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Structural Studies on a Photosystem II Reaction-Center Complex Consisting of D-1 and D-2 Polypeptides and Cytochrome *b*-559 by Resonance Raman Spectroscopy and High-Performance Liquid Chromatography

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Resonance Raman spectra of chlorophyll *a* and β-carotene moieties have been observed from a photosystem II reaction-center complex containing the D-1 and D-2 polypeptides and cytochrome *b*-559. Examination of the CC double-bond and the keto CO stretching regions of chlorophyll *a* moiety has indicated that the Mg atom is five-coordinate and the keto CO is hydrogen-bonded. The resonance Raman spectrum of β-carotene moiety has suggested that this carotenoid molecule takes the all-*trans* form. This suggestion has been supported by an HPLC analysis of the extracted carotenoid.

The entity of the photosystem II reaction center (RC) of higher plants is known to be a pigment-protein complex having six polypeptide subunits, namely, 47- and 43-kDa polypeptides, two polypeptides of about 30 kDa (called D-1 and D-2), and two polypeptides of cytochrome *b*-559.[1,2] One of the present authors (KS) has recently reported isolation of a photosystem II RC complex consisting of the D-1 and D-2 polypeptides and cytochrome *b*-559,[3] which he believes is the site of primary photochemistry.[3-5]

The purpose of the present study is to obtain as much structural information as possible for the newly isolated RC complex by resonance Raman and HPLC techniques.

The RC complex consisting of the D-1 and D-2 polypeptides and cytochrome *b*-559 was prepared from spinach.[3] The concentration of chlorophyll *a* (Chl *a*) in the solution used for resonance Raman measurements was estimated to be 2.0×10^{-4} mol dm^{-3}. Resonance Raman spectra were observed at 77 K.

Extraction of β-Car from the RC complex and thylakoid membranes and the subsequent HPLC analyses of the extracts were performed by using a $Ca(OH)_2$ column and 0.3% acetone/hexane as the eluent.[6]

When excited at 441.6 nm (nearly resonant with the Soret band of Chl *a*), the

RC complex gave the Raman bands of Chl *a* with a sufficiently high signal-to-noise ratio in addition to a few strong β-carotene (β-Car) bands (Fig. 1a), whereas with 488.0 and 514.5 nm excitation only the β-Car bands were selectively observed (Fig. 1b). Comparison of the observed spectra with the existing Raman data of chlorophylls[7] and those of various isomers of β-Car[8,9] supported the earlier conclusion on the chemical composition of the RC complex.[3] This was further confirmed by our HPLC analysis of the extracted carotenoid, as described below.

We focus our attention on the state of Chl *a* and the structure of β-Car in the RC complex.

It has been pointed out by a group (MF & MT) in the the present authors[10] that the Raman bands of Chl *a* in the CC double-bond stretching region are sensitive to the coordinaiton number (five or six) of the Mg atom. At room temperature, the five-coordinate species (with one axial ligand) shows three Raman bands at 1612-1606, 1554-1551, and 1529-1527 cm^{-1}, whereas these bands shift, respectively, to 1599-1596, 1548-1545, and 1521-1518 cm^{-1} for the six-coordinate species (with two axial ligands). More recently we have confirmed that the above structure-spectrum correlation holds also at 77 K, although some bands shift to slightly higher wavenumbers. The RC complex gives rise to two bands at 1615 and 1552 cm^{-1} (Fig. 1a), which demonstrate that the Mg atom of Chl *a* in the RC complex is five-coordinate. In Fig. 1a the region of 1535-1515 cm^{-1} gives no information on the state of Chl *a* because of the overlapping of a strong β-Car band.

The behavior of the carbonyl stretching bands of Chl *a* in various environments has been extensively studied using infrared[11,12] and resonance Raman[7,13,14] spectroscopy. Although the structure-spectrum correlation is rather complicated in this case, it seems most reasonable to classify the keto CO band at 1672 cm^{-1} observed for the RC complex (Fig. 1a) to the hydrogen-bonded case on the following grounds. (1) The wavenumber of 1672 cm^{-1} falls in the region of 1673-1668 cm^{-1} assigned to the hydrogen-bonded case from room-temperature measurements.[14] (2) The 1672-cm^{-1} band is definitely lower in wavenumber than the corresponding bands in acetone (1679 cm^{-1}) and in tetrahydrofuran (1681 cm^{-1}) where Chl *a* must be monomeric and free from hydrogen bonding. (3) According to an infrared study by Cotton *et al.*[12] in ethanol solution at 80 K, the free and hydrogen-bonded keto CO stretches are located, respectively, at 1694 and 1663 cm^{-1}. We have observed a Raman band at 1657 cm^{-1} which is regarded as corresponding to the 1663-cm^{-1} infrared band. These data suggest that the keto CO group of Chl *a* in the RC complex is hydrogen-bonded but this hydrogen bonding would not be a strong one.

It may be worth noting that the 1672 cm^{-1} band of the RC complex has a rather simple shape, in marked contrast with a more complex feature observed in this region for antenna Chl *a* in intact chloroplasts of various origins.[15] This suggests that the five Chl *a* molecules in the RC complex are located in environments similar to one another to such an extent that their differences are difficult to observe by Raman spectroscopy.

It is interesting to determine the isomeric form of β-Car in the RC complex, since the carotenoids in the RC's of *Rhodopseudomonas sphaeroides* 2.4.1 and G1C are known to take the centrally mono-*cis* form.[8,9] Comparing the spectra in Fig. 1b with those of various isomers of β-Car previously reported by one of the

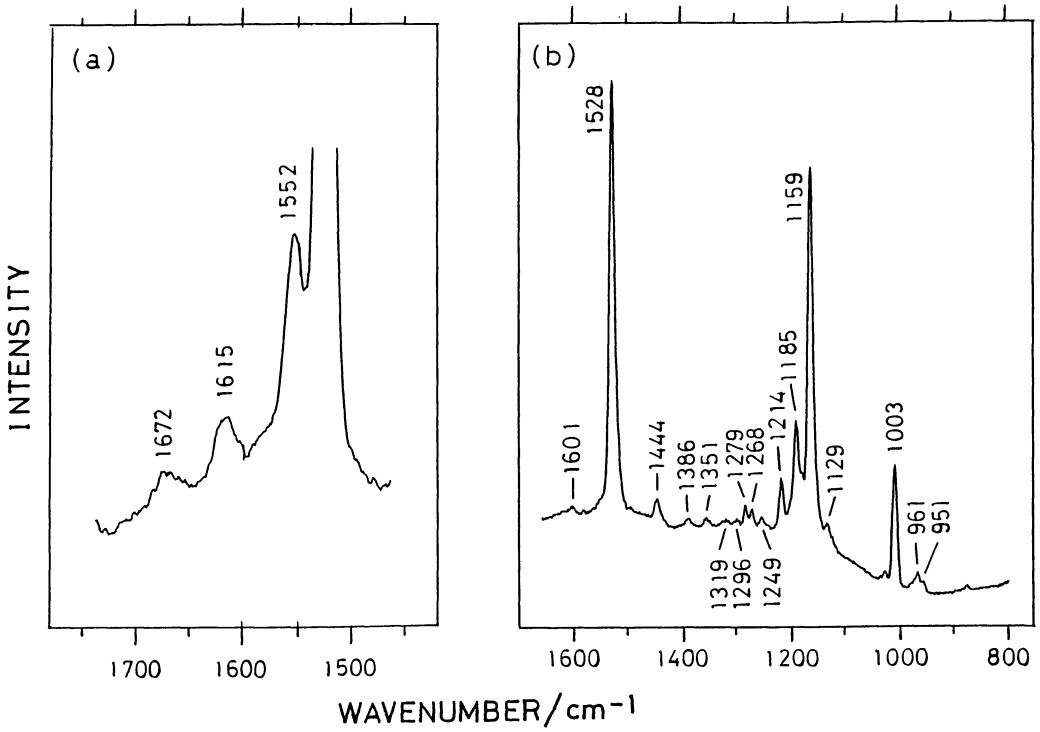

Fig. 1. Resonance Raman spectra of the RC complex at 77 K. (a) Excited at 441.6 nm (the bands at 1672, 1615, and 1552 cm^{-1} are due to Chl *a*); (b) excited at 488.0 nm (only β-Car bands are observed).

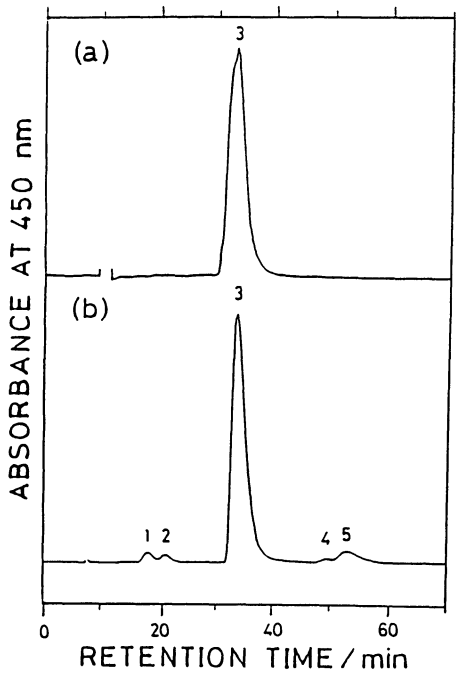

Fig. 2. HPLC elution patterns (detected at 450 nm) of the β-Car isomers extracted from (a) the RC complex and (b) thylakoid membranes. Peaks in (b) are assignable as follows (see Ref. 16): 1, 15-*cis*; 2, 13-*cis*; 3, all-*trans*; 4, 9,9'-di-*cis*; 5, 9-*cis*.

present authors (YK),[9] we find at once that the all-*trans* form is the isomer most likely to exist in the RC complex. The weak bands at 1386, 1351, 1279, 1268, 1214, and 1185 cm^{-1} in Fig. 1b correspond well with the bands of the all-*trans* form. However, it is difficult to exclude completely some other possibilities, for example, existence of the 7-*cis* and 9-*cis* forms whose Raman spectra are similar to that of the all-*trans*. Analysis of the extracted carotenoid with high-performance liquid chromatography (HPLC) is expected to give further information on the isomeric form of the carotenoid *in vivo*. The HPLC analysis of the extracted carotenoid clearly shows a single peak in the elution pattern (Fig. 2a), which undoubtedly corresponds to all-*trans* β-Car. On the other hand, the elution pattern of the carotenoids extracted from thylakoid membranes (Fig. 2b) has a few minor peaks in addition to the main peak due to all-*trans* β-Car. We conclude therefore that β-Car in the RC center complex under study is different in its isomeric form from the carotenoids (sphaeroidene and neurosporene) in the bacterial RC's.

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