extract is straightforward after incubation of the lipid dispersion at 4 °C: there was no apparent endothermic transition over the temperature range −10 to 60 °C. Both NMR and ESR data suggest that in the absence of membrane proteins the lipid phase transition is lowered by about 20 °C; it is probably obscured by the water peak. The effect of membrane protein is to shift the phase transition temperature to higher values. Such a shift has been observed for some membranes, e.g., the cell membranes of *Mycoplasma laidlawii* (Steim et al., 1969), brush border membranes (Hauser et al., 1982; Mütsch et al., 1983), rat liver mitochondrial membranes (Blazyk & Steim, 1972), submitochondrial particles (Blazyk & Newman, 1980), and sarcoplasmic reticulum (R. Ghosh and J . Seelig, unpublished results), but not for others, e.g., model membranes reconstituted from a single pure lipid and a single membrane protein. In these model systems, the transition apppeared to be broadened in the presence of membrane protein, but the T_c temperature was little affected even at lipid/protein ratios present in biological membranes. This was observed for reconstituted membranes of Ca^{2+}-ATPase (Seelig et al., 1981), cytochrome *c* oxidase (Tamm & Seelig, 1983), and rhodopsin (Bienvenue et al., 1982). No explanation can be offered for this phenomenon presently; it must await further structural studies.

The value of the chemical shielding anisotropy $\Delta\sigma$ at 30 °C is in good agreement with that of protein-free lipid bilayers made from the lipid extract. It is also in good agreement with $\Delta\sigma$ values measured for bilayers of pure phosphatidylethanolamine and phosphatidylglycerol in the liquid-crystalline state. This indicates that the motionally averaged conformation of the phosphate group in pure phosphatidylethanolamine or phosphatidylglycerol bilayers and in mixed bilayers of the two phospholipids containing about 10% cardiolipin is the same; furthermore, lipid–protein interactions have no detectable effect on this average conformation.

There is a difference in the temperature dependence of $\Delta\sigma$ between chromatophore membranes and the lipid extract. For chromatophore membranes, $|\Delta\sigma|$ shows a marked increase below about 10 °C (Figure 5), and this change in $\Delta\sigma$ coincides with the onset temperature of the lipid phase transition observed by DSC. The increase in $|\Delta\sigma|$ has been shown to reflect accurately thermal transitions (Seelig, 1978) and corresponds presumably to an increase in the C(1)–C(2) order parameter of the glycerol backbone (Gally et al., 1976). For the extracted lipids, the onset temperature for the increase in $|\Delta\sigma|$ occurs at −10 °C (Figure 5), but the plateau region of the gel phase

is not observable due to substantial line broadening (see below). If this increase in $|\Delta\sigma|$ indeed indicates a lipid phase transition, then we can conclude that in the protein-free lipid bilayer this transition occurs at temperatures below about −10 °C, i.e., about 20 °C lower than in chromatophore membranes. This conclusion is not unreasonable considering the lipid and fatty acid composition of the chromatophore membrane (Snozzi & Bachofen, 1982). Since 16:1 and 18:1 fatty acyl chains account for ~75% of the hydrocarbon chains in the membrane, the predominant phospholipid would be phosphatidylethanolamine with these two unsaturated chains. Dipalmitoleoyl- and dioleoylphosphatidylethanolamine were shown to have gel to liquid-crystal phase transitions at −33.5 and −16 °C, respectively. From this, the phase transition of the protein-free lipid extract is expected to be at temperatures well below −10 °C.

The isotropic component observed in the ^{31}P NMR spectra of chromatophore membranes amounts to less than 10% of the observed spectrum. Its intensity appears to remain fairly constant between 25 and −20 °C at which temperature the phospholipid head groups become completely immobilized. Isotropic structures have been frequently observed in the ^{31}P NMR spectra of natural membranes (de Kruijff et al., 1978; Burnell et al., 1980) and also in reconstituted systems (Rajan et al., 1981), but their molecular origin is a matter of some controversy. In view of the similarity of the T_1 relaxation time of the isotropic component and the anisotropic bilayer components and the fact that the isotropic peak also occurs in the spectra of extracted lipids (Figures 3 and 4B), this component is likely to be a lipid. However, we cannot distinguish between an isotropic lipid structure within the bilayer (Wieslander et al., 1980) and a special head-group conformation giving rise to a single ^{31}P signal. In the latter case, some lipid phosphate groups must have a structure such that magic-angle rotation would lead to the collapse of $\Delta\sigma$ to a singlet (Thayer & Kohler, 1982).

The origin of the second anisotropic component ($\Delta\sigma \simeq -25$ ppm) in the ^{31}P NMR spectrum is unknown. Its unambiguous assignment requires more work.

The ESR spectra of 5-doxylstearic acid incorporated in chromatophore membranes are characteristic of rapid, anisotropic motion. This is also true for the label incorporated in lipid bilayers made from the lipid extract. The ESR results are consistent with the label being present in a lipid bilayer undergoing fast rotation ($>10^8$ s^{-1}) about the molecular long axis or the bilayer normal. This interpretation is also consistent with the ^{31}P NMR data obtained for the bulk phospholipid. In contrast to ^{31}P NMR, the ESR spectra show no isotropic component. The explanation for this is that the molecular motion responsible for the isotropic component of the ^{31}P NMR spectrum is not fast enough on the ESR time scale. It therefore does not contribute to the averaging of the hyperfine splitting tensor [cf. Schreier-Muccillo et al. (1973)].

The order parameter S_{33} is about 20% higher in chromatophore membranes than in dispersions of the lipid extract (Figure 8B), at least at temperatures below ~50 °C. The effect of membrane proteins upon the lipids is therefore to increase the order of the hydrocarbon chain packing. At the growth temperature (30 °C), the order parameter S_{33} is 0.75 and 0.62 for chromatophore membranes and the protein-free lipid bilayer, respectively. A comparison with other biological membranes shows that these values are among the highest observed. They are comparable to those measured in other bacterial membranes, e.g., in red membranes and extracted lipids thereof from *Halobacterium cutirubrum* (Esser & Lanyi, 1973) and in purple membranes from *Halobacterium halobium* (Chignell & Chignell, 1975). At the growth temperature (37 °C) of the latter bacterium, the maximum hyperfine splitting, $A_\parallel$ = 29.5 G, is in close agreement with $A_\parallel$ = 30 G measured at the growth temperature (30 °C) of *R. rubrum*. This holds for other equal relative temperatures referred to the growth temperature. Thus, we can conclude that the packing of the hydrocarbon chains in chromatophore membranes from *R. rubrum* is equivalent to that in the purple membrane of *H. halobium*. The order parameter of the lipid extract from *R. rubrum* at the growth temperature (30 °C), S_{33} = 0.62, is comparable to that of dipalmitoylphosphatidylcholine at 30 °C. This indicates that there is tight packing and a high order even in the protein-free lipid bilayer. In the presence of protein, this packing order is further increased as evident from Figure 8.

A comparison of the ESR results with the DSC and NMR data shows that the lipid phase transition manifests itself as a discontinuity in the temperature dependence of $A_\parallel$ (Figure 8A). Moreover, the onset temperature of the lipid phase transition coincides with the point where the rotational motion about the long axis of the spin-label molecule is sufficiently reduced so that $A_\perp$ can no longer be directly derived from the ESR spectrum. This is true for chromatophore membranes as well as lipid bilayers made from the lipid extract (Figure 8B). Consequently, the order parameter S_{33} could not be derived from the ESR spectra (Figure 8B) at temperatures equal to or smaller than the onset temperature of the lipid phase transition. These minimum temperatures in the S_{33} vs. temperature plot (10 and −10 °C for chromatophore membranes and protein-free lipid bilayers, respectively) are in good agreement with the ^{31}P NMR data.

There are further discontinuities in the temperature dependence of $A_\parallel$ (Figure 8A) which are apparently not correlated to thermal events. This illustrates the inherent difficulty of ESR spin-labeling in detecting lipid phase changes in membranes. Such additional discontinuities may reflect protein denaturation or more general changes in the lateral packing of membrane components and in turn a redistribution of the spin-label between different membrane domains [cf. Birrel et al. (1978)].

Biological Significance of the Physical State of Membrane Lipids in Chromatophore Membrane Vesicles. The photophosphorylation in the chromatophore membrane requires the transfer of an electron across several intrinsic membrane proteins. These events occur on the time scale of 0.1–10 ms (Meinhardt & Crofts, 1982) and have been postulated to involve rotational and lateral diffusion of proteins in the plane of the membrane. Clearly, as the rate of lateral diffusion in the liquid-crystalline phase is usually 100 times faster than in the gel phase, it is most useful to determine the physical state of the chromatophore membrane lipids. The results of this study have shown that the chromatophore membrane lipids exist mainly in the liquid-crystalline bilayer state. ESR studies show that the fatty acyl chains are highly ordered, possibly as much as those of dipalmitoylphosphatidylcholine at temperatures close to the gel to liquid-crystal phase transition. The ^{31}P T_1 relaxation measurements indicate that the phosphate group reorientation, although rapid enough to average the chemical shielding tensor, lies in the slow motional regime ($\tau > 1$ ns). The values of the ^{31}P T_2 relaxation times derived from second-moment calculations are similar to those derived for dipalmitoylphosphatidylcholine bilayers entering the gel phase. These data taken together show that the chromatophore membrane is rather "rigid". Assuming that lipid motional time

scales extend to protein rotational and lateral diffusion, the data presented suggest that these protein motions are probably too slow to play an important role in electron-transport events [cf. Cherry (1979)]. This would favor an aggregate-like ordered structure of the photosynthetic unit. In support of this suggestion, Mar et al. (1981) observed that in the chromatophore membrane of *R. rubrum* S1 (wild type) the reaction center is immobilized on the millisecond time scale, as measured by time-resolved fluorescence recovery of the intrinsic chromophore (bacteriochlorophyll) in membranes where the antennae bacteriochlorophyll had been photobleached. An aggregate-like structure of the photosynthetic unit has also been suggested previously on the basis of X-ray diffraction measurements of the intact membrane (Kataoka et al., 1981).

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