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Lipid–Protein Associations in Chromatophores from the Photosynthetic Bacterium *Rhodopseudomonas sphaeroides*†

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ABSTRACT: Lipid–protein interactions were examined in chromatophores isolated from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* using lipid spin-labels. The chromatophores contain fluid bilayer and a significant amount of lipid immobilized by membrane proteins. For a typical preparation of cells grown under 600 ft-c illumination, 59% of the spin-labeled fatty acids were bound. Essentially the entire length of the 18-carbon fatty acid chain was immobilized, judging from results obtained with the spin-label at the 7, 12, and 16 positions. The amount immobilized varies directly with the bacteriochlorophyll content of the chromatophore material, suggesting that a significant fraction of the lipid spin-labels is immobilized on the hydrophobic surfaces of the chlorophyll-binding proteins. Changing the lipid spin-label head group from a negatively charged carboxyl group to a positively charged quarternary amine greatly decreased the amount of immobilized lipid. The changes in immobilized lipid with light level and polar head group suggest that the anntenna bacteriochlorophyll-binding proteins preferentially associate with negatively charged lipids.

Biological membranes consist largely of protein and lipid. The lipid forms a two-dimensional fluid bilayer in which integral membrane proteins are embedded. It is known that lipid–protein interactions are important to membrane function (Griffith & Jost, 1978; Gennis & Jonas, 1977). These can be viewed as consisting of a hydrophobic interaction between the lipid chains and nonpolar protein residues and an ionic interaction between lipid polar head groups and the protein at the aqueous interface. The available evidence suggests that the hydrophobic lipid tails are immobilized by binding to hydrophobic surfaces of integral membrane proteins and that the immobilized lipid forms a boundary layer or annulus around the protein (Jost et al., 1973a). This layer is apparently required for activity. The boundary layer lipid does not exhibit the usual segmental motion seen in fluid bilayer (Jost et al.,

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1973a). This generalization is drawn from spin-labeling studies on proteins isolated from mammalian membranes with detergents and subsequently incorporated in artificial phospholipid vesicles. No quantitative information is available on intact membranes or integral membrane proteins which have not been exposed to detergents during isolation.

For several reasons, it seemed worthwhile to quantitate bound lipid and fluid bilayer in the photosynthetic membranes (chromatophores) of the photosynthetic bacterium *Rhodopseudomonas sphaeroides*. In the first place, little information is currently available on the physical state of lipids in photosynthetic membranes. Secondly, it is fairly easy to obtain homogeneous preparations of photosynthetic membranes from this bacterium without the use of detergents. In cell extracts of *Rp. sphaeroides* the photosynthetic membranes appear as vesicles, the chromatophores, about 60 nm in diameter. Chromatophores can be separated from both soluble material and cell-wall outer-envelope material (Niederman & Gibson, 1978).

Although polyacrylamide gel electrophoresis of chromatophore material solubilized in sodium dodecyl sulfate ($NaDodSO_4$)[1] reveals a dozen or so polypeptides (Takemoto & Huang Kao, 1977), more than 50% of chromatophore protein can be accounted for by two proteins: reaction-center protein and a protein apparently associated with light harvesting bacteriochlorophyll (LH-BChl). The reaction-center protein has a molecular weight of about 80 000 (Okamura et al., 1974) and accounts for 20–25% of chromatophore protein (Clayton & Haselkorn, 1972). This protein with its associated pigments and prosthetic groups is responsible for the photochemistry. Only a small fraction of the BChl in chromatophore material is in the reaction centers; the bulk of the pigment collects and transfers energy to reaction centers. This light-harvesting BChl is apparently associated with the second major protein component. This is a very small polypeptide, at least in $NaDodSO_4$-solubilized material, with a molecular weight of around 10 000 (Clayton & Clayton, 1972). For brevity, we shall refer to this protein as LH protein. It can constitute up to 50% of the chromatophore protein (Fraker & Kaplan, 1972). It has been known for some time that the specific BChl content of chromatophore material of *Rp. sphaeroides* can be experimentally manipulated by varying the light intensity in which the cells are grown: the higher the light intensity, the smaller the specific BChl content (Worden & Sistrom, 1964). More recently, it has been shown that the amounts of LH-BChl (Aagaard & Sistrom, 1972) and of LH protein (Takemoto & Huang Kao, 1977) vary in concert with the specific BChl content. Thus, simply by varying the light intensity during growth, it is possible to obtain chromatophore membrane preparations which differ primarily in their contents of a single kind of protein. This approach has enabled us to monitor lipid–protein interactions in the intact chromatophores and to characterize, at least in a tentative way, the lipid-binding properties of a specific protein without isolation.

Materials and Methods

Growth Conditions. Rhodopseudomonas sphaeroides strain Ga (Griffiths & Stanier, 1956) was grown on photosynthetic medium M22 of Sistrom (1977) with malic acid replacing lactic acid and supplemented with 0.1% (w/v) casamino acids. Illumination of 8500 and 600 ft-c (measured with a Weston light meter) was provided by banks of six 300-W and four 150-W flood lamps, respectively. One 75-W flood lamp was used for 20 ft-c illumination. The cultures were bubbled with 95% N_2–5% CO_2. Cells were harvested during exponential growth at a concentration of $\sim 1.6 \times 10^9$ bacteria/mL.

Chromatophore Preparation. The bacteria were harvested according to the method of Fraker and Kaplan (1971) and passed through a French pressure cell twice at 20 000 psi. The cell extracts were centrifuged at 27 000*g* for 15 min and the pellet was discarded. The supernatant was centrifuged at 150 000*g* for 1.5 h and the supernatant discarded. The crude chromatophores were resuspended in 0.1 M sodium phosphate (pH 7.6) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and layered on top of a sucrose density gradient [20–40% (w/v) in the same buffer]. The gradients were centrifuged at 65 000*g* for 12 h. This separated the pigmented material into two fractions, a denser turbid band and a less dense, transparent and highly pigmented band. The lower band was discarded. The upper band was collected, centrifuged, resuspended in 0.1 M sodium phosphate (pH 7.6) containing 1 mM EDTA and 0.25 M sucrose, and dialyzed against the same buffer.

The phosphate–EDTA buffer was chosen to maximize the yield and purity of the chromatophore preparations (Fraker & Kaplan, 1971; Niederman & Gibson, 1978). The most likely source of contamination in chromatophore preparations is cell-wall outer-envelope material. We used the content of 2-keto-3-deoxyoctonate (KDO) as a measure of outer-envelope contamination. Chromatophores from cells grown at 600 ft-c contained 2 μg of KDO/mg of chromatophore protein, and chromatophores from cells grown at 8500 ft-c contained 4 μg of KDO/mg of protein. Ding and Kaplan (1976) report that outer-envelope preparations from aerobically grown (nonpigmented) *Rp. sphaeroides* contained approximately 90 μg of KDO/mg of protein. This value agrees roughly with those reported by Osborn et al. (1972) for outer-envelope material from *Salmonella typhimurium*. On the basis of these figures, we conclude that not more than 2–4% of the protein in our chromatophore preparations comes from the outer envelope.

The ratio of absorbance at 260 nm to that at 280 nm was very nearly equal to 1, indicating minimal contamination by RNA.

Chromatophore Analyses. Lipid phosphorus was determined by the method of Lowry and Tinsley (1974) after extraction of the lipids from the protein with chloroform–methanol (Bligh & Dyer, 1959). The results are expressed as phospholipid by multiplying lipid phosphorous by 25. Chromatophore protein was estimated by the method of Lowry et al. (1951). BChl was measured by the method of Cohen-Bazire et al. (1957) using a value of 59 for the millimolar extinction coefficient of BChl (Cohen-Bazire & Sistrom, 1966). KDO was determined using the method of Weissbach and Hurwitz (1959) and the micromolar extinction coefficient of Osborn (1963). Absorption spectra were recorded on a Cary Model 14R spectrophotometer.

Lipid-Depleted Chromatophores. The phospholipid to protein ratio of most chromatophore preparations was 0.33 mg of phospholipid/mg of protein. In order to prepare lipid-depleted chromatophores, a procedure was followed similar to that previously applied to cytochrome oxidase by Yu et al. (1975). Seventeen milliliters of a chromatophore preparation containing 2 mg of protein/mL (from bacteria grown at a light intensity of 600 ft-c) was solubilized in 1% sodium cholate. Enough saturated ammonium sulfate was then added to bring the preparation to 35% of saturation. The mixture was stirred for 1 h at 0 °C and centrifuged at 12 000*g* for 10 min. The

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; LH-BChl, light harvesting bacteriochlorophyll; KDO, 2-keto-3-deoxyoctonate; EDTA, ethylenediaminetetraacetic acid; ESR, electron spin resonance.

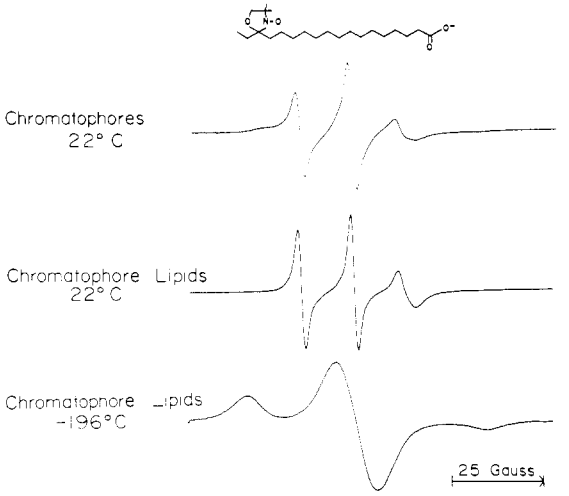

FIGURE 1: ESR spectra of the spin-label, 16-doxylstearic acid, diffused into chromatophores of *Rhodopseudomonas sphaeroides* and the lipids extracted from these chromatophores. The arrow in the top spectrum indicates the immobilized component which appears in the spectrum of the label in chromatophores at room temperature but is absent in the chromatophore lipids spectrum. A reference spectrum of the label in the corresponding lipids at −196 °C is shown at the bottom. The chromatophores were isolated from bacteria grown under an illumination of 600 ft-c.

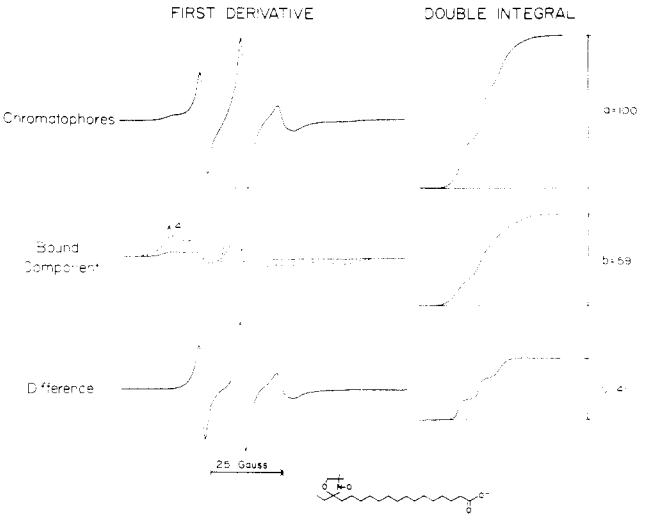

FIGURE 2: An example of a spectral subtraction to obtain the amount of spin-label immobilized. The top ESR spectrum is the same as the top spectrum of Figure 1. The center spectrum is the bound component obtained from lipid-depleted chromatophores. The bottom spectrum is the difference between the composite chromatophore spectrum and the bound component. In the spectral subtraction, incremental amounts of the bound component are subtracted from the composite chromatophore spectrum until the end point (bottom spectrum) is reached. In this case, the end point was reached when 59 ± 3% of the absorption, determined by double integration of the first derivative spectra, was removed. The ESR spectra and double integrals are scaled to reflect the relative populations of immobilized and fluid bilayer spin-labels determined to be present in the chromatophore spectrum. The dashed line bound component is scaled at four times higher intensity to show spectral detail.

pellet was resolubilized in 1 mL of 0.1 M sodium phosphate (pH 7.6) containing 1% sodium cholate. The preparation was again brought to 35% saturation with saturated ammonium sulfate, stirred on ice for 1 h, and then centrifuged at 12 000*g* for 10 min. The pellet was resuspended in 0.1 M sodium phosphate (pH 7.6) containing 1 mM EDTA and 0.25 M sucrose and dialyzed extensively against this buffer. The phospholipid to protein ratio (mg of phospholipid/mg of protein) of the delipidated preparation was 0.08.

Spin Labeling. 12-Doxyl- and 16-doxylstearic acid were obtained from Syva (Palo Alto, Calif.); 7-doxylstearic acid was prepared according to the method of Waggoner et al. (1969). 16-Doxyloctadecyltrimethylammonium methanesulfonate was the gift of Dr. J. F. W. Keana. Stock solutions of the spin labels were made in ethanol (5 mg/mL). For each experiment, 0.5 μL of the stock solution of spin-label was dried down under a stream of nitrogen in a small vial. Chromatophores containing 0.6 mg of protein were added to the vial, and the mixture was bath sonicated (0 °C) for 15 min. The spin-labeled chromatophores were centrifuged at 75 000*g* for 1 h, forming a pellet. ESR spectra of the pellets (without resuspension) were then recorded.

The lipid-depleted chromatophores were spin-labeled in the following way: 1 μL of the stock solution of spin-label was dried down in a 1-mL homogenizer. Delipidated chromatophores containing 4 mg of protein were added, and the preparation was homogenized for several minutes and then transferred to a 1-mL conical centrifuge tube. The sample was centrifuged at 12 000*g* for 10 min; ESR spectra were recorded on the resulting pellet.

ESR spectra were recorded on a Varian E-Line 9.5-GHz spectrometer interfaced to a 32K Varian 620L/100 computer. Spectra were analyzed by the procedure of Klopfenstein et al. (1972). Spin–spin exchange in the spin-labeled preparations was determined to be negligible using the criteria developed by Jost et al. (1977).

Results

The Partitioning of Fatty Acid Spin-Labels in Photosynthetic Membranes. ESR spectra of the spin-label 16-doxylstearic acid in chromatophores from *Rp. sphaeroides* and chromatophore lipids are shown in Figure 1. In the chromatophore lipids at room temperature, the spin-label is in a single environment consisting of a fluid bilayer. The chromatophore spectrum, on the other hand, is composite; some of the labels are in a fluid bilayer, but the rest of the labels are in a more immobilized environment (indicated by the arrow in the top spectrum of Figure 1). For comparative purposes, an ESR spectrum of the same label in chromatophore lipids at −196 °C, where the lipid chains are essentially rigid, is also shown.

The percentage of the absorption due to immobilized spin-label in the composite chromatophore spectrum of Figure 1 was estimated in the following way. First a spectrum of the bound component was obtained (middle spectrum, Figure 2) by incorporating the spin-label in lipid-depleted chromatophores. This spectrum was then subtracted from the chromatophore spectrum in increments until the difference spectrum was that of the spin-label in a fluid environment (bottom spectrum of Figure 2). The total absorption represented by each first derivative spectrum in Figure 2 was obtained by two integrations, the first to obtain the absorption spectrum and the second to determine the area under the absorption spectrum. In the case of the chromatophores obtained from bacteria grown at 600 ft-c, the end point in the spectral titration was reached when 59% of the total absorption was removed. Thus, 59 ± 3% of the labels are immobilized on the hydrophobic surfaces of membrane proteins present in the chromatophores. The remaining 41% of the labels are in the fluid bilayer of the membranes. The lipid immobilization is a direct result of the binding to protein. Photopigments can alter the motion of the lipid spin-labels (Oettmeier et al., 1976) but do not cause the pronounced immobilization observed in these preparations. The spectral line shapes do not distinguish between spin-labels bound to protein and spin-labels bound to protein complexed with photopigment.

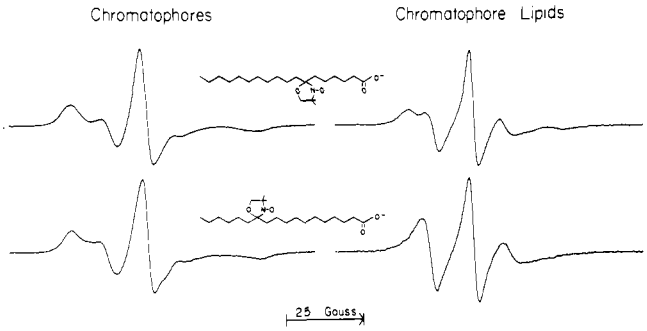

FIGURE 3: ESR spectra with the spin-label at different positions along the fatty acid chains. The top and bottom rows are 22 °C ESR spectra of the 7- and 12-doxylstearic acid labels in chromatophores and lipids extracted from the chromatophores. The chromatophores were prepared as for Figure 1.

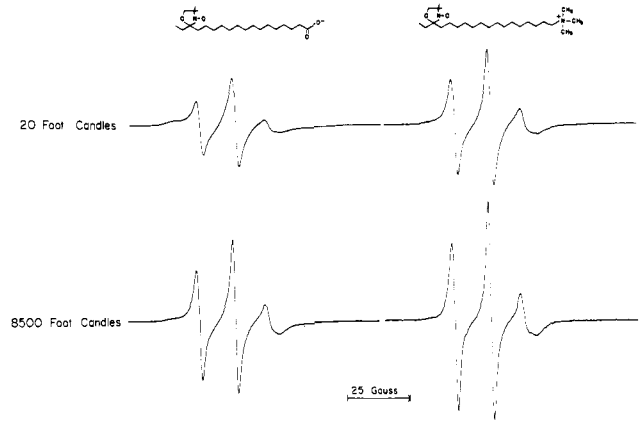

FIGURE 4: The effects of two different light levels during bacterial growth and two different polar head groups on the distribution of spin-label between the protein sites and fluid bilayer regions in chromatophores. All four ESR spectra were recorded at 22 °C and are scaled to the same value of the double integral.

TABLE I: Composition of Chromatophore Material and Percentages of Fatty Acid Spin-Label Immobilized.

light intensity during growth (ft-c)	specific BChl content[a]	wt ratio of phospholipid to protein	LH-BChl (A_{800}/mg of protein)	16-doxylstearic acid immobil (%)
20	11.0	0.31	6.5	68
600	8.1	0.33	3.6	59
8500	2.1	0.34	0.7	48

[a] nm of BChl/100 μg of protein.

It is of interest to see if labels with the same head group but differing in the position of the label along the fatty acid chain are immobilized to the same extent. For this experiment, 7- and 12-doxylstearic acids were incorporated in chromatophores, chromatophore lipids, and lipid-depleted chromatophores. ESR spectra of these labels in a preparation of chromatophores similar to that used for Figure 1 and in chromatophore lipids are shown in Figure 3. Spectral subtractions in these cases are more difficult than with 16-doxylstearic acid because features of the composite and difference spectra become quite similar as the spin-label is moved closer to the polar head group of the stearic acid molecule. This similarity of line shapes makes end-point recognition difficult, especially in the case of 7-doxylstearic acid, and tends to lead to an overestimation of the amount of spin-label immobilized. Spectral subtractions performed with the 7- and 12-doxylstearic acids indicate that 60 to 75% of the labels were immobilized. Since 59% of the 16-doxylstearic acids were observed to be immobilized, the entire length of the fatty acid chains is evidently restricted when the spin-labels bind to the chromatophore proteins.

Partitioning of the Fatty Acid Spin Labels as a Function of Specific BChl Content. The chromatophore preparations used to obtain Figures 1–3 were obtained from bacteria grown under moderate light intensity, 600 ft-c. Bacteria were also grown under very dim (20 ft-c) and very bright (8500 ft-c) light levels. Chromatophores from these bacteria were spin-labeled with 16-doxylstearic acid, and the resulting ESR spectra are shown on the left side of Figure 4. The spectra are scaled to the same total absorption (i.e., they represent the same number of spin-labels) and illustrate the extent to which the ESR line shapes are dependent on the specific BChl content of the chromatophores. Subtraction of the bound components from these ESR spectra yielded the following results. In chromatophores from bacteria grown in a light intensity of 20 ft-c, 68% of the spin-labels were immobilized; in chromatophores from bacteria grown in a light intensity of 8500 ft-c, 48% of the labels were bound. These data are summarized in Table I.

Partitioning of Positively Charged Lipid Spin-Labels as a Function of Specific BChl Content. The chromatophore preparations were routinely prepared in a buffer of pH 7.6. At this pH the doxylstearic acid labels are for the most part negatively charged (Sanson et al., 1976). The question arises whether the relatively large percentage of these labels which is immobilized is due in part to a preference for negatively charged lipids on the part of the membrane proteins. To answer this question, the two chromatophore preparations exhibiting the largest difference in 16-doxylstearic acid binding (20 and 8500 ft-c preparations) were labeled with the positively charged 16-doxyloctadecyltrimethylammonium methanesulfonate. The resulting ESR spectra are shown in Figure 4 (right side). Computer subtractions indicate that in chromatophores from cells grown in a light intensity of 20 ft-c 35% of the positively charged labels were immobilized. In chromatophores from bacteria grown in a light intensity of 8500 ft-c only 25% of the labels were immobilized. For both chromatophore preparations, the percentage of immobilized spin-label was reduced by almost 50% when the positively charged label was used in place of the negatively charged 16-doxylstearic acid.

Discussion

The behavior of the fatty acid spin-labels indicates the presence of fluid bilayer regions in *Rp. sphaeroides* chromatophores and significant amounts of lipid immobilized on the hydrophobic surfaces of membrane proteins. In chromatophores from bacteria grown under 600 ft-c illumination, approximately 40% of the 16-doxylstearic acid is intercalated into fluid bilayer regions and 60% is bound to chromatophore proteins.[2] The lipid spin-labels exchange between the bilayer and bound sites and the aqueous regions. the sharp three-line aqueous peaks normally observed were suppressed by concentrating the membranes and thus reducing the water content. The exchange rate is slow on the ESR time scale (exchange

[2] Single-chain lipid spin-labels are much more readily incorporated into these chromatophore preparations than are phospholipid spin-labels because of the very low monomeric solubility of phospholipids in water. In one experiment, we have incorporated a spin-labeled phosphatidylcholine (made from lysophosphatidylcholine and 16-doxylstearic acid) by fusion with the chromatophores from bacteria grown under 600 ft-c light intensity. The results are similar. The percent of the phospholipid spin-label bound to the protein falls between that observed for the fatty acid and the quaternary amine spin labels.

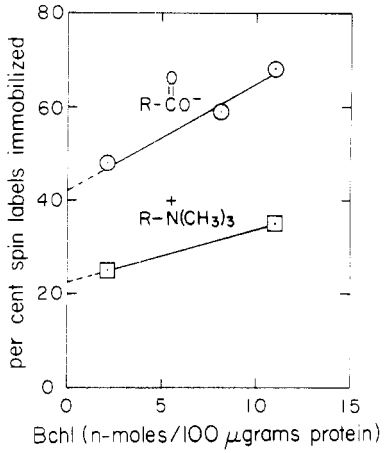

FIGURE 5: The effect of bacteriochlorophyll content on the amount of spin-label immobilized by chromatophore proteins: (○) 16-doxylstearic acid; (□) 16-doxyloctadecyltrimethylammonium methanesulfonate.

frequency $<10^7$ s^{-1}). Very similar results were obtained with chromatophores of *Rhodospirillum rubrum* grown under the same conditions.

The large amount of immobilized lipid present underscores the importance of lipid–protein associations in these photosynthetic membranes. In view of the high precentage of immobilized lipid, it is interesting that these membranes form closed vesicles sufficiently tight to maintain the pH gradient generated by the light-driven proton pump (Crofts et al., 1974). In the specialized purple membrane of *Halobacterium halobium* the percent of immobilized lipid is evidently even greater (Chignell & Chignell, 1975; Jost et al., 1978). These purple patches, however, exist as organized planar structures within the red membrane of *Halobacterium halobium* and do not by themselves form closed vesicles.

In *Rp. sphaeroides* chromatophores there is extensive immobilization along the length of the lipid chains interacting with the membrane proteins. This is shown by the fact that the percent lipid immobilized is independent of the position of the label. It is also indicated by the line shape of the immobilized spectral components (Figure 3). The spectral line shape with the label attached at the 7, 12, and 16 positions of the 18-carbon fatty acid chain indicates that there is very little increase in segmental motion toward the hydrocarbon terminus, in marked contrast with the behavior of the same spin labels in fluid bilayer regions. A similar immobilization along the lipid chains has been observed in the cytochrome oxidase–lipid vesicles from mitochondria (Jost et al., 1973b).

The percent lipid bound varies markedly with charge of the polar head group. This is readily seen by comparing the heights of the top two spectra of Figure 4. Computer analysis of these data indicates that 68% of the negatively charged fatty acid spin-labels are immobilized in chromatophores from *Rp. sphaeroides* grown under 20 ft-c illumination, whereas only about 35% of the corresponding quaternary amine spin-labels are bound in a separate aliquot of the same preparation. Thus, there is a significant preference for negatively charged over positively charged lipid on the part of chromatophore proteins. The same effect is observed in chromatophores from bacteria grown at high light intensities (8500 ft-c; see Figure 4). In this case, 48% of the negatively charged lipid is immobilized, whereas only 25% of the positively charged lipid is bound to chromatophore proteins. Because only the head group was changed, these relative binding data are relatively independent of any perturbations introduced by the spin-labels at the other ends of the lipid chains.

The major phospholipids of *Rp. sphaeroides* are phosphatidylcholine (23% of total lipid phosphorus), phosphatidylethanolamine (35%), and phosphatidylglycerol (34%). At pH 7.6, the first two are neutral (zwitterionic) and the phosphatidylglycerol carries a negative charge. The minor lipid components are evidently cardiolipin (4%) and phosphatidic acid (3%) (Gorchein, 1958) and are also negatively charged. There are no positively charged lipids present. Both the spin-labeling and the lipid-composition data suggest a nonrandom distribution of charged amino acids over the surfaces of integral membrane proteins. For example, a net concentration of positively charged amino acids at the polar interface of the membrane adjacent to the lipid-binding sites could account for the preferential binding of the negatively charged spin-label.

As shown in Table I, the amount of lipid spin-label bound also varies with the specific BChl content of the chromatophore material: the higher the BChl content the greater the amount of bound lipid. This is clear from a comparison of the two spectra of the fatty acid spin-label in the left column of Figure 4. It is also true for the corresponding positively charged lipid data in the right column of this figure. A trivial explanation would be that at higher light levels more lipid is present. However, we find that the phospholipid to protein ratio is very nearly constant. Since it has been reported that the relative amounts of the three major phospholipids do not change significantly with changes in light intensity (Lascelles & Szilágyi, 1965), it seems likely that the change in immobilized lipid reflects changes in the protein composition. It has been well established that progressively lower light levels lead to the synthesis of greater relative proportions of antenna BChl-binding protein (Takemoto & Huang Kao, 1977).[3] We have used the absorbance at 800 nm as a measure of the amount of antenna BChl in our preparations (Sistrom, 1964). The results in Table I show that there is a marked change in the amount of antenna BChl and presumably in the amount of LH protein. In contrast, the amount of reaction center as a percentage of total chromatophore protein remains relatively constant in *Rp. sphaeroides* (Aagaard & Sistrom, 1972). Since the amount of spin-label bound increases with decreasing light levels, we conclude that part of the spin-label is bound to LH proteins.

Plotting the percent spin-labels immobilized as a function of the BChl content provides further insight into the binding of lipid by LH-BChl protein. Figure 5 shows this plot for both the negatively and positively charged spin-labels. Extrapolation to zero BChl content provides a measure of the specificity of lipid spin-label binding. The intersection of the upper curve with the vertical axis of Figure 5 gives roughly 43% as the amount of fatty acid spin-label bound to proteins other than LH-BChl protein. Thus, at the highest BChl content examined (11 nmol/100 μg of protein), the fraction of immobilized fatty acid associated specifically with the BChl antenna protein is at least $(68 - 43)/68 \approx 37\%$.[4] The extrapolated value for the lower curve of Figure 5 is considerably smaller (23%). Although there is no reason a priori to expect specific binding of

[3] In order to verify this result, we have performed polyacrylamide gel electrophoresis on some of these chromatophore preparations. The intensities of the bands stained with Coomassie blue were consistent with the data of Takemoto and Huang Kao (1977).

[4] The method of estimation neglects the reduction in binding to proteins other than LH protein as the LH protein concentration increases. Thus, the final number is probably an underestimate of the amount of binding to LH protein. From the intercepts, proteins other than the LH-BChl protein also, on the average, bind the negatively charged spin-label to a greater extent than the positive charged spin-label. However, since the remaining population is heterogeneous, we focus our attention on the properties of the LH-BChl protein.

either the fatty acid or quaternary amine spin-labels to LH-BChl proteins, the results in Figures 4 and 5 suggest that the LH-BChl protein does bind negatively charged lipids to a greater extent than it binds positively charged lipids.

In addition, the fact that the fraction of immobilized spin-label increases as the relative amount of LH protein increases, at relatively constant phospholipid/protein ratio, indicates that the LH protein immobilizes more lipid per unit weight of protein than do the average chromatophore proteins. This is consistent with the relatively small size of the LH protein.

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