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Towards a vibrational analysis of spheroidene. Resonance Raman spectroscopy of ^{13}C-labelled spheroidenes in petroleum ether and in the *Rhodobacter sphaeroides* reaction centre

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Abstract

We report resonance Raman spectra of the carotenoid spheroidene and its 14′-^{13}C and 15′-^{13}C substituted analogues in petroleum ether and bound to the reaction centre of *Rhodobacter sphaeroides* R26. The spectra in petroleum ether correspond to planar all-*trans* spheroidene while those of the reaction centres are consistent with a nonplanar 15,15′-*cis* spheroidene. The effect of ^{13}C labelling is largest in the carbon-carbon double-bond stretching region. The 15′-^{13}C substitution of the reaction centre bound spheroidene, however, hardly changes the C=C band as compared to that for the natural abundance spheroidene apart from a new weak band at 1508 cm^{-1}. This observation has been interpreted as a decoupling of the C_{15}=$C_{15'}$ stretch from the other double-bond stretches in combination with a small intrinsic Raman intensity of this local mode for 15,15′-*cis* spheroidene.

Key words: Vibrational analysis; Photosynthesis; Carotenoid; Spheroidene structure; Resonance Raman; Reaction center; (*Rb. sphaeroides*)

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1. Introduction

Carotenoids in photosynthetic reaction centres (RCs) take part in light harvesting and protect the bacteriochlorophyll pigments from photodestruction [1]. In anaerobically grown *Rhodobacter sphaeroides* wild-type strain 2.4.1, the RC-bound carotenoid is spheroidene (Fig. 1). Understanding the role of this carotenoid in photophysical terms requires a precise knowledge of the structure of spheroidene in the RC. The crystallization and X-ray diffraction of the *Rhodobacter sphaeroides* RC [2–5] provided a breakthrough in the structure determination of the RC. The X-ray analysis located the bound spheroidene molecule on the M-subunit side of the reaction centre in close proximity to one of the accessory bacteriochlorophylls and approx. 11 Å away from the primary electron donor. The stereochemistry of the carotenoid has been determined from these studies to be consistent with a 15,15′-*cis* configuration of the spheroidene molecule. A *cis*-configuration was suggested as early as 1976 [6] and was subsequently supported by proton NMR and resonance Raman data [7] which also suggested that the molecule may be distorted away from planarity near the ends of the conjugated π-electron chain. Recently, a MAS NMR investigation of specifically ^{13}C-enriched spheroidenes provided additional evidence for a 15,15′-*cis* configuration of the carotenoid in the protein [8].

Although spectroscopic methods have already been used extensively for the determination of the absolute configurations of carotenoids bound to photosynthetic pigment-protein complexes, the assignments of the spectroscopic observables to particular molecular features is not always straightforward. Indeed, the spec-

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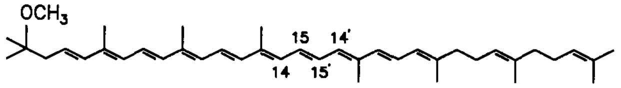

spheroidene

Fig. 1. The molecular structure of all-*trans* spheroidene.

troscopic assignments of the configuration of the carotenoid in the photosynthetic bacterial reaction centre have been controversial and hotly debated in the literature. (See Ref. [1] for a review of this work.) Whether or not the bound carotenoid is distorted away from planarity and what effect these distortions have on the spectroscopic properties of the molecule are still unclear. An examination of these issues requires a systematic correlation of the structural features of carotenoids with their spectroscopic behaviour.

In principle, a resonance Raman study of spheroidene would allow such an examination through a vibrational analysis of that molecule. This type of study, albeit elaborate, is feasible for large molecules, as has been demonstrated for retinals and rhodopsins by Lugtenburg, Mathies and co-workers [9]. We have embarked on a project that comprises (i) the synthesis of spheroidenes that are ^{2}H or ^{13}C labeled at specific positions, (ii) the reconstitution of these spheroidenes into the RC complex of the carotenoidless mutant *Rb. sphaeroides* R26, and (iii) resonance Raman spectroscopy of these spheroidenes both in solution and bound to the RC. A reliable interpretation of the Raman spectra requires the study of a large number of isotopically-labeled compounds. Here we present the first resonance Raman data for natural abundance spheroidene and its 14'-^{13}C and 15'-^{13}C substituted analogues. Well-resolved Raman spectra have been obtained between 130 and 1700 cm^{-1}. In petroleum ether the spheroidenes are planar and in the all-*trans* configuration. The Raman intensity is largely concentrated in three bands. For the RC-bound spheroidenes the spectra support the 15,15'-*cis* configuration. They are rich in lines, in particular also below 1000 cm^{-1} where essentially no scattered light is detected for the spheroidenes in petroleum ether. This is a clear indication of the non-planarity of the RC-bound spheroidene. For all-*trans* spheroidene in solution, a significant redistribution of intensity takes place in the carbon-carbon double-bond stretching region upon 15'-^{13}C substitution. For the RC-bound spheroidene, however, the C=C stretching band remains virtually unaltered upon this substitution apart from a new weak band at 1508 cm^{-1}. This low frequency band most probably derives from the C_{15}=$^{13}C_{15'}$ stretching mode which, apparently, hardly contributes to the Raman intensity in this region for the 15,15'-*cis* RC-bound spheroidene.

2. Experimental

The synthesis of the 14'-^{13}C and 15'-^{13}C spheroidenes has been described previously [10]; the same holds for the reconstitution procedure [8].

The spectra from solutions of the all-*trans* spheroidenes in petroleum ether were measured at about 100 K. Spectra of spheroidenes reconstituted in the *Rhodobacter sphaeroides* reaction centre were obtained at 1.2 K from a glass prepared by adding glycerol to the RC/Tris buffer solution in a ratio 3 : 1.

Resonance Raman scattering was induced by the 496.5 nm line of a Spectra Physics model 2016 Ar^{+} laser and collected at an angle of 90°. The Raman spectra were recorded using a Spex 1403 double monochromator and an EG&G OMA-Vision-CCD multichannel analyser. For the spectra between 130 and 700 cm^{-1} gratings with 1800 gr/mm were used, for the others gratings with 600 gr/mm. The spectral resolution ranged from 0.7 cm^{-1} to 2.5 cm^{-1}, depending on the gratings. Simultaneous recording of a Raman spectrum and the spectrum of a neon calibration lamp enabled us to determine the position of the Raman lines with an accuracy of at least $\pm$ 1.5 cm^{-1}. For the spectra of the RC-bound spheroidenes between 450 and 1700 cm^{-1} in Fig. 3 the fluorescence background has been subtracted in order to facilitate comparison of the spectra with those in Fig. 2.

3. Results and discussion

Resonance Raman spectra are presented in Figs. 2 and 3. The spectra obtained for the RC-bound spheroidene are rich in well-resolved lines while for the all-*trans* spheroidenes in petroleum ether the Raman intensity is largely concentrated in three bands. Although a detailed assignment of the Raman lines is lacking as yet, general agreement exists as regards the local modes that contribute to the spectra in the various frequency ranges [11]. For methyl-substituted polyene chains, bands below 1000 cm^{-1} correspond to out-of-plane bendings and torsions, a band at about 1005 cm^{-1} to the coupled in-plane methyl-rocking mode, those in the fingerprint region between 1100 and 1400 cm^{-1} to carbon-carbon single bond stretches and in-plane carbon-hydrogen bendings, and those between 1500 and 1600 cm^{-1} to carbon-carbon double-bond stretches.

For the RC-bound spheroidenes, a large number of transitions show up below 1000 cm^{-1} including two bands of medium intensity at about 280 and 484 cm^{-1} corresponding to torsional modes and several lines that cluster into bands around 955 cm^{-1} corresponding to hydrogen out-of-plane vibrations. On the other hand, essentially no intensity is present in this region for the planar all-*trans* molecules in petroleum ether. The intensity of the Raman bands is determined by the carbon-carbon stretching character of the corresponding normal modes because the excitation is resonant with the polyene $\pi\pi^*$ transition. For a planar polyene chromophore symmetry precludes the coupling of out-of-plane and in-plane vibrations. Consequently, out-of-plane vibrations are not resonance enhanced in this case. Apparently, the protein matrix induces a distortion of the spheroidene molecule from the planar geometry in the ground state, resonant excited state or both. Further experiments on selectively ^2H substituted spheroidenes are planned to localize the position of the distortion along the polyene chain.

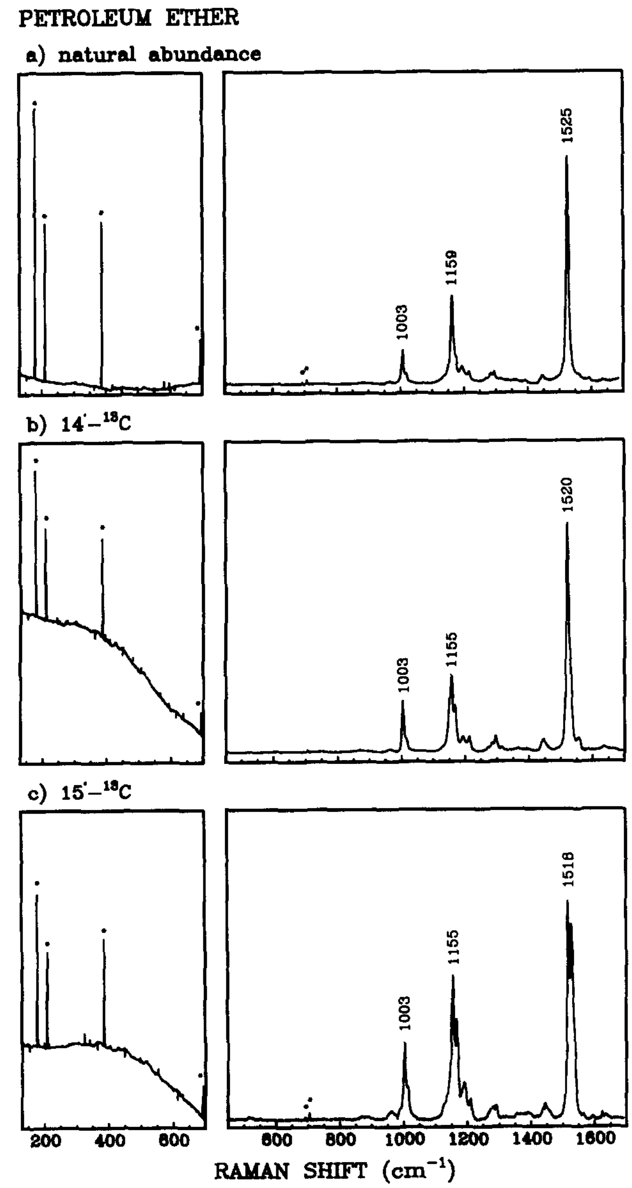

Fig. 2. Resonance Raman spectra of spheroidene, 14'-^{13}C spheroidene and 15'-^{13}C spheroidene in petroleum ether. The lines marked with an asterisk are laser plasma lines.

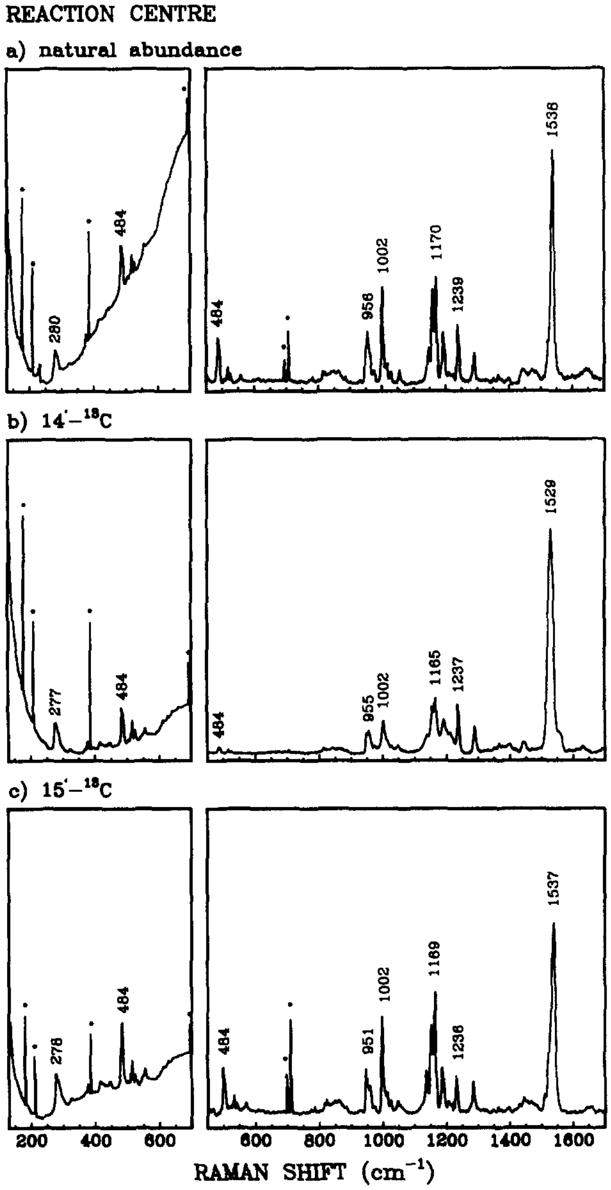

Fig. 3. Resonance Raman spectra of spheroidene, 14'-^{13}C spheroidene and 15'-^{13}C spheroidene bound to the *Rhodobacter sphaeroides* R26 reaction centre. For the spectra between 450 and 1700 cm^{-1} the fluorescence background has been subtracted. The lines marked with an asterisk are laser plasma lines.

The fingerprint region has played a key role in the discussion of the configuration of RC-bound carotenoids. The intensity distribution in the 1150–1300 cm^{-1} region of the Raman spectra of RC-bound spheroidenes (Fig. 3) is clearly different from that of the spectra in solution. Six bands of medium intensity can easily be recognized in the RC spectra, including a prominent band at about 1239 cm^{-1}. Such a band seems to be common in Raman spectra of RC-bound carotenoids. It has been observed for neurosporene in *Rb. sphaeroides* G1C [12–14], for spheroidene in *Rb. sphaeroides* strains Y and 2.4.1 [14], and for spirilloxanthin in *R. rubrum* S1 [14]. Koyama et al. [12,13] observed a band around 1240 cm^{-1} in the resonance Raman spectra of a few *cis* isomers of β-carotene. Comparison of the fingerprint region for these *cis* β-carotene isomers with that for the RC-bound carotenoids led them to the conclusion of a central *cis* double bond for the latter. Significant differences nevertheless exist between the fingerprint region of the resonance Raman spectra of 15,15′-*cis* β-carotene and that of RC-bound spheroidenes. For example, the most intense band in the spectrum of Fig. 3a occurs at 1170 cm^{-1}, whereas there is no band at that position in the spectrum of 15,15′-*cis* β-carotene. An interpretation of the spectra of spheroidene in the fingerprint region has to await a normal coordinate analysis.

The most intense band in the spectra of Figs. 2 and 3 is to be found in the C=C stretching region and corresponds to several overlapping modes. Particularly those modes that contain in-phase contributions of C=C stretching coordinates in the central part of the conjugated chain dominate the Raman intensity because bond-order changes upon electronic excitation are largest there [15]. The band maximum shifts from 1525 to 1538 cm^{-1} on going from the solution to the RC. Such a shift is generally observed on going from all-*trans* to *cis* polyenes and derives from the decreased conjugation resulting in an increased force constant [9,14]. The spectral changes upon ^{13}C labelling of spheroidene at the 14′ or 15′ position are largest in this region, as indicated in detail in Fig. 4. For the all-*trans* spheroidene in petroleum ether the effect is largest for ^{13}C substitution at the 15′ position which is to be expected because of the more central position of the C_{15}=$C_{15'}$ bond compared to that of the $C_{14'}$=$C_{13'}$ bond. A mode splits off at 1518 cm^{-1}. For a local C=C normal mode the shift would be 30 cm^{-1}. The experimentally observed 7 cm^{-1} refers to the mixed character of the C=C stretching normal modes. For the RC-bound spheroidene the spectrum changes in a different way, consistent with its 15,15′-*cis* configuration. For 14′-^{13}C substitution the intensity redistribution is more pronounced than in petroleum ether whereas for 15′-^{13}C substitution the Raman band remains virtually unchanged apart from a small shoulder at 1508 cm^{-1}. This new band most probably represents the local C_{15}=$^{13}C_{15'}$ stretch. A normal mode analysis for 15,15′-*cis* β-carotene [16] suggests that, as compared to the all-*trans* isomer, the C_{15}=$C_{15'}$ stretch decouples from the other C=C stretches. If that is true for spheroidene, the minor intensity of the 1508 cm^{-1} band indicates a small intrinsic Raman intensity of the C_{15}=$C_{15'}$ stretching mode. This mode then hardly contributes to the intensity of the 1538 cm^{-1} band in the resonance Raman spectrum of spheroidene bound to the *Rhodobacter sphaeroides* RC.

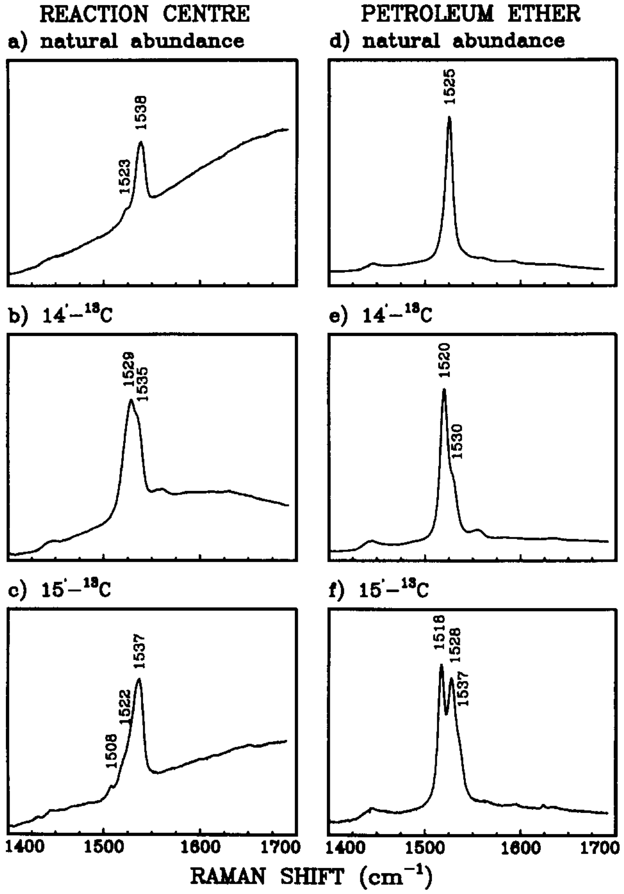

Fig. 4. Enlarged view of the C=C stretching region in the spectra of Figs. 2 and 3.

In conclusion, the resonance Raman data reported here present a first step towards a vibrational analysis and a correlation with the structural determination of RC-bound spheroidene. More, specifically labeled, spheroidenes, particularly ^{2}H substituted ones, will be studied in order to obtain a reliable normal coordinate analysis.

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