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Structure of spheroidene in the photosynthetic reaction center from Y *Rhodobacter sphaeroides*

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The structure of the reaction center of Y *Rhodobacter sphaeroides* has been solved at 3 Å resolution, using the atomic coordinates of the reaction center from the carotenoidless mutant R26 *Rhodobacter sphaeroides*. The structure has been refined by a simulated annealing with the computer program X-PLOR, leading to a crystallographic R factor of 0.22 using reflections between 8 and 3 Å. The spheroidene molecule which is bound to the Y reaction center has been fitted in the electron density map as a 15-*cis* isomer with a highly asymmetric structure. The *cis*-bond is located at proximity from ring I of the accessory bacteriochlorophyll on the inactive M side. The nature of the *cis*-bond was confirmed by resonance Raman spectra obtained from Y reaction center crystals. The structure of spheroidene in Y reaction center is compared to that proposed for 1,2-dihydroneurosporene in *Rhodopseudomonas viridis* reaction center crystals.

Photosynthesis; Reaction center; X-ray crystallography; Spheroidene; (*Rhodobacter sphaeroides*)

1. INTRODUCTION

Photosynthetic reaction centres from purple bacteria generally contain one firmly bound carotenoid molecule. This pigment does not participate directly in electron transfer, but it performs an essential role of photoprotection mainly by quenching photogenerated triplet state of the reaction center (RC) 'special pair' (for a recent review see [1]). As evidenced by resonance Raman spectroscopy, this carotenoid differs in its chain configuration [2,3] and conformation [4] from the bulk carotenoid which is bound to the light-harvesting complexes; owing to the carotenoid being bound to protein it adopts a *cis*-configuration, although the exact nature has been a matter of controversy [2,3]. A thorough reexamination of the resonance Raman results, together with new NMR data, led Lutz et al. [4] to propose that the carotenoid has a central 15-*cis* configuration with additional out of plane twisting(s) occurring outside the central region.

Recently, the structure of the carotenoid, 1,2-dihydroneurosporene (see fig.1), which is bound to the RC of *Rhodopseudomonas viridis* has been described from X-ray crystallography data [5]. In the structure refined at 2.3 Å [6,7], the carotenoid is located between the B and C helices of the M subunit close to the accessory bacteriochlorophyll; the electron density was fitted with a 13′-*cis* isomer, with an out-of-plane twist occurring in the C14′-C15′ part of the molecule. In wild-type 241 *Rhodobacter sphaeroides* RC structure, the carotenoid (here spheroidene, see fig.1) was reported [8] to be located at the same site as in *Rps. viridis* RC [6]. The resolution was however insufficient to position unequivocally the *cis*-bond, which has been placed in the model at the C15 position.

We have recently solved the structure of the RC of another wild-type strain, Y *Rb. sphaeroides*. A peculiar feature of this strain is that the metal which is interacting with the quinones in the RC is easily exchanged in vivo; thus RCs containing predominantly Mn^{2+} instead of Fe^{2+} could be isolated [9] and crystallized [10]. The Y RC also contains a bound carotenoid, spheroidene, which is absent from mutant R26 RC. Linear dichroism was demonstrated for spheroidene in the crystals, as well as for the other pigments; photoinduced electron transfer was shown to be functional from the primary donor to the secondary quinone [11]. In this paper we present structural data concerning the localization and the conformation of the spheroidene molecule in Y RC; we compare these results with those published for 1,2-dihydroneurosporene in the *Rps. viridis* RC structure.

2. MATERIALS AND METHODS

Crystallization of *Rb. sphaeroides* Y RC was performed as described in [11,12]. Orthorhombic crystals grew as long rods with a diamond-shaped cross-section; the space group is $P2_12_12_1$ with parameters a = 143.7; b = 139.8; c = 78.65 Å.

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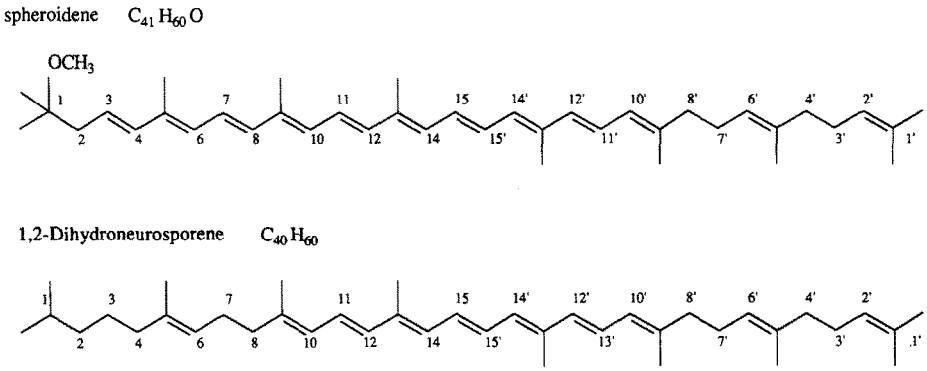

Fig.1. Formulae of spheroidene (top) and 1,2-dihydroneurosporene (bottom).

For resonance Raman spectroscopy, crystals were transferred from their mother liquor to a cover slide and were rapidly frozen in liquid N_2 before transfer to a liquid helium cryostat; experiments were performed as described in [4].

Best crystals diffract to 2.8 Å resolution. Using synchrotron radiation at LURE (Orsay, France) shortens exposure time considerably. An oscillation rotation camera was used and average exposure time was 15 min per film. The X-ray wavelength was 1.40 Å; a dozen crystals were necessary to collect a full set of data at 18°C. Films were scanned with an Optronics microdensitometer and analyzed with DENZO, a program kindly provided by Z. Otwinowski (Yale University, USA), which includes a profile-fitting procedure. Further data processing used the CCP4 software package (Daresbury, England). After scaling the various batches corresponding to each crystal, a set of 26000 independent reflections was obtained. The structure has been solved by simulated annealing with the computer program X-PLOR [13]. The molecule was entirely rebuilt, using difference Fourier in which 30 residues were omitted at a time. Pigments were repositioned by Fourier difference, one pair of analogous molecules being removed at a time.

3. RESULTS AND DISCUSSION

The known structure of R26 *Rb. sphaeroides* RC [14] which crystallizes with the same space group and with isomorphous unit cells was used as a starting point for refinement. Using X-PLOR program [13] one complete set of cycles was applied to the three polypeptide chains and to all pigments except spheroidene and quinones. This led to a crystallographic R factor of 22% with a set of data at 3 Å resolution. Sequence determination of Y RC polypeptides is in progress but not complete (Astier, C. and Ajlani, G., personal communication); in its absence we are temporarily using instead, the known sequence [15] of the RC polypeptides from *Rhodobacter sphaeroides* wild-type, 241 strain; close similarity is expected. At the end of the refinement, a difference Fourier map, in which residues surrounding spheroidene were also removed, showed without ambiguity the location of spheroidene (fig.2) and both quinones (not shown).

The electron density corresponding to spheroidene, closely surrounded by hydrophobic residues from B and C helices of M subunit, has a bent cylindrical shape. Consequently it is not possible to fit a fully extended, all-*trans* molecule. The bending of the electron

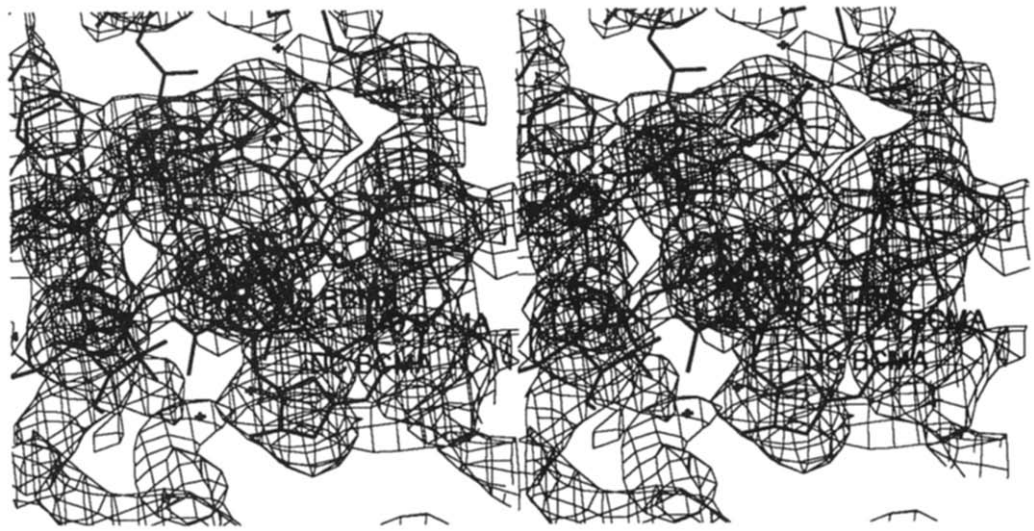

Fig.2. Stereoplot of the electron density map, around the spheroidene *cis*-bond (NS C15-C16). Nitrogen atoms of accessory Bchl BCMA are identified; NB, ring I; NC, ring II; ND, ring III.

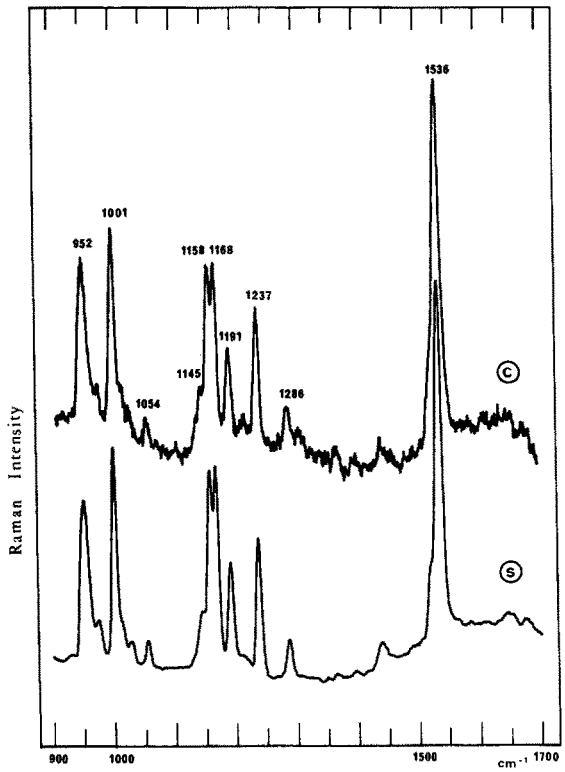

Fig.3. Resonance Raman spectra (900–1700 cm^{-1} region) of spheroidene present in reaction centers from *Rb. sphaeroides* strain Y. Excitation: 496.5 nm, sample at 25 K. Spectral resolution, 7 cm^{-1}; S, solution; C, single crystal.

density corresponding to spheroidene clearly implies that a *cis*-bond is present at close proximity of ring I of the accessory bacteriochlorophyll on the M side.

In order to build spheroidene in the electron density, we started from a 15-15′ *cis* configuration, on the basis of resonance Raman spectra obtained from Y RC crystals. These spectra indeed were identical to those previously obtained from RCs of the same strain in detergent solution [2], which have been shown to be characteristic of a distorted 15-15′ *cis* molecule [4] (fig.3). The crystal and solution spectra in particular present a specific band at 1237 cm^{-1}. This band, which arises from coupled CH bending and CC stretching of the 15CH = 15′CH group [16], has been observed for 15-*cis* isomers only [17]. It is absent from resonance Raman spectra of 13-*cis* isomers of C40 carotenoids obtained in equivalent conditions, while the latter isomers yield a strong Raman band at 1138 cm^{-1} [3,16,17], which is absent from the RC spectra.

The electron density corresponding to the two extremities of the spheroidene molecule was not well defined. The central region of spheroidene (C7–C9′) only could be fitted in the first Fourier difference map. This prevents the assignment of the methoxy terminal group on either side. However, the C1–C15 part of the molecule being conjugated from C3 to C15 may be expected to be planar. The other moiety has single bonds at C7′–C8′, C3′–C4′, which allows for a more flexible conformation. This implies a quite asymmetrical structure, which can be fitted in only one way in the cavity defined by the side chains around the carotenoid molecule (fig.4).

For comparing this structural model with that obtained from *Rps. viridis* RC structure, the pigment arrays have been superimposed so as to get the best fit between the four Bchls (fig.5). This points out the similar location of the carotenoid in these two RCs, despite the different position of the *cis*-bond. Note that the C1 end of the spheroidene molecule is on the side of the C1′ end of 1,2-dihydroneurosporene molecule. In both cases, the part of the molecule which is the closest one from ring I of the accessory Bchl is the bent part including the *cis*-bond.

Functional properties of these carotenoids are known to differ: unlike spheroidene, 1,2-dihydroneurosporene is unable to quench the dimer triplet state by forming

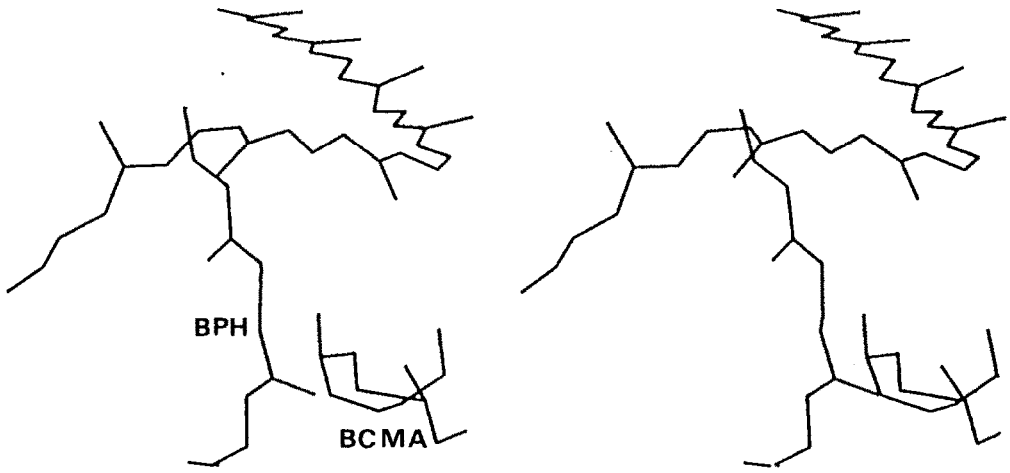

Fig.4. Stereoview of spheroidene showing the spheroidene molecule. The pseudo-twofold symmetry axis is approximately vertical and in the plane of the figure.

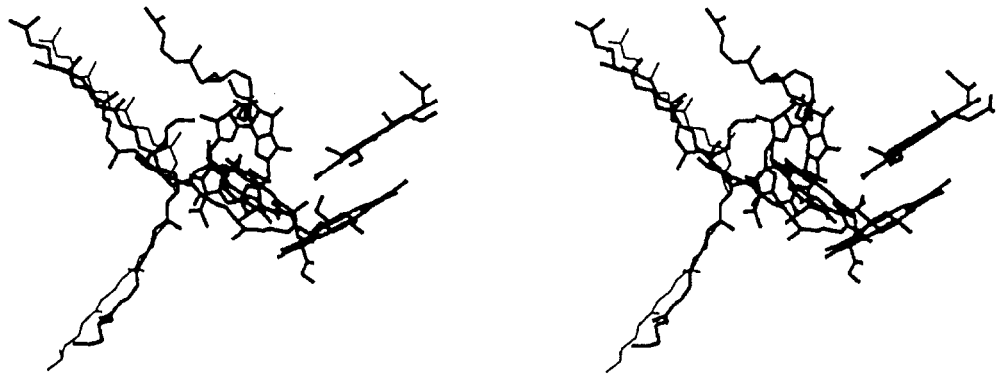

Fig.5. Stereoview showing the accessory Bchls, the Bchl dimer and spheroidene (bold traces); the pseudo twofold symmetry axis is approximately perpendicular to the plane of the figure. 1,2-Dihydroneurosporene has been superimposed as described in text (thin line).

a carotenoid triplet [1]. Two possible explanations have been proposed, based either on structural differences (e.g. different distances from the Bchl dimer), or on unfavourable energetics for the formation of 1,2-dihydroneurosporene triplet state [1]. At the present stage of our structure determination, the rather similar location of the two carotenoids in the RC structures is in favour of the second hypothesis.

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