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Resonance Raman studies of the conformations of all-*trans* carotenoids in light-harvesting systems of photosynthetic bacteria

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Comparison of the resonance Raman spectra of carotenoids in vivo and in vitro has revealed that in some species of photosynthetic bacteria the major fraction of carotenoids associated with the light-harvesting systems has forms distorted (twisted) from the planar all-*trans* conformation. These distorted forms are kept in isolated and purified light-harvesting bacteriochlorophyll-protein complexes.

Carotenoids in photosynthetic organellae are antenna pigments which absorb light and transfer their energy ultimately to the reaction center. In addition, they quench singlet oxygen and the triplet state of chlorophyll, thus protecting the photosynthetic apparatus against photosensitized oxidation. In photosynthetic bacteria, carotenoids mainly exist in intracytoplasmic membranes as the pigment-protein complexes. A very small fraction of carotenoids is associated with the reaction center complex (one mol of carotenoids per one mol of reaction center) [1]. The majority is associated with the light-harvesting bacteriochlorophyll-protein complexes.

Resonance Raman spectroscopy is a powerful tool for studying the in vivo state of carotenoids. Using the excitation light in the 450–550 nm region the vibrational spectra of carotenoids can be exclusively obtained from the photosynthetic membranes and pigment-protein complexes. This technique has clearly shown that the carotenoid molecule in the reaction center complex of photosynthetic bacteria takes a *cis* configuration [2–4]. It has also indicated that the major fraction of carotenoids contained in the light-harvesting systems exists in an essentially all-*trans* configuration [2,3]. However, neither detailed structural analysis nor information on the state and environment of these all-*trans* carotenoids in the photosynthetic membranes and pigment-protein complexes has been reported yet.

In this study we compared the resonance Raman spectra of carotenoids existing in the membranes and pigment-protein complexes of several strains of photosynthetic bacteria with those of carotenoids extracted from them. We present new information on the conformation of all-*trans* carotenoids associated with the light-harvesting systems of photosynthetic bacteria.

Sources of photosynthetic bacteria were cultured phototrophically for 3–4 days in the media suitable for each bacterial strain [5,6]. Intracytoplasmic membranes and pigment-protein complexes were prepared as previously reported [6]. Carotenoids were extracted with acetone from the membranes, which had been treated with an 85% (vol/vol) aqueous solution of methanol to remove bacteriochlorophyll.

Raman spectra were measured at room temperature using a rotating cell. The Raman spectrometer consisted of a double monochromator (Spex 1401) and a photon counting system. Visible lines

of an Ar^+ laser (Coherent CR-4) were used for excitation. The laser power was less than 70 mW at the sample point. Plasma emissions from the laser tube were separated with a filter monochromator (Anaspec 300S).

In Fig. 1 are shown the Raman spectra of the intracytoplasmic membranes from (a) *Chromatium vinosum,* (c) *Rhodopseudomonas palustris,* and (e) *Rhodospirillum rubrum.* Since the 488.0 nm line was used for excitation, all the Raman bands arose from carotenoids in the membranes. The Raman spectra of carotenoids (extracted from the membranes) in acetone are also shown in Figs. 1b, d and f. For each spectrum, two strong Raman bands are commonly observed at 1515–1508 and 1152–1148 cm^{-1}, which are assigned to a C=C and a C–C stretching mode, respectively. From

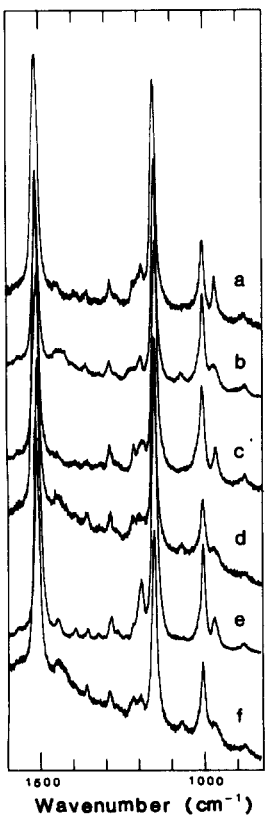

Fig. 1. Resonance Raman spectra of carotenoids in intracytoplasmic membranes from (a) *C. vinosum,* (c) *R. palustris,* (e) *R. rubrum* and extracted carotenoids in acetone from the membranes of (b) *C. vinosum,* (d) *R. palustris,* (f) *R. rubrum,* measured at room temperature using a rotating cell. The 488.0 nm line of an Ar^+ laser was used for excitation.

the resonance Raman studies, it has been concluded that most carotenoids in the membranes of *R. rubrum* take all-*trans* configuration [3]. Similarly, the Raman spectra of carotenoids in the membranes from *C. vinosum* and *R. palustris* (Fig. 1a and c) are essentially those of all-*trans* carotenoids. For *R. rubrum,* the frequency of the C=C stretching band (at about 1510 cm^{-1}) of extracted carotenoids is higher than that of carotenoids in the membranes (Fig. 1e and f). As Lutz et al. suggested [3], this frequency difference indicates that the content of *cis*-isomers is higher in extracted carotenoids than in the membranes, due to the quick isomerization after extraction [7].

Although the spectra of carotenoids in the membranes and those of extracted carotenoids in acetone are roughly the same, we can easily distinguish the two kinds of spectra. In this study, we focus our attention on the relative intensity of the Raman band at approx. 960 cm^{-1}, i.e., the intensities of this band are relatively different between the spectra of the membranes and those of extracted carotenoids. In the Raman spectra measured with other laser lines (457.9, 476.5, 496.5, and 514.5 nm), the differences in the relative intensities of the 960 cm^{-1} band were always observed. Thus, the difference in the relative intensity of this band should not be attributed to the difference of the resonance conditions between the in vivo and in vitro samples, although the absorption maxima of the membranes shift to longer wavelengths by approx. 20 nm from those of extracted carotenoids. The intensity difference of the 960 cm^{-1} band also should not be attributed to the contamination of *cis*-carotenoids into in vitro samples, because their contamination may increase the 960 cm^{-1} band intensity, but never decreases it [8,9]. As discussed below, the intensity of this band gives information on the in vivo conformations of all-*trans* carotenoids.

According to the normal coordinate calculations, the 965 and 955 cm^{-1} bands of β carotene were assigned to the CH out-of-plane waggings in the C_{11}=C_{12} (and $C_{12'}$=$C_{11'}$) and C_7=C_8 ($C_{8'}$=$C_{7'}$) parts, respectively [10] (Fig. 2a). For carotenoids in the membrane from *C. vinosum,* this assignment was confirmed by the frequency shifts by ^{13}C and ^{2}H substitution [11]. Because each of these out-of-plane modes has the local symmetry of 'un-

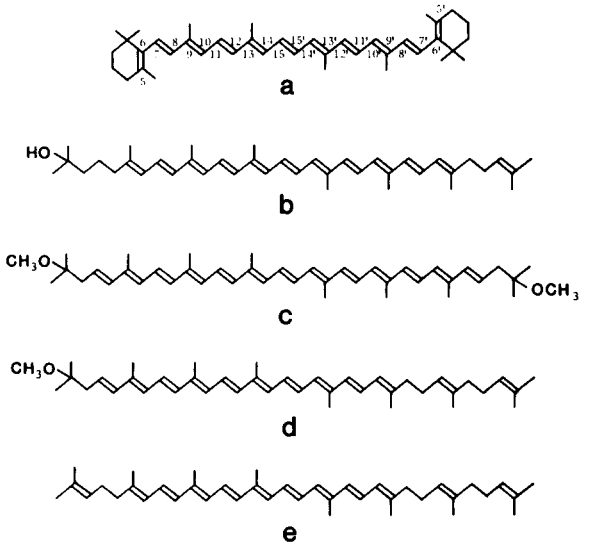

Fig. 2. Structural formulas of β carotene and carotenoids which are major components in the photosynthetic bacteria used in this study. (a) β Carotene; (b) rhodopsin; (c) spirilloxanthin; (d) spheroidene; and (e) neurosporene.

gerade' for the inversion with respect to the center of the relevant C=C double bond, the Raman intensities intrinsic to these modes must be very small, if the polyene chains are planar. It has been pointed out 'that the relative intensities of the out-of-plane wagging modes in the 970–940 cm^{-1} of not only the all-*trans*, but also various *cis* isomers of β carotene must be associated with the distortion around the C_{11}=C_{12} ($C_{12'}$=$C_{11'}$) and C_7=C_8 ($C_{8'}$ = $C_{7'}$) bonds from the planar structure [8,10].

Under the culture conditions used in this study, spirilloxanthin is a dominant component of carotenoids for *R. rubrum* (more than 90%) and *R. palustris* (more than 70%). *C. vinosum* contains rhodopin (50%) and spirilloxanthin (30%). Their conjugated systems are almost the same as β carotene, i.e., symmetrical for the central C_{15}=$C_{15'}$ bond (Fig. 2a, b, and c). Furthermore, the ends of conjugated parts hardly affect the resonance Raman spectra measured with the visible excitation light. Hence, we can extend the above interpretation of the intensities of the Raman bands at 970–940 cm^{-1} of β carotene to those of carotenoids in the bacteria used in this study. Accordingly, the fact that carotenoids in the membranes from *C. vinosum*, *R. palustris*, and *R. rubrum* exhibit the relatively intense Raman band at approx. 960 cm^{-1} suggests that carotenoids in the membranes of these bacteria have polyene chains distorted from the planar all-*trans* form.

Similar relationships between the resonance Raman intensities of the CH out-of-plane modes and the distortion about the C=C bonds have been also reported in other molecules. The intensity of the 960 cm^{-1} Raman band of *trans*-stilbene was found to be enhanced in going from solid to liquid, and it has been concluded that the molecule suffers an out-of-plane distortion in the liquid phase [12]. Three intense Raman bands of the CH out-of-plane modes of bathorhodopsin suggest a distorted polyene chain of retinal bound to a protein moiety [13].

In Fig. 3 are shown the resonance Raman spectra of carotenoids in the membranes from (a)

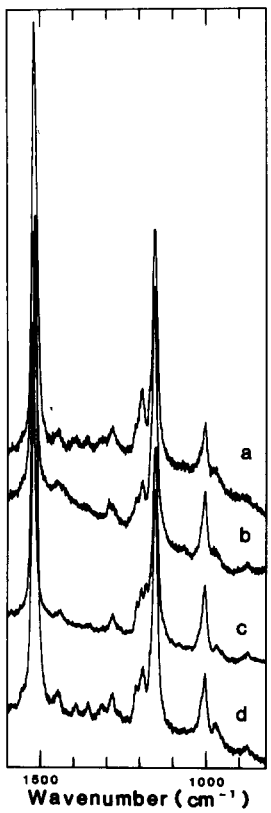

Fig. 3. Resonance Raman spectra of carotenoids in intracytoplasmic membranes from (a) *R. sphaeroides*; (c) *R. capsulata*; and (d) green mutant of *R. sphaeroides*. (b) Resonance Raman spectrum of carotenoids extracted in acetone from *R. sphaeroides*. Conditions were the same as in Fig. 1.

Rhodopseudomonas sphaeroides, (c) *Rhodopseudomonas capsulata,* and (d) a green mutant of *R. sphaeroides.* These spectra are also characteristic of all-*trans* carotenoids. In contrast to the Raman spectra of carotenoids in the membranes shown in Fig. 1, the Raman bands at approx. 960 cm^{-1} of carotenoids in the membranes of these bacterial strains are as weak as those of carotenoids in acetone (Fig. 3b). According to the above interpretation, carotenoids in the membranes of these bacteria take an almost planar all-*trans* form. However, the main carotenoid species of these bacteria are spheroidene (80%) and spheroidenone (15%) in the wild types, and methoxyneurosporene (40%) and neurosporene (40%) in the green mutant. Their conjugated polyene systems are not identical with that of β carotene, being asymmetrical about the central C_{15}=$C_{15'}$ bond (Fig. 2d and e; although structural formulas of spheroidenone and methoxyneurosporene are not shown, their conjugated systems are identical with those of spheroidene and neurosporene, respectively). As a result, it is possible that these carotenoids have vibrational modes different from those of β carotene. If this is the case, we cannot draw any conclusion about the distortion.

The resonance Raman spectra of carotenoids in the reaction center–B890 complex and B800–850 complex from *C. vinosum* are shown in Fig. 4. The relatively intense band at approx. 960 cm^{-1} is clearly observed for each complex. This indicates that all-*trans* carotenoids are distorted also in the isolated and purified complexes, i.e., conformations of carotenoids in vivo are already determined when they are incorporated into the pigment-protein complexes. In addition, the distorted forms of carotenoids were not affected by the isolation procedures such as detergent treatments. This suggests that carotenoids do not exist in the protein-lipid interface in the membranes, but they are surrounded by the hydrophobic regions of the protein subunits, probably α-helical domains which span the lipid bilayer [14–16].

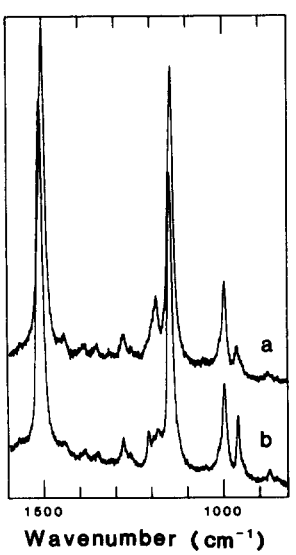

Fig. 4. Resonance Raman spectra of carotenoids in (a) reaction center-B890 complex and (b) B800–850 complex from *C. vinosum.* Conditions were the same as in Fig. 1.

The relative intensities of the 960 cm^{-1} band are different between the reaction center–B890 complex and B800–850 complex from *C. vinosum* (Fig. 4). The spectrum of the former in this region is similar to those of the membranes from *R. palustris* and *R. rubrum* (Fig. 1c and e), while the spectrum of the latter is similar to those of the membranes from *C. vinosum* (Fig. 1a). It is noted that spirilloxanthin is dominant in the reaction center–B890 complex from *C. vinosum* [17] and in the membranes from *R. palustris* and *R. rubrum,* and that rhodopin is dominant in the B800–850 complex [17] and the membranes from *C. vinosum.* Thus, the relative intensities of the 960 cm^{-1} band seem to be specific to the carotenoids species and the conformations of all-*trans* carotenoids in vivo are probably determined by individual carotenoids.

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