Surface-Enhanced Resonance Raman Scattering Spectroscopy of Bacterial Photosynthetic Membranes: Orientation of the Carotenoids of *Rhodobacter sphaeroides* 2.4.1†

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ABSTRACT: Surface-enhanced resonance Raman scattering (SERRS) spectra were obtained from carotenoids, in the all-trans configuration, located on the antenna complexes of *Rhodobacter sphaeroides* 2.4.1 membranes. Since resonance Raman (RR) spectra are barely detectable at the concentration that SERRS signals saturate, SERRS represents a very sensitive means of detecting pigments in biological systems. Prominent SERRS spectra of sphaeroidenone were detected in chromatophores (cytoplasmic side out) but not in spheroplast-derived vesicles (periplasmic side out), demonstrating that the carotenoid is asymmetrically located on the cytoplasmic side of the cell membrane. Comparison of peak frequencies from SERRS and RR spectral data suggests that the carotenoids are oriented into the membrane with the methoxy end of the isoprenoid chains located closest to the cytoplasmic side of the intracytoplasmic membrane. This work not only shows that SERRS spectroscopy can provide information on the location of a chromophore in a biological membrane but also for the first time demonstrates that SERRS data can be used to ascertain the orientation of a chromophore within the membrane. This observation greatly increases the potential of this technique for structural analysis of intact membranes at the molecular level.

Photosynthetic bacteria use light to drive a series of photochemical reactions leading to the storage of chemical energy necessary for biological activity. For this purpose, the organisms have developed a complex, well-organized photosynthetic apparatus. Antenna pigments absorb light and transfer the resultant excitation energy to a special bacteriochlorophyll (BChl)[1] protein complex called the reaction center. The primary photochemical act (charge separation) takes place within the reaction center, generating a cation on the donor side and an anion on the acceptor side. Primary charge separation and subsequent electron transfer steps across the photosynthetic membrane are vectorial in character and require proper placement of each redox component involved in the process. Consequently, knowledge of the location and orientation of all chromophores involved is a prerequisite for a full understanding of the photosynthetic process.

X-ray crystal structures of the bacterial reaction center show the location of the chromophores within the complex (Deisenhofer et al., 1985; Chang et al., 1986; Allen et al., 1987, 1988). However, the situation is different for the two major antenna complexes of photosynthetic bacteria, B880 (or B875) and B800-850. The basic unit of B875 in *Rhodobacter sphaeroides* 2.4.1 is composed of two small polypeptides (B875-α and B875-β apoproteins), two BChl, and two carotenoids (Broglie et al., 1980; Picorel & Gingras, 1988). The basic unit of B800-850 is thought to consist of four small polypeptides (two B800-850-α and two B800-850-β apoproteins), six BChl, and three carotenoids (Kramer et al., 1984). Although the chemical composition and biophysical properties of the two antenna complexes are well characterized (Zuber, 1985), detailed structural knowledge and the location of the chromophores are not known. Crystals of B800-850 from *Rb. sphaeroides* have been obtained (Allen et al., 1985), but X-ray structures are not available.

Surface-enhanced resonance Raman scattering (SERRS) spectroscopy is being developed as a tool for investigating the structure of membrane surfaces (Seibert & Cotton, 1985; Nabiev et al., 1985a; Picorel et al., 1987, 1988; Seibert et al., 1988). Two unique features of this technique make its application as a membrane surface probe very promising. First of all, resonance Raman scattering from an absorbate on the surface of an anodized SERRS-active metal (Ag, Au, and Cu) electrode under potentiostated conditions is greatly enhanced over resonance Raman scattering from the sample in bulk solution (Van Duyne, 1979). Second, the SERRS effect is extremely distance sensitive (Metiu & Das, 1984) and suitable for detecting the presence of chromophores located close to the surface of biological membranes (Picorel et al., 1988). For recent reviews on SERRS and its application to biological molecules, see Koglin and Sequaris (1986) and Cotton (1988). In this paper we report results obtained from SERRS spectroscopy in combination with resonance Raman (RR) spectroscopy to probe for the location and orientation of carotenoids in membranes isolated from *Rb. sphaeroides* 2.4.1 grown either

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[1] Abbreviations: BChl, bacteriochlorophyll; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; RR, resonance Raman; SDV, spheroplast-derived vesicles; SERRS, surface-enhanced resonance Raman scattering; Tris, tris(hydroxymethyl)aminomethane.

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in the light or in the dark. The results provide evidence for the localization of the carotenoids on the cytoplasmic side of the intracytoplasmic cell membrane and show that the methoxy end of the carotenoid molecules lies closer to the surface of the membrane.

Experimental Procedures

Biological Materials. Rhodobacter sphaeroides 2.4.1 wild type (ATCC 17023) and the R26.1 carotenoidless mutant were grown at 30 °C in the medium of Lascelles (1956) supplemented with 2 g/L yeast extract. The carotenoidless strain was grown anaerobically in the light. Wild type was grown either anaerobically in the light or aerobically in the dark, depending on the intended use of the cells. Photosynthetic cultures (for preparation of chromatophores) were grown in flat Roux bottles illuminated on one side with 150-W floodlamps (GE) located 30 cm from the center of the bottles. Cells were harvested in early stationary phase. Dark-grown cultures (for preparation of chromatophores) were cultured in 1-L flasks filled to 80% capacity on a rotatory shaker (Lab-Line Orbit Environ-Shaker, Lab-Line Instruments, Melrose Park, IL) at 200 rpm, and cells were harvested in the stationary phase. Dark-grown cells (for preparation of spheroplast-derived vesicles) were obtained as described above, but the flasks were filled to 40% capacity. The cells were harvested in the early stationary phase when the cultures reached a Bchl concentration of about 2.0 nmol/mg dry weight of cells. Low-pigmented cultures (low concentration of intracytoplasmic membranes) are required for obtaining good spheroplast-derived vesicles preparations with minimal chromatophore contamination. Cells were harvested by centrifugation at 5000*g* for 10 min at 4 °C and washed once in 200 mM Tris-HCl, pH 8.0.

Preparation of Chromatophores. Chromatophores were released from bacterial cells suspended in 50 mM Tris-HCl, pH 8.0, by two passages through a precooled French pressure cell at 20000 psi. Unbroken cells and large debris were removed by centrifugation at 20000*g* for 20 min. The supernatant was centrifuged at 120000*g* for 90 min, and the resulting pellet (the chromatophore fraction) was washed once with the same buffer. Chromatophores were then layered onto a 30–55% (w/v) linear sucrose density gradient prepared in 10 mM Tris-HCl, pH 8.0. After a 15-h centrifugation at 150000*g* in a Beckman SW 41 Ti rotor, the main pigmented band at about 38% (w/v) sucrose was collected, diluted about four times with 10 mM Tris-HCl, pH 8.0, and spun down at 120000*g* for 90 min. The pellet was resuspended in 50 mM Tris-HCl, pH 8.0. All procedures were done at 4 °C.

Preparation of Spheroplast-Derived Vesicles. Spheroplast-derived vesicles (SDV) were prepared by modification of the methods described by Takemoto and Bachmann (1979) and Hellingwerf et al. (1975). To a dark-grown cell suspension of a final optical density of 20 (1-cm path length) at 660 nm, the following reactants were added at the indicated final concentrations: 200 mM Tris-HCl, pH 8.0; 25% (w/v) sucrose; 6 mM EDTA previously neutralized at pH 7.3 with NaOH; 0.5 mg/mL egg lysozyme (Sigma, EC 3.2.1.17, grade I). Following a 2-h incubation at 35 °C in a slowly agitated water bath, magnesium sulfate crystals (up to 50 mM) were added to the suspension and incubated for 15 min at room temperature with gentle stirring. Spheroplasts were harvested by centrifugation at low speed (7000*g*) for 10 min. The pigmented pellet was suspended in 10 mM Tris-HCl buffer supplemented with 0.02% (w/v) Brij 58 (Sigma) and 10 mM magnesium sulfate by use of a small, soft paintbrush. A few grains of DNase I (Sigma DN-25) and RNase III-A (Sigma) were added, and the material was incubated for 30 min with gentle stirring. The crude suspension was then centrifuged at 3000*g* for 5 min, and the resulting supernatant was centrifuged at 50000*g* for 20 min. The SDV fraction, recovered as a pellet, was then washed twice with 10 mM Tris-HCl buffer, resuspended in the same buffer with a small, soft paintbrush, and then layered onto a 30–55% (w/v) linear sucrose density gradient made up in the same buffer. After a 12-h centrifugation at 150000*g*, the lower part of the broad pigmented band around 40% (w/v) sucrose was recovered, diluted about four times in 10 mM Tris-HCl (pH 8.0), and centrifuged at 50000*g* for 30 min. The pellet (purified SDV) was resuspended in the same buffer and kept in the dark until use during the next 2–3 days. All centrifugations were carried out at 4 °C.

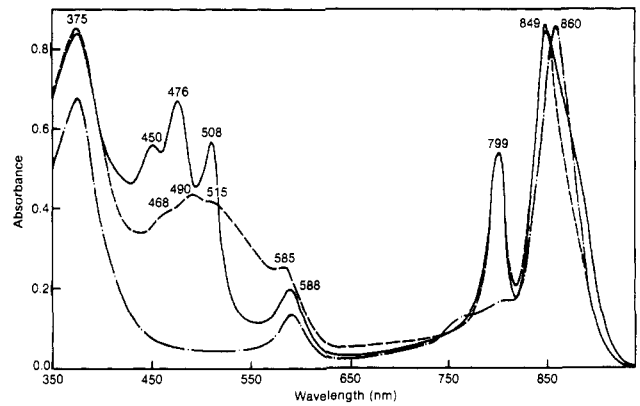

FIGURE 1: Absorption spectra of chromatophores isolated from *Rb. sphaeroides* 2.4.1 (wild type), grown anaerobically in the light (—) and aerobically in the dark (– –). A similar spectrum is presented from the carotenoidless R26.1 mutant (–·–) grown anaerobically in the light. The spectra were normalized at their highest peak in the near-infrared. Chromatophores were resuspended in 10 mM Tris-HCl (pH 8.0).

Assays. Carotenoids and BChl were extracted with a mixture of acetone/methanol (7/2) and assayed spectrophotometrically with an ϵ^{1cm}_{1mM} = 150 for sphaeroidene at 456 nm, ϵ^{1cm}_{1mM} = 120 for sphaeroidenone at 482 nm (Shneour, 1962), and ϵ^{1cm}_{1mM} = 70 for the BChl at 770 nm (Picorel et al., 1988).

Spectroscopic Methods. Absorption spectra were recorded with a Cary 17D or an HP 8450A spectrophotometer using 1-cm path-length cuvettes. RR and SERRS spectra (16 scans per spectrum) were obtained at room temperature as described previously (Seibert & Cotton, 1985; Picorel et al., 1988; Seibert et al., 1988) excitation being with the 457.9-, 488.0-, and 514.5-nm lines of an argon ion laser (INNOVA 90-5, Coherent Inc., Palo Alto, CA) or the 530.9-nm line of a krypton laser (INNOVA 100) at 30-mW power. The Raman spectral resolution was 5–8 cm^{-1} in the 530.9–457.9-nm excitation range.

Results

The absorption spectra (Figure 1) of chromatophores isolated from *Rb. sphaeroides* wild type grown either in the light or in the dark show three characteristic bands in the near-infrared region due to BChl associated with B800-850 and B875 antenna complexes. In our preparations B800-850 complex is more abundant than B875 and is characterized by prominent peaks at 799 and 849 nm. B875 is seen as a shoulder at around 880 nm. The three absorption bands in the 450–530-nm region correspond to electronic transitions in the carotenoids, the other class of chromophores found in the antenna complexes. The difference between the two spectra in this region is due to the presence of different car-

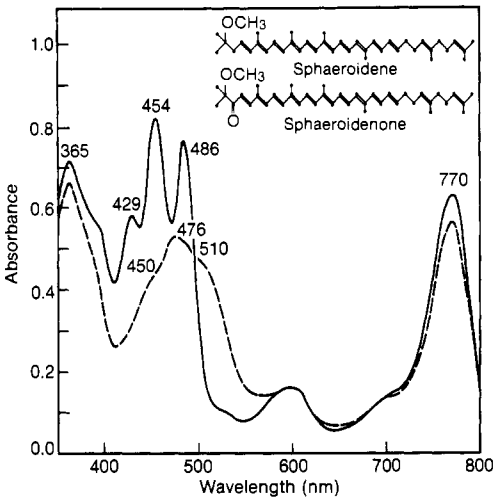

FIGURE 2: Acetone/methanol (7/2) extracts of chromatophores from *Rb. sphaeroides* wild type, grown anaerobically in the light [sphaeroidene and BChl (—)] or aerobically in the dark [sphaeroidenone and BChl (--)]. The structures shown are those of sphaeroidene and sphaeroidenone.

otenoids in chromatophores isolated from light- or dark-grown cells. Sphaeroidene is found in light-grown cells, and sphaeroidenone is induced in dark-grown cells (Cogdell & Crofts, 1978; Shneour, 1962). Figure 1 also shows the absorption spectrum of chromatophores from the R26.1 mutant, which lacks colored carotenoids. The shape of this spectrum differs from that of the wild type in the near-infrared region although the mutant contains both types of antenna complexes (Davidson & Cogdell, 1981; Theiler et al., 1984). Furthermore, the spectrum lacks the three prominent absorption bands in the visible region since, as mentioned above, colored carotenoids are absent in the mutant. Figure 2 shows the absorption spectra of pigments extracted with a mixture of acetone/methanol (7/2) from either light- or dark-grown wild-type cells. Monomeric BChl absorbs at 770 nm, and the carotenoids absorb in the 425–520-nm region. Note that the sphaeroidene bands are sharper and blue-shifted compared to those of sphaeroidenone. Figure 2 also shows the chemical structure of the two carotenoids (Boucher et al., 1977). Both molecules contain asymmetric isoprenoid chains with conjugated double bonds. Sphaeroidenone arises from the oxidation of sphaeroidene and is characterized by the addition of an oxo group at the methoxy end of the molecule.

Figure 3 shows RR spectra of chromatophores from either light- (A) or dark- (B) grown cells induced by excitation into the $\pi \rightarrow \pi^*$ transitions of the carotenoids at several excitation wavelengths (457.9–530.9 nm). Each spectrum displays three main peaks centered around 1518 (ν_1), 1154 (ν_2), and 1004 (ν_3) cm^{-1} in light-grown cells and at 1512, 1150, and 1001 cm^{-1} in dark-grown cells (spectra excited at 457.9 nm). No peaks were observed in chromatophores isolated from the carotenoidless mutant. The frequencies of the peaks and the observation that no peaks are observed in the R26.1 mutant demonstrate that the RR spectra arise from sphaeroidene or sphaeroidenone present in chromatophores. The peak frequencies of the bands from dark-grown cells are shifted to lower values compared to those from light-grown cells due to slight differences in chemical structure of the two carotenoids. Furthermore, the intensity ratios of the peaks vary depending on the excitation wavelength. The ratio changes are due to differences in the resonance enhancement arising from the individual vibronic bands of the carotenoids within the lowest electronic transition. However, the frequencies of the peaks

Table I: Intensity Ratios of the 1156–1151-cm^{-1} (ν_2)/1520–1511-cm^{-1} (ν_1) RR and SERRS Bands of Chromatophores from Light- (Sphaeroidene) and Dark- (Sphaeroidenone) Grown Cells[a]

type of Raman spectroscopy	ν_2/ν_1 intensity ratios					
	light-grown cells			dark-grown cells		
	457.9[b]	488.0	514.5	457.9	488.0	514.5
RR	0.8	0.4	0.9	0.7	0.7	0.7
SERRS	1.1	0.9	1.0	0.9	1.0	1.2

[a] The data were obtained from Figures 3 and 4. [b] Excitation wavelength in nanometers.

Table II: Intensity Ratios of the 1004–1002-cm^{-1} (ν_3)/1520–1511-cm^{-1} (ν_1) RR and SERRS Bands of Chromatophores from Light- (Sphaeroidene) and Dark- (Sphaeroidenone) Grown Cells[a]

type of Raman spectroscopy	ν_3/ν_1 intensity ratios					
	light-grown cells			dark-grown cells		
	457.9[b]	488.0	514.5	457.9	488.0	514.5
RR	0.17	0.05	0.18	0.13	0.13	0.13
SERRS	0.28	0.23	0.25	0.24	0.24	0.25

[a] The data were obtained from Figures 3 and 4. [b] Excitation wavelength in nanometers.

for each set of spectra remain constant and are independent of the excitation wavelength. No peaks characteristic of BChl, the most abundant pigment in chromatophores, were detected with excitation in the above-mentioned spectral region. This is expected since BChl absorbs little light at these wavelengths.

As shown in Figure 4, SERRS spectra could be detected with chromatophores from both light- (A) and dark- (B) grown cells. Major peaks were detected at 1520 (ν_1), 1153 (ν_2), and 1004 (ν_3) cm^{-1} due to the sphaeroidene and at 1520, 1157, and 1001 cm^{-1} due to the sphaeroidenone. The difference in frequencies between the two sets of data is within the experimental error of the instrument. No major peaks were observed in chromatophores isolated from the R26.1 mutant. The frequencies of the RR and SERRS peaks are quite similar (although the shift to lower frequence seen in the RR spectrum of sphaeroidenone is not observed in the SERRS spectrum—see Discussion), suggesting a large resonance contribution to the enhancement. Note that the RR contribution (the companion spectra at 488.0 nm) to the SERRS spectra was barely detectable at this carotenoid concentration, demonstrating that the surface enhancement is large (see supplementary material). This illustrates the extreme sensitivity associated with the SERRS technique. In addition to the three major peaks associated with carotenoids, other minor peaks are clearly seen in the spectra of Figure 4. At least some of these additional peaks could be due to other chromophores present on, or near, the cytoplasmic surface of the photosynthetic membrane. The origin of these minor peaks will be the subject of future study.

Table I depicts RR and SERRS band intensity ratios of the ν_2/ν_1 bands of light- and dark-grown cells. Comparisons of the intensity ratios show that some SERRS bands are preferentially enhanced. This observation is most apparent in the case of sphaeroidene with 488.0-nm light and of sphaeroidenone with 514.5-nm light. Similar observation are seen in Table II for ν_3/ν_1 ratios. With some chromophores, this type of data could be used to ascertain orientational information; however, as will be discussed later, this is not the case with carotenoids.

To determine on which side of the photosynthetic membrane the carotenoids are located, we compared SERRS spectra obtained from chromatophores and SDV. SDV prepared from

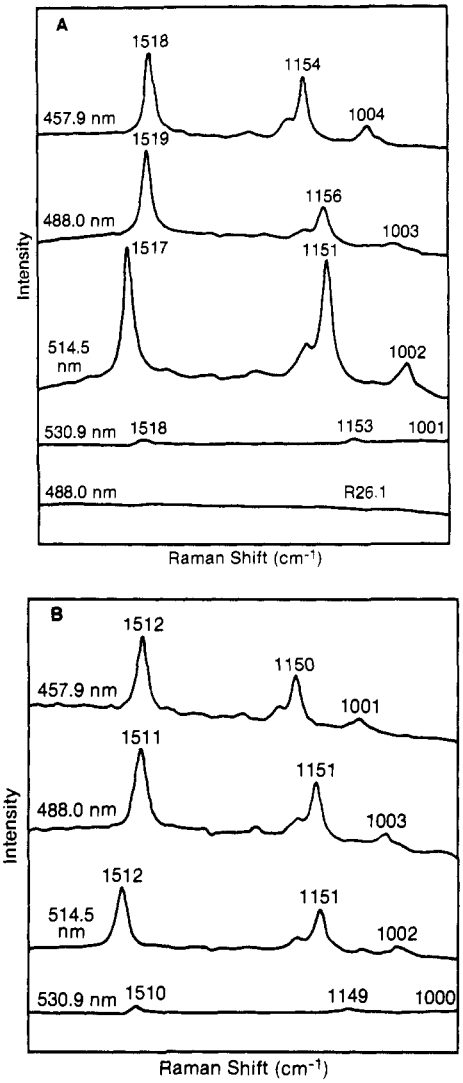

FIGURE 3: Resonance Raman spectra of chromatophores isolated from wild-type and R26.1 mutant cells of *Rb. sphaeroides*. Laser excitation wavelengths were 457.9, 488.0, 514.5, and 530.9 nm. (A) Light-grown cells; (B) dark-grown cells. The spectrum at the bottom of (A) is of chromatophores from R26.1. The frequencies of the peaks are reported in cm^{-1}. The concentration of carotenoid was 14 μg/mL for the spectra in (A) and 12 μg/mL for the spectra in (B). The spectra of (B) are magnified 2-fold. Sample buffer, 20 mM HEPES (pH 7.5).

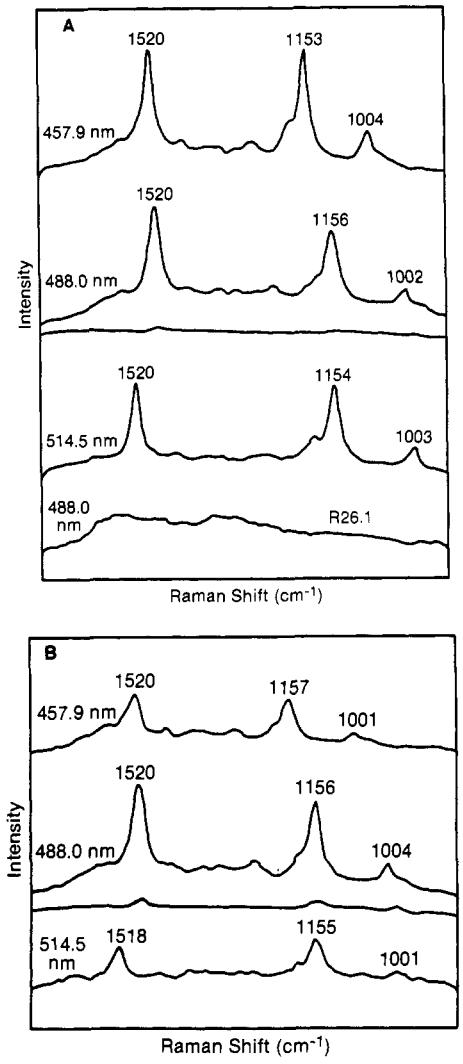

FIGURE 4: SERRS spectra of chromatophores isolated from wild-type and R26.1 mutant cells of *Rb. sphaeroides* excited at 457.9, 488.0, and 514.5 nm. (A) Light-grown cells; (B) dark-grown cells. The trace accompanying the spectra excited at 488.0 nm is a RR spectrum of the sample in the presence of a polished Ag electrode prior to anodization. The spectrum at the bottom of (A) is that of chromatophores from R26.1. The carotenoid concentration was 0.2 (A) and 0.3 (B) μg/mL. The spectra of (B) are magnified 2-fold. Sample buffer, 20 mM HEPES (pH 7.5)–100 mM Na_2SO_4.

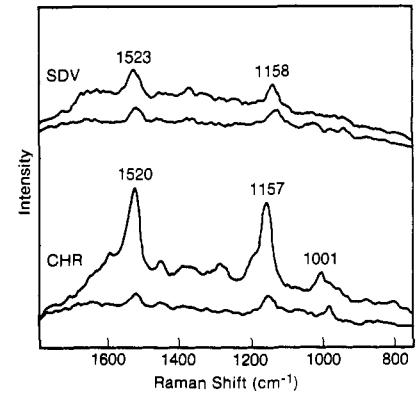

FIGURE 5: SERRS spectra obtained from spheroplast-derived vesicles (SDV) and chromatophores (CHR) of *Rb. sphaeroides*. Cells for both samples were grown in the dark. Little SERRS signal corresponding to sphaeroidenone is detected in the SDV preparation compared to that of the CHR preparation. The accompanying trace in each case is a background RR spectrum taken prior to anodization of the Ag electrode. The concentration of carotenoid was 0.37 and 0.33 μg/mL for SDV and CHR preparations, respectively. λ_{ex} = 457.9 nm.

Rb. sphaeroides are well characterized structurally (Lommen & Takemoto, 1978; Michels & Konings, 1978). They have the opposite orientation (periplasmic side out) compared to chromatophores (cytoplasmic side out) and are devoid of cell wall. Figure 5 shows SERRS spectra obtained from spheroplast-derived vesicles and chromatophores purified from dark-grown cells. As can be seen, very small SERRS signals were detected in SDV, but large SERRS signals were detected with chromatophores. A quantitative analysis of the spectra shows that the SDV preparation exhibits about 10% of the carotenoid SERRS signal as compared to the chromatophore preparation. Enzymatic assays for vesicle sideness have shown that a small amount of chromatophore material always contaminates SDV preparations from *Rb. sphaeroides* (Lommen & Takemoto, 1978; Takemoto & Bachmann, 1979). This contamination most likely is the source of the small amount of carotenoid SERRS signal observed in our SDV preparation. SDV preparations were also checked at other concentrations (data now shown), but larger SERRS signals were not observed. These results show that sphaeroidenone is located on,

or near, the cytoplasmic surface of the intracytoplasmic membrane.

Discussion

In this study RR and SERRS signals originating from carotenoids were detected in chromatophores isolated from *Rb. sphaeroides* 2.4.1 grown either anaerobically in the light or aerobically in the dark. Several lines of evidence suggest that we are detecting carotenoids from antenna complex rather than the carotenoid found in the reaction center. First, the antenna complexes represent more than 95% of the pigment content of chromatophores, and thus, we would expect those pigments to dominate the spectrum. Second, the spectra correspond to a carotenoid in the all-trans configuration, the configuration assumed by carotenoids in all bacterial antenna complexes analyzed thus far (Lutz et al., 1978). The carotenoid present in the reaction center assumes a cis configuration (Lutz et al., 1978, 1988; Koyama et al., 1982; Boucher & Gingras, 1984). Third, our previous mutant studies with *Rhodospirillum rubrum* determined that antenna complex is the source of SERRS carotenoid signals in chromatophores of this species (Picorel et al., 1988). Thus, we conclude that the antenna complexes are the source of the carotenoid SERRS signals in *Rb. sphaeroides*. Unfortunately, our data are not able to distinguish the extent to which each type of antenna complex contributes to the observed RR and SERRS signals. The BChl-to-carotenoid ratios for B800-850 and B875 complexes are 2:1 (Evans et al., 1988; Radcliffe et al., 1984) and 1:1 (Broglie et al., 1980; Picorel & Gringras, 1988), respectively, but the former is much more abundant in the membranes used in this work (Figure 1). Further comparative analysis using the wild-type and light-harvesting mutants, such as RC^+ $B800\text{-}850^-$ $B875^+$ Car^+ and RC^- $B800\text{-}850^+$ $B875^-$ Car^+ (van Dorsen et al., 1988), will determine the proportional contribution of each antenna complex to the total RR and SERRS signals observed in *Rb. sphaeroides* 2.4.1.

Although much is known about the spectroscopic, biochemical, and functional properties of the carotenoids present in bacterial light-harvesting antenna complexes (Cogdell & Frank, 1987), their precise locations in the protein complexes and within the membrane are largely unknown. On the basis of the unique distance sensitivity of the SERRS technique, it is now possible not only to show where carotenoids are located in the photosynthetic membrane but also to infer orientational information about the asymmetric carotenoids found in this species.

From the studies of the effect of the 33-kDa protein on a surface-enhanced Raman signal related to Mn in spinach membranes (Seibert et al., 1988) and determinations of the thickness of the 33-kDa protein in situ (Seibert et al., 1987), we can estimate that the range of sensitivity of SERRS in these preparations is <10 Å (i.e., the distance from the surface of the anodized Ag electrode that still yields a SERRS signal). The SERRS effect is a function of chromophore concentration and distance (Cotton et al., 1986; Kim et al., 1989). In these samples, the concentration is quite low, and therefore, the signal decreases rapidly with distance from the Ag surface. This is consistent with the results of Nabiev et al. (1985b), indicating a maximum range of sensitivity on the order of 5–10 Å. Thus, the distance represented by <20% of the 50-Å thickness of a chromatophore membrane is sufficient to reduce a carotenoid SERRS signal to below detection limits. The data in Figure 5 show as a consequence that the carotenoids of *Rb. sphaeroides* are located asymmetrically on, or very near, the cytoplasmic surface of the bacterial membrane.

Although it is often possible to obtain orientational or bonding information from a comparison of SERRS and RR spectral peak intensity ratios, this is not the case with carotenoids. The modes observed in the carotenoid spectra are delocalized throughout the conjugated chain. Hence, increased relative intensity of a particular band in the SERRS spectrum as compared to that in the RR spectrum cannot be ascribed to the proximity of a specific functional group or region of the carotenoid molecule to the Ag surface. Nevertheless, some relative intensity differences are observed for ν_1 and ν_2, as shown in Table I, where a significant enhancement in ν_2 occurs relative to ν_1 in the SERRS spectra. In Table II, a similar trend is seen for the weaker ν_3 band as compared to ν_1. These changes may reflect a perturbation of the excited state of the carotenoid, resulting from its interaction with the Ag surface. This interaction could change the contribution of the individual vibronic states to the resonance enhancement. Further evidence for a change in the vibronic structure of the absorption band may be obtained from a comparison of SERRS and RR excitation profiles. This study is now in progress.

On the other hand, this paper does show that in certain cases it is possible to obtain orientational information on asymmetrical carotenoid molecules, such as sphaeroidene and sphaeroidenone, by comparing SERRS and RR peak frequency data. The frequencies of ν_1 (assigned to C=C symmetrical stretching), ν_2 (C–C symmetrical stretching and C=C—C bending), and ν_3 (CH_3 rocking) are unchanged in SERRS spectrum of sphaeroidene (Figure 4A) compared to its RR spectrum (Figure 3A). This similarity suggests that the SERRS spectrum is dominated by resonance enhancement without significant perturbation of the ground electronic state of the sphaeroidene from its interaction with the Ag surface. In contrast, a shift of ca. +9 cm^{-1} is observed for ν_1 in the SERRS spectrum of sphaeroidenone compared to its RR spectrum[2] (Figures 3B and 4B). This shift may result from the interaction of the oxo group with the Ag surface in a manner that disrupts its conjugation with the polyene chain. The interaction could produce a twisting of the carbonyl out of the plane of the conjugated system or restrict the delocalization of π electrons in some other manner. For this to occur, the methoxy end of the sphaeroidenone molecule must be very close to the cytoplasmic surface of the membrane and possibly exposed. The other end of the molecule is buried deep in the antenna complexes. This agrees with the model proposed by Picorel et al. (1988) for the B880 complex of *Rs. rubrum*, and it is also consistent with the linear dichroism results of Breton (1974) indicating that the carotenoids of *Rb. sphaeroides* are pointing out of the membrane plane. It is only in partially agreement with the model proposed by Kramer et al. (1984) for the B800-850 complex of *Rb. sphaeroides* on the basis of fluorescence polarization spectroscopy. Kramer's model suggests that one-third of the carotenoids are bound parallel to the plane of the membrane, but Breton and Nabedryk (1987) have disputed this convincingly in more recent work.

This paper represents one of the few examples of the application of SERRS spectroscopy to a biological membrane problem. Our conclusion that sphaeroidenone (and most likely sphaeroidene also) is located on the cytoplasmic side of the intracytoplasmic membrane in *Rb. sphaeroides* is the first such report for *Rb. sphaeroides* and is consistent with our previous work with *Rs. rubrum* antenna complexes (Picorel et al.,

[2] The shift in ν_1 (and ν_2) to lower frequency in the RR spectra on introduction of the oxo group to sphaeroidene (compare panels A and B of Figure 3) is due to a higher degree of delocalization of the π electrons in the isoprenoid chain of sphaeroidenone (Marlin, 1985).

1988). However, the unique contribution of this study is the determination of the specific orientation of a carotenoid molecule within the photosynthetic chromatophore. This work clearly shows that SERRS spectroscopy is a particularly valuable technique for studying the surface topography of biological membranes. Finally, membranes have not, as yet, been crystallized to obtain X-ray structures as have soluble and some intrinsic membrane proteins (Deisenhofer et al., 1985; Chang et al., 1986; Allen et al., 1987). Thus, SERRS spectroscopy represents a potentially powerful new technique for inferring structural information in intact membranes.

Supplementary Material Available

Two figures showing chromatophore concentration saturation curves for RR and SERRS signals and demonstrating that about 2% of the biological material is required to elicit maximal SERRS signals compared to that required to elicit maximum RR signals and one figure showing that little photobleaching of the sample material occurs during the time it takes to obtain a SERRS spectrum (5 pages). Ordering information is given on any current masthead page.

Registry No. Sphaeroidenone, 13836-70-9; sphaeroidene, 13836-61-8.

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