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ON THE STATE OF CAROTENOIDS BOUND TO REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA: A RESONANCE RAMAN STUDY

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Summary

The carotenoids bound to reaction centers of wild, Ga and GlC strains of *Rhodopseudomonas spheroides*, of *Rhodospirillum rubrum*, strain S1 and of *Rhodopseudomonas viridis*, yield very similar, but unusual resonance Raman spectra. Through a comparison with resonance Raman spectra of 15,15′-*cis*-β-carotene, these carotenoids are shown to assume cis conformations, while the corresponding chromatophores contain all-trans forms only. These cis conformations likely are identical for all the carotenoids studied. They remain unaffected by variations of temperature from 20 to 300 K as well as by the redox state of *P*-870. They are unstable, being rapidly isomerised towards the all-trans forms when extracted from the reaction centers. The possible nature of these conformers is discussed on the basis of their electronic and vibrational spectra.

Introduction

Recently Lutz et al. showed that spheroidene present in preparations of reaction centers of *Rhodopseudomonas spheroides*, strain Y, yields specific resonance Raman spectra, very different from those of bulk spheroidene of the chromatophore [1]. Lutz et al. tentatively interpreted these differences as resulting from different conformations of the two pools of molecules, bulk spheroidene undoubtedly assuming an all-trans conformation. These observations demonstrated that spheroidene is an intrinsic constituent of the reaction centers of *Rps. spheroides*, as other experiments showed simultaneously [2]. A cis conformation, if general among carotenoids bound to reaction centers, could be the origin of their playing a specific role, the most obvious one being their photoprotective effect, most recently demonstrated for reaction centers

of *Rhodospirillum rubrum* [3]. The present work was thus intended to test the validity of the interpretation of Lutz et al., by comparing resonance Raman spectra of spheroidene bound to reaction centers with those of 15,15'-*cis*-β-carotene. Further, the generality of the property was investigated by working on another wild strain of *R. spheroides* (2.4.1) and its Ga and GlC mutant strains, containing different carotenoids, as well as on other *Athiorhodaceae*, namely *Rhodospirillum rubrum* and *Rhodopseudomonas viridis*.

Material and Techniques

Samples. Previously described methods were followed in preparing chromatophores and reaction centers of *Rps spheroides*, strain Y [4] *Rps. spheroides*, strain R 26 [5], 2.4.1, Ga and GlC [6], of chromatophores and reaction centers of *R. rubrum* [7] and of membranes and reaction centers of *Rps. viridis* [8].

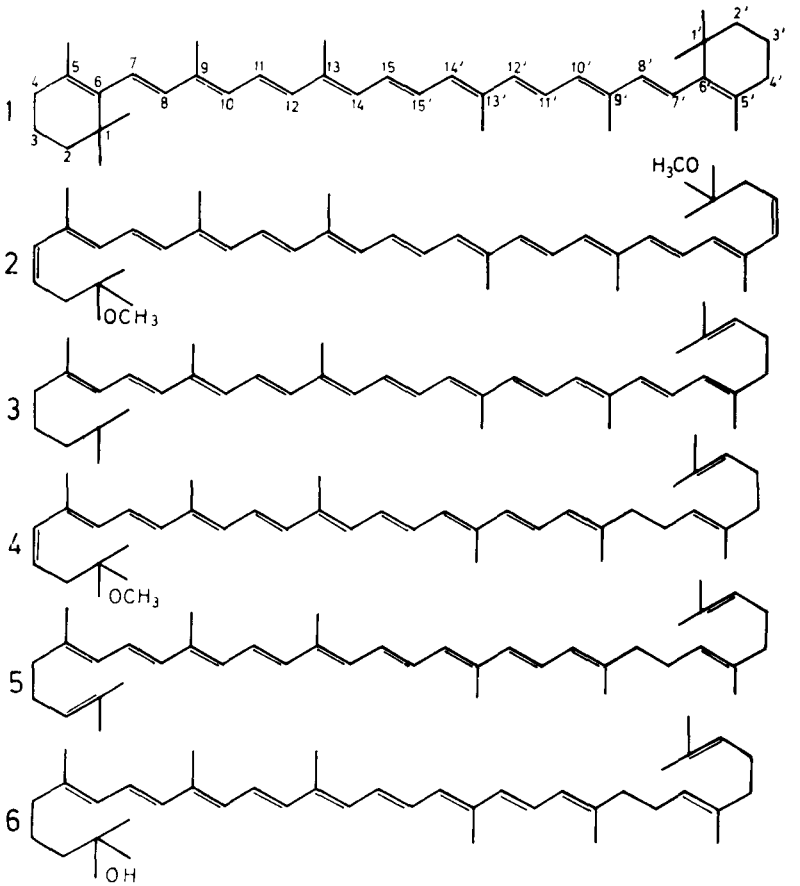

Fig. 1. Structural formulae of carotenoids studied in this work. (1) β carotene. (2) spirilloxanthin, present in reaction centers of *R. rubrum* S1. (3) 1,2-dihydrolycopene, in *Rps. viridis*. (4) spheroidene, in *Rps.* spheroides Y and 2.4.1. (5) neurosporene, in *Rps. spheroides* GlC. (6) chloroxanthin, in *Rps. spheroides* Ga. Conformations given to the ends of the chains are arbitrary.

The 15.15'-cis isomer of β-carotene (purity 98%) was a gift from Hoffmann La Roche S.A.; the all-trans form was extracted from spinach and purified by column chromatography [9].

Carotenoids were extracted from chromatophores and reaction centers and chromatographed according to the methods of Shneour [10].

Rapid extraction of all pigments from reaction centers was achieved by treatment with acetone and petroleum ether [11] in dim light. A droplet of petroleum ether containing the dissolved pigments was quickly frozen down to 77 K. The whole process took less than 30 s.

Resonance Raman spectra. Apparatus and techniques have been previously described [12,13]. A Jobin-Yvon HG 2S spectrometer was alternatively used in some experiments. Spectral resolution ranged from 3 to 8 cm^{-1}. Emission lines between 454.5 and 514.5 nm of an Argon Laser (Spectra Physics) were used to selectively induce resonant Raman scattering from carotenoids.

Room temperature spectra were obtained using grazing excitation on thoroughly degassed suspensions or solutions [14]. Cooling of samples results in higher quality spectra and prevents thermal degradation. Low temperature spectra were obtained using grazing excitation on droplets of concentrated suspensions or centrifugation pellets of chromatophores and reaction centers, and of solutions of free pigments in acetone, hexane or petroleum ether. These droplets (about 5 μl) were cast on microscope coverslips, then frozen in liquid nitrogen and further cooled down to ca 30 K by a flow of gaseous helium circulating in a cryostat (13).

Differential absorption spectra. The sums of two to four absorption spectra of reaction centers of the R 26, carotenoidless mutant of *Rps. spheroides* were subtracted with various relative weights, from the same number of spectra of reaction centers of the wild type, using a PDP 12 computer (Digital Equipment Corp.).

Results and Discussion

Resonance Raman spectra of spheroidene

Resonance Raman spectra of spheroidene (Fig. 1) bound to reaction centers of *Rps. spheroides*, strains Y or 2.4.1 (wild types), yield marked discrepancies with those of bulk spheroidene of the chromatophore (Figs. 2 and 3 and Tables I and II). Most of these discrepancies were observed in preresonance as well as in resonance on any of the visible bands of the carotenoid. Spheroidene of the reaction center at 30 K is thus mainly characterized by: (i) a +10 cm^{-1} shift and an alteration of the structure of the band located at 1540 cm^{-1} (ν_1); (ii) a decrease in intensity relatively to that of the ν_3 band at 1006 cm^{-1} and a splitting into two components at 1161 and 1171 cm^{-1} of the ν_2 band observed at 1159 cm^{-1} for chromatophores; (iii) a strong increase in relative intensity of a band at 956 cm^{-1}, present as a very weak shoulder only in spectra from chromatophores; (iv) additional or strongly enhanced bands at 1241 (intense), 1058 (weak) and 490 cm^{-1} (medium). (v) several other spectral changes in the lower frequencies region particularly among a group of weak bands in the 750—900 cm^{-1} range.

None of these discrepancies can be ascribed to the presence of small amounts

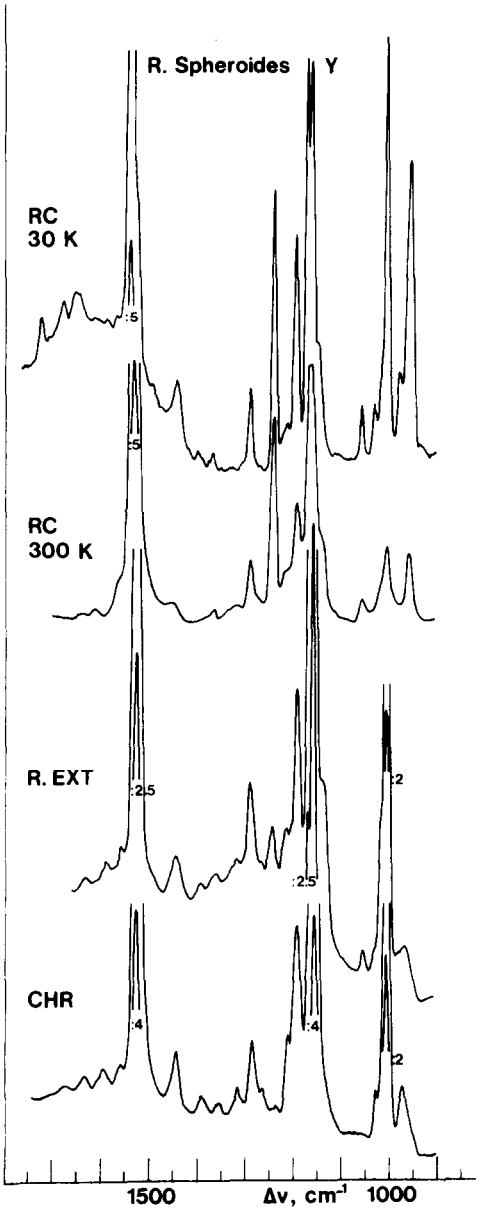

Fig. 2. Resonance Raman spectra of spheroidene from *Rps. spheroides* Y (wild type), 900—1700 cm^{-1} region, excitation 496.5 nm, resolution at 1000 cm^{-1}: 6 cm^{-1}. RG 30 K, in reaction centers at 30 K. RC 300 K, in reaction centers at room temperature, background subtracted. R. EXT, in petroleum ether solution, rapidly extracted from reaction centers and frozen at 30 K (see text). Note the shoulder at 1172 cm^{-1} on the ν_2 band. CHR: in chromatophores 30 K. Instrumental sensitivities in recording the strongest bands have been divided by the factors indicated; base lines approximately correspond to the lowest points of the spectra.

of spheroidenone in the chromatophores. This carotenoid indeed yields resonance Raman spectra almost indiscernable, in a given environment, from those of spheroidene.

The oxidation state of *P*-870 apparently has no influence on the resonance

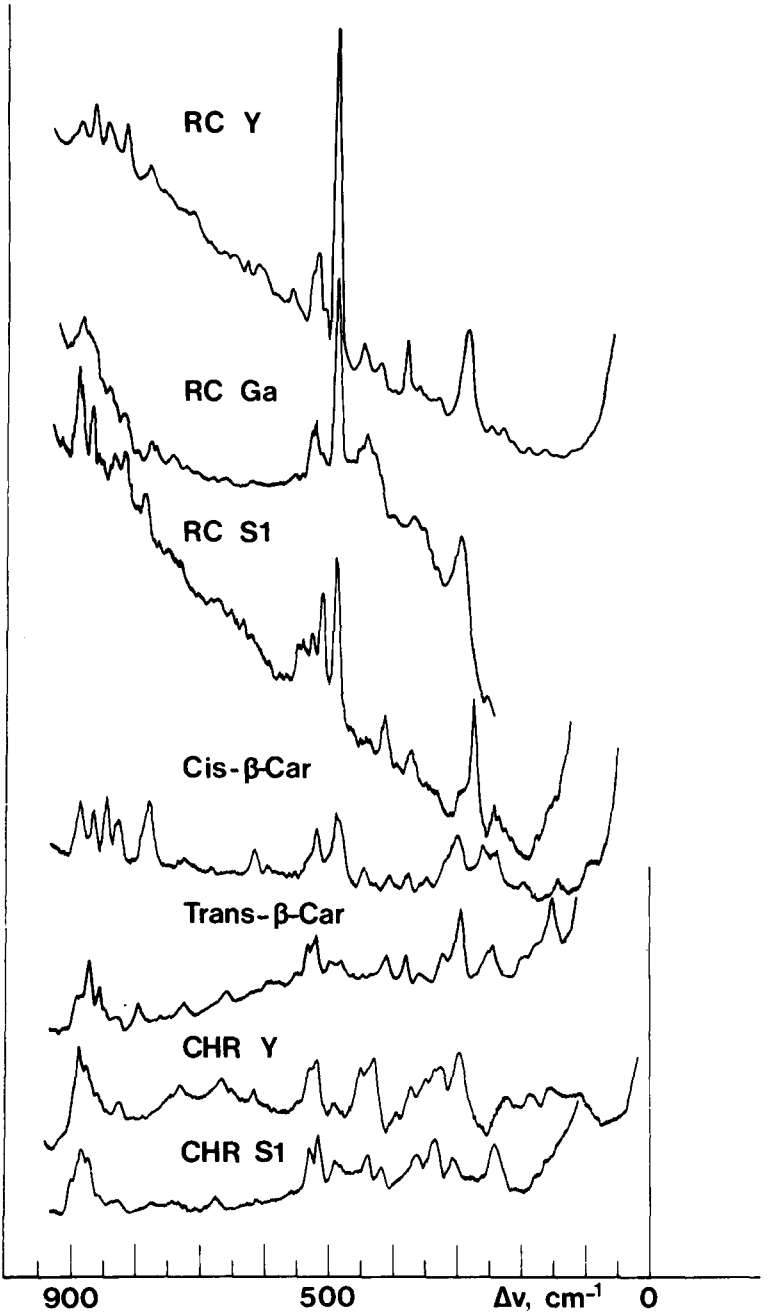

Fig. 3. Resonance Raman spectra of carotenoids at 30 K, 0—900 cm^{-1} region, excitation 496.5 nm, resolution at 900 cm^{-1} : 6 cm^{-1}. RCY spheroidene in reaction centers of *Rps. spheroides* Y. RCGa, chloroxanthin in reaction centers of *Rps. spheroides* Ga. RCS1, spirilloxanthin in reaction centers of *R. rubrum* S1. Cis-β-car, 15,15′-*cis*-β carotene in cyclohexane. Trans-β-car, *all-trans*-carotene in cyclohexane. CHRY, spheroidene in chromatophores of *Rps. spheroides* Y. CHRS1, spirilloxanthin in chromatophores of *R. rubrum* S1. Same instrumental sensitivities as in Figs. 2, 4 and 8, respectively, except for *all-trans*-β-carotene and for chromatophores of *Rps. spheroides* Y here reproduced with sensitivies 1.5 and 2.5 times higher than the 1000—1600 cm^{-1} regions in Figs. 4 and 2, respectively.

TABLE I

FREQUENCIES (cm^{-1}) OF RESONANCE RAMAN BANDS OBSERVED FOR CAROTENOIDS AT 35 K, 800—1700 cm^{-1} REGION

Excitation range 454.5—514.5 mm. Indications of relative intensities are valid for resonance on the 0—0 level of the ^1B-^1A band. Overall uncertainty on frequencies: of strong bands, ±2 cm^{-1}; of weakest bands, ±5 cm^{-1}. CHR, chromatographores. EXT, pigment extracted from reaction centers, all-trans conformation; RC, reaction centers; w, weak relative intensity; m, medium; s, strong; v, very; e, extremely; b, broad; sh, shoulder.

β-Carotene		Neurosporene		Spheroidene		Spirilloxanthin	
All-trans-	15,15′-*cis-*	EXT	RC	CHR	RC	CHR	RC
				1680 ew	1680 w		
					1657 w		
		1640 ew	1635 ew	1640 ew	1648 w	1640 ew	
	1604 vw	1600 w	1600 vw	1600 vw	1612 ew	1614 w	1610 vw
1595, vw,b					1590 ew		1589 vw
1565 wsh	1568 wsh	1575 ew		1565 wsh	1570 wsh	1575 vw	1555 w
1542 sh	1540 vs		1542 vs		1540 vs		1528 vs
1530 vs	1525 sh	1533 vs	1531 vssh	1530 vs	1525 sh	1514 vs	
					1495 vw	1487 wsh	1495 vw
1450 w	1445 w	1450 w	1444 w	1450 w	1444 w	1448 w	1447 w
	1407 vwb			1407 vwsh	1402 vw		1400 vw
1392 vw	1398 vw	1395 vw	1395 vw	1397 vw		1396 vw	
1358 vw	1363 vw	1358 vw	1366 ew	1363 vw	1368 vw	1360 vw	1370 ew
			1330 ew		1334 ew		1353 ew
1323 w,b	1319 vw	1322 vw	1316 ew	1323 vw	1320 ew	1329 vw	1325 vw
1305 vw	1307 vw	1298 vw				1305 ew	1307 vw
1285 vw	1285 vwsh	1285 vw	1290 w	1290 w	1291 w	1289 w	1291 w
1274 vw	1270 vwsh	1270 vw	1275 vwsh	1269 ewsh	1270 ewsh	1265 vw	1273 ew
	1245 s						
	1237 s		1241 s	1240 ew	1241 s	1241 ew	1240 m
1216 w	1221 w	1215 wsh	1217 w	1218 wsh	1215 vw	1220 ewsh	1205 sh
1194 m	1197 s	1203 sh	1204 w	1195 m	1195 s	1194 s	1196 m
1177 wm	1180 vw	1187 wsh	1187 vwsh				
			1172 sh		1171 s		
1160 vs	1160 vs	1164 s	1162 s	1160 vs	1161 s	1153 vs	1162 vs
1138 m	1136 w	1135 wsh	~1140 vwsh	~1135 wsh	1150 msh	1132 wsh	1148 msh
	1056 w		1058 vw		1058 w		1060 w
1024 wsh	1026 w	1035 vwsh	1030 wsh	1035 wsh	1031 w	1034 vwsh	1035 wsh
						1018 mwsh	
1010 s	1006 s	1010 s	1008 s	1005 s	1005 s	1004 s	1005 s
965 w	970 sh	965 w	979 vw	978 w	978 w	970 m	960 s
955 v	957 s,b		957 ms	955 vwsh	956 s	947 wsh	
	939 w	———	———		937 vw		
	918 w	*	*			902 w	
893 vw				893 vw,b	892 w	887 w	892 w
874 vw						875 w	873 w
858 vw				865 vw	869 w	860 vw	862 w
850 ew	849 w				849 w		840 vw
830 ew	831 w			835 vw	829 w	827 ew	823 vw

* Not studied below 950 cm^{-1}.

Raman spectra of spheroidene bound to the reaction center. Reaction centers treated with dithiothreitol and untreated indeed yield identical spectra for bound spheroidene. The excitation light beam keeps *P*-870 oxidized in the untreated centers [1].

Resonance Raman spectra of spheroidene of reaction centers at room tem-

TABLE II

FREQUENCIES (cm^{-1}) OF RESONANCE RAMAN BANDS OBSERVED FOR CAROTENOIDS AT 35 K, 0—800 cm^{-1} REGION

See legend of Table I.

β-Carotene		Spheroidene		Spirilloxanthin	
All-trans	15,15'-cis-	CHR	RC	CHR	RC
798 ew	784 w		785 ew		790 vw
?765 ew			755 ew	775 ew	
730 vw	730 ew,b	~735 ew,b	715 ew		
663 vw		675 ew,b	655 ew	680 vw	
			630 ew		
	618 ew	615 ew	610 ew,b		
			561 ew		545 w,b
533 w		530 vwsh	529 sh	532 w	528 w
521 w	520 w	518 vw	521 w	519 w	
490 vw,b	487 w	488 vw	490 ms	490 vw	492 wm
	447 vw	453 vw	450 vw		445 ew
437 ew		439 vw		440 vw	
418 vw	405 vw		423 vw	418 vw	416 w
380 vw	378 vw	370 ew	381 w		375 vw
360 ew	355 ew		360 ew	365 vw	
322 vw		330 ew	330 ew	335 w	
	302 w,b	309 vw		305 vw	295 vwsh
294 w	260 w	~280 ew,b	280 w		276 w
245 vw,b	242 w	240 vw	242 ew	242 w	245 vw
		230 vw	230 ew	225 vwsh	
195 vw	197 ew				
?175 vw		~180 ew	190 ew		175 ew
150 w	142 ew		165 ew	~150 ew	
	90 vw				

perature exhibit the same characteristics at those obtained at low temperatures, down to 25 K (Fig. 2). Larger bandwidths in room temperature spectra do not allow the two components of the ν_2 band to be resolved. However, its complexity is clearly shown by a "square" shape.

Extensive alterations of the resonance Raman spectrum of spheroidene can only result from a change in symmetry of the molecule and/or from an extensive alteration of π electron repartition along the polyene chain. Such an alteration can result from a change in conformation of the polyene backbone, or from π electronic interaction with adjacent molecules. The latter hypothesis appears less probable than the former, in view of the important spectral alterations observed: resonance Raman spectra of carotenoids generally are very weakly sensitive to environment [14,15], and spheroidene has only weak effects on other pigments of the reaction centers (ref. 1, and see below, electronic absorption spectra). Resonance Raman spectra of bulk spheroidene of the chromatophores do not differ from those of a C_{40} carotenoid in its all-trans conformation. In the former hypothesis, spheroidene bound to reaction centers should then adopt some cis conformation.

Resonance Raman spectra of 15,15'-cis-β-carotene

In order to check the validity of this hypothesis, we ran resonance Raman

spectra of 15,15′-*cis*-β-carotene (central monocis form) and compared them to those of the all-trans form, at low temperature (Figs. 3 and 4 and Table I and II). Several weak or very weak bands are observed on spectra of both forms, in addition to the main bands. They are of general occurrence for carotenoids [13,16] and most probably correspond to fundamental modes, theoretically predicted, but not observed previously [17—19].

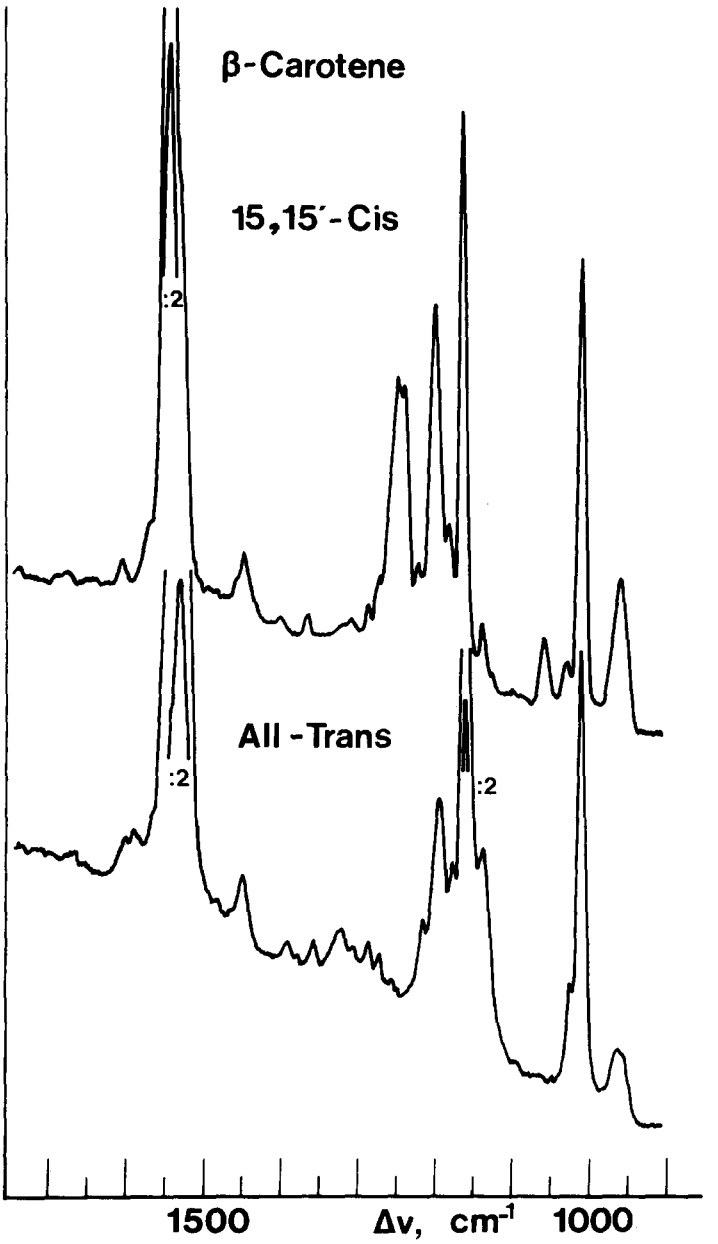

Fig. 4. Resonance Raman spectra of β-carotene at 30 K, 900—1700 cm^{-1} region, same conditions and remarks as in Fig. 2, Top: 15,15′-cis isomer; bottom: all-trans isomer, cyclohexane solutions.

The main differences between these spectra are the following: the frequency of the ν_1 band, located at 1540 cm^{-1} for the cis isomer, is 11 cm^{-1} higher than for the all-trans from, and its structure is modified. The corresponding normal modes primarily involve stretching of C=C bonds of the polyene chain [20]. The increase in frequency resulting from bending of the chain can be explained, as is the well-known effect of chain length [18,20], in terms of an increase in force constant resulting from a decrease in delocalization of π electrons of the C=C bonds over the whole chain.

The intensity of the ν_2 band at 1160 cm^{-1} decreases for the cis form relatively to that of the ν_3 band, but its structure and frequency are not altered.

The relative intensity of the 955 cm^{-1} component of the broad band at 970—955 cm^{-1} increases markedly. This band has been attributed to out of plane bending of C—H groups of the chain [20], but may also involve C—CH_3 stretching [21]. Lower molecular symmetry of the cis form may result in higher activity of these modes. An additional weak band is observed at 1057 cm^{-1} and may have a similar origin.

An additional pair of strong bands occurs at 1237 and 1245 cm^{-1}. These occur in the so called fingerprint region (1100—1400 cm^{-1}) of vibrational spectra of polyenes known to contain bands sensitive to both the nature of the end groups and the conformation of the chain. The corresponding modes should involve admixtures of C—C stretching, of C=C—C bending, and perhaps of in plane C—H bending [20,21].

Half a dozen weak bands in the 750—900 cm^{-1} range are very sensitive to isomerisation of the chain. Among them, a band at 784 cm^{-1} characterizes the cis form. This band may correlate with a strong infrared band at 779 cm^{-1}, which occurrence has been shown by Lunde and Zechmeister [22] to depend on the presence of a non-methylated, cis double bond in the molecule. Resonance Raman spectra of retinal isomers bring support to the idea that the same mode may be active in resonance Raman, depending on the same criterion: among the all-trans, 13-cis, 9-cis and 11-cis isomers, the latter only yields a Raman band at 768 cm^{-1} [23].

Finally, a marked increase in intensity is observed for a band at 487 cm^{-1} in resonance Raman spectra of 15,15'-*cis*-β-carotene, in the region of bending and torsional modes.

It is immediately apparent from these data that resonance Raman spectra of the 15,15'-cis isomer differ from those of the all-trans form by features very similar to those which differentiate spectra of spheroidene bound to reaction centers and of bulk spheroidene of the chromatophore.

Spheroidene bound to reaction centers of *R. spheroides*, strains Y and 2.4.1 thus adopts a cis conformation.

Extraction of cis-spheroidene

Additional evidence that the specific resonance Raman spectra of spheroidene bound to reaction centers are not due to pigment-pigment π electronic interactions is provided by resonance Raman spectra of pigments extracted from the reaction center. Extraction and chromatography of spheroidene by conventional methods [10] yields the all-trans form only. However, a rapid extraction (30 s) of all the pigments of the reaction center by petroleum ether,

in subdued light, followed by immediate freezing at 77 K allowed to obtain the resonance Raman spectrum of Fig. 2. Some features of the native form of spheroidene are still present, namely bands at 1242, 1172, 1057 and 490 cm^{-1}. This demonstrates that spheroidene bound to reaction centers assumes some very unstable, cis conformation which isomerises very rapidly towards the all-trans form in normal conditions. We note that the Raman spectrum of the conformer trapped by quick extraction slightly differs from that of the native form by a different ratio of the intensities of bands at 1241 and a 1058 cm^{-1} (in vivo, 5; extract, 2). This effect is not trivially due to the shift in electronic bands accompanying the extraction. It is thus indicative either of further strain exerted on the cis form by binding to the protein or of the existence of an intermediate form between the native cis and the all-trans conformations.

Electronic absorption spectra

Being given that spheroidene bound to reaction centers assumes a cis conformation, it may exhibit a $^1C \leftarrow {}^1A$ absorption in the near ultraviolet enhanced with respect to that of the all-trans form, although not necessarily [24]. A two-component band is expected from the nature of the end groups of the molecule [24]. From the position of the visible bands of the all-trans form in the chromatophore, the cis band of lower energy may be expected around 358—360 nm, whatever the isomer considered [24]. Difference absorption spectra between reaction centers of wild and of carotenoidless mutant strains persistently exhibit a series of positive bands in the 300—420 nm region in addition to the $^1B \leftarrow {}^1A$ transition of spheroidene (Fig. 5). The first of these bands, superimposed upon a vibrational sublevel of the $^1B \leftarrow {}^1A$ transition occurs at 409 ± 1 nm and has a variable extinction, depending on the sample. It most

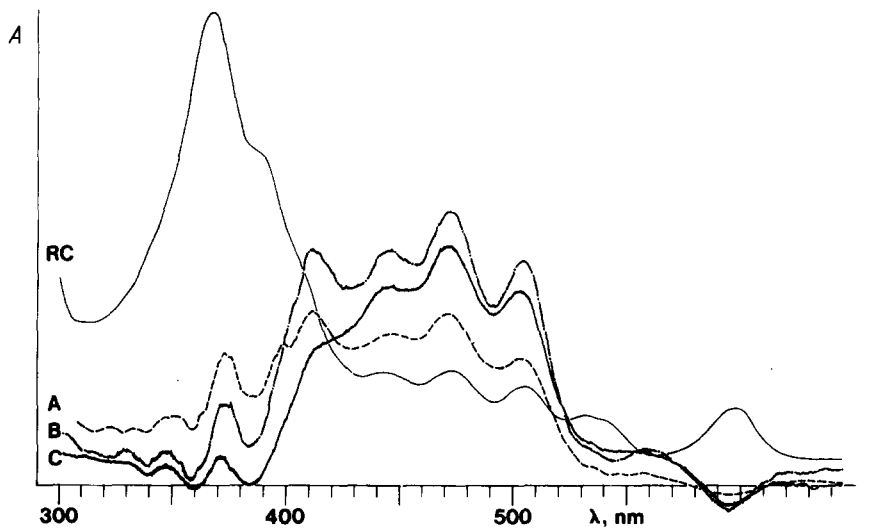

Fig. 5. Differential absorption spectra of reaction centers of *Rps. spheroides* Y minus reaction centers of *Rps. spheroides* R26, room temperature, 300—650 nm region. A, B and C are the results from experiments on three different cultures of each type. The Q_x band of bacteriochlorophyll in Y reaction centers was overcompensated by 10% in Expt. A and by 20% in Expt. B and C. RC, absorption spectrum of reaction centers of *Rps. spheroides* Y.

likely arises from an impurity, although the B^- transition of bacteriochlorophyll could contribute [25]. Another, also most probably artifactual, positive absorption band was occasionally observed at 395 nm. Reaction centers of the wild type exhibit a B_x band of bacteriochlorophyll and bacteriopheophytin at 365 nm approximatively 15% higher than that of R-26 reaction centers, with respect to the Q_x and Q_y bands. This effect likely is induced by the presence of spheroidene. A similar, but weaker hyperchromic effect may affect the B_y transitions around 387 nm. Undercompensating the B_x bands, that is, compensating the Q_x band by factors lower than 1.2, causes a positive band to appear around 370 nm which position depends on the degree of compensation. Overcompensating the B_x induces a negative band to appear at 360 nm. The 360—370 nm pair of bands should thus originate in a slight redshift, of about 1.5 nm, of the B_x band, likely induced by the presence of spheroidene. A persistent positive band is observed at 348 ± 3 nm together with a weaker, more elusive one at 328 ± 3 nm. Although of relatively short wavelength, these bands may represent the $^1C \leftarrow {}^1A$ transition of spheroidene. The extinction coefficient of the 348 nm band is approximately $7 \cdot 10^3\ M^{-1} \cdot cm^{-1}$.

We note that the components of the $^1B \leftarrow {}^1A$ transition of spheroidene bound to reaction centers have lower extinction coefficients, and are blue shifted by 5 to 10 nm with respect to those of bulk spheroidene, as expected for a cis form [24]. Thus, electronic absorption spectra, although not demonstrative of, are consistent with spheroidene assuming a cis conformation in reaction centers.

The 387 and 393 nm bands recently observed by Boucher et al. upon addition of spheroidene to reaction centers of *R. rubrum*, strain G9, most probably cannot be ascribed to the $^1C \leftarrow {}^1A$ transition of a cis isomer of spheroidene, in view of both their low energies and very high absorbances [3].

General occurrence of cis forms in reactions centers

Rhodopseudomonas spheroides. Reaction centers of the mutants strains Ga and GlC of *Rps. spheroides* respectively contain chloroxanthin and neurosporene in place of spheroidene (Fig. 1). These carotenoids have one conjugated double bond less than spheroidene. Yet GlC reaction centers yield resonance Raman spectra of neurosporene extremely close to those of spheroidene yielded by wild reaction centers (Fig. 6 and Table I). The ν_1 band of neurosporene is bicomponent, and the ν_2 band has a 1162 cm^{-1} component stronger than the 1172 cm^{-1} one. This may be explained by a small amount of the all-trans form being present in the preparation. Similarly resonance Raman spectra of chloroxanthin in Ga reaction centers yield all the features characteristic of a cis form, but with weaker relative intensities with respect to the main ν_1—ν_3 bands. The ν_2 band does not exhibit the splitting observed for spheroidene. Again, the presence of a certain amount of the all-trans form in the preparation studies accounts for the discrepancies observed.

Rhodopseudomonas viridis. Preparations of reaction centers of *Rps. viridis* contain a carotenoid [26], most probably 1,2-dihydrolycopene [27], which absorption bands are largely masked by the spectra of two *c*-type cytochromes, present in amount 4—5:1 with respect to *P*-960 [28]. However, when both *c*-553 and *c*-558 are in their oxidized states, and under illumination at 496.5 or

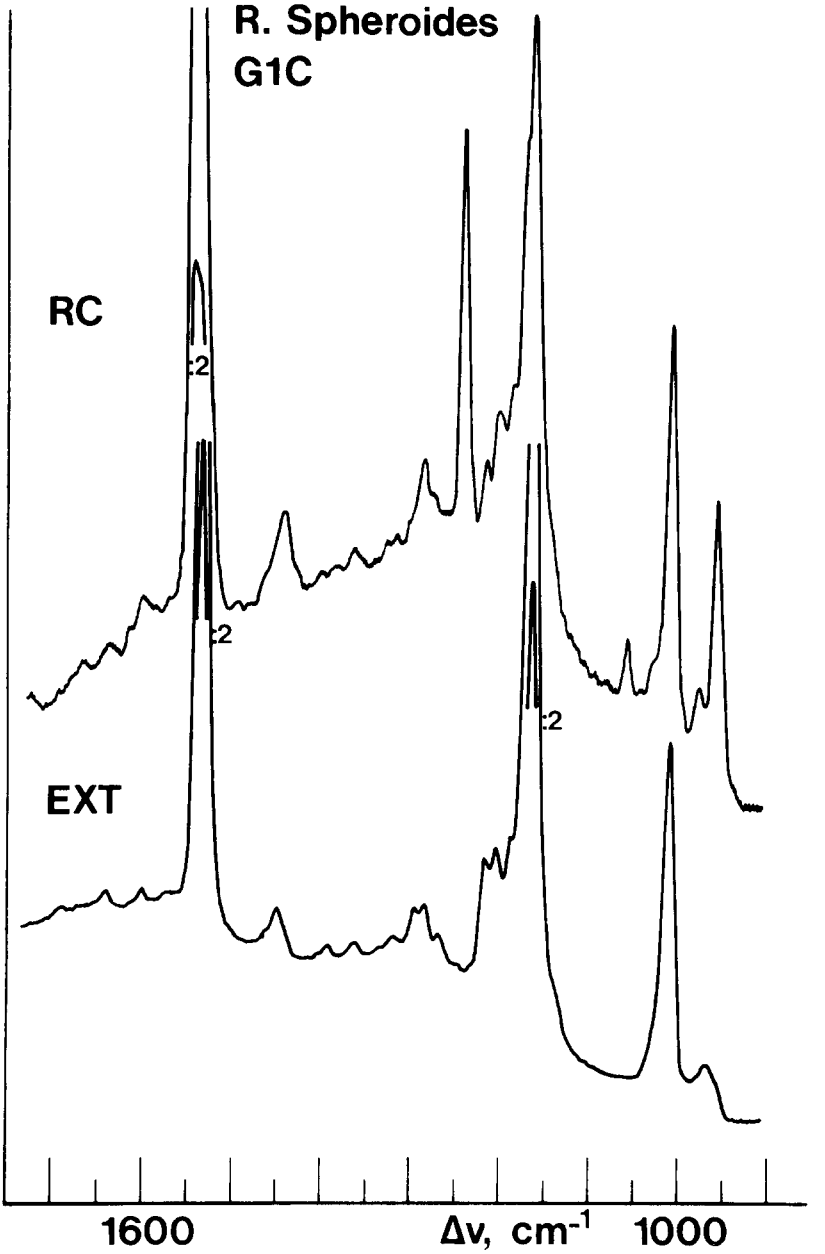

Fig. 6. Resonance Raman spectra of neurosporene at 30 K in reaction centers (RC) of *Rps. spheroides*, strain G1C, and in a petroleum-ether extract (EXT) from these reaction centers, kept 5 mm at 0°C in dim light prior to freezing. Same conditions and remarks as in Fig. 2.

514.5 nm, these reaction centers yield resonance Raman spectra containing almost only bands from the carotenoid (Fig. 7). Again, the features characteristic of a cis form are present in these spectra. This demonstrates that reaction centers of *Rps. viridis* intrinsically contain a carotenoid.

Rhodospirillum rubrum. Reaction centers of *R. rubrum* contain a molecule

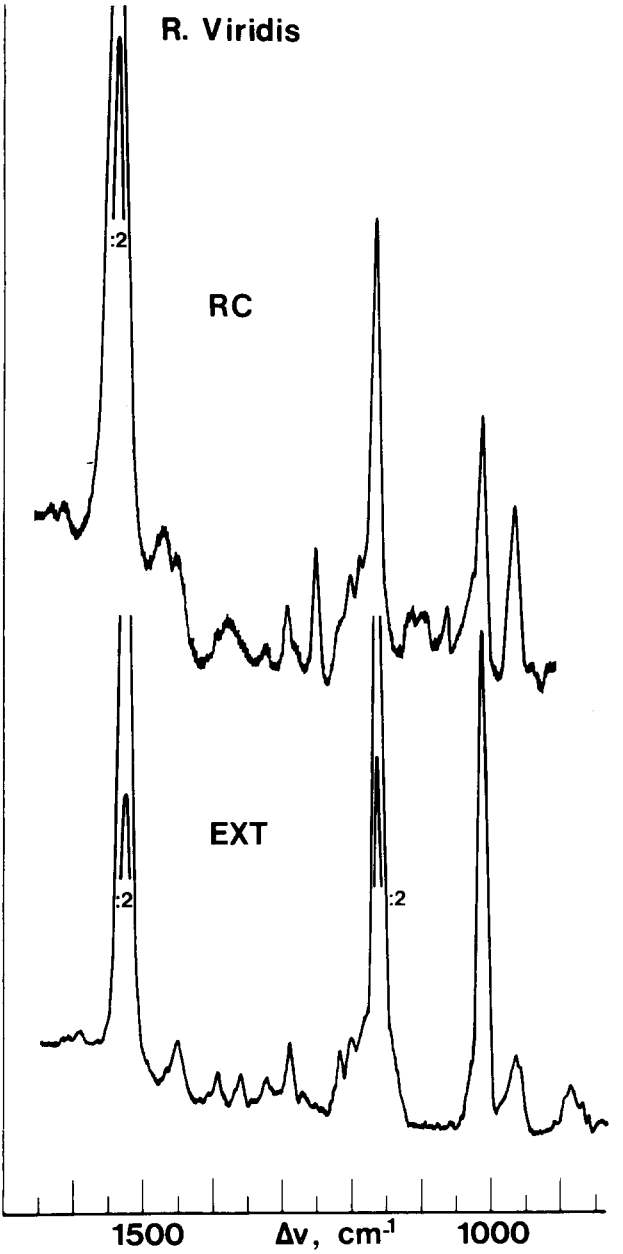

Fig. 7. Resonance Raman spectra of 1,2-dihydrolycopene at 30 K in reaction centers (RC) of *Rps. viridis*, and a petroleum-ether extract (EXT) from these reaction centers, kept 5 mn at 0°C in dim light prior to freezing. Same conditions and remarks as in Fig. 2.

of spirilloxanthin. Chromatophores contain several types of carotenoids, but spirilloxanthin should largely predominate in cultures older than one day [29]. Resonance Raman spectra of spirilloxanthin in both environments differ by a set of features very similar to that observed for other bacteria (Tables I and II, Fig. 8). For spirilloxanthin in reaction centers the ν_1 band is shifted by +15

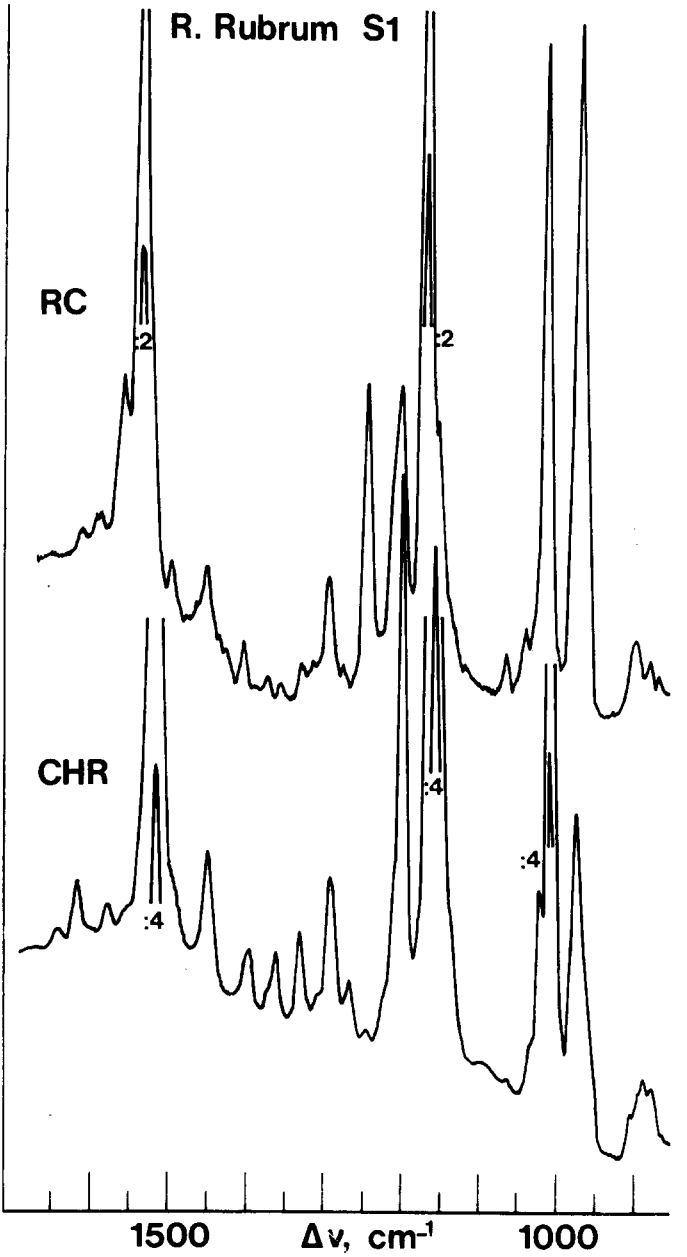

Fig. 8. Resonance Raman spectra of spirilloxanthin at 30 K in reaction centers (RC) and chromatophores (CHR) of *R. rubrum* S1. Same conditions and remarks as in Fig. 2.

cm^{-1}; the 956 cm^{-1} band has the same intensity as the ν_3 band; the ν_2 band is shifted by +9 cm^{-1}. Extraction of spirilloxanthin from the reaction center leads to the same conclusion as for the other carotenoids: the native form bound to reaction centers is unstable and is quickly isomerised. The equilibrium form obtained in petroleum ether yields resonance Raman spectra slightly differing from those observed on chromatophores. Its ν_1 band is

found at 1522 cm^{-1} (+8 cm^{-1}), a ν_2 satellite at 1193 cm^{-1} is weaker and a ν_2 shoulder at 1137 cm^{-1} is stronger. Similar observations were made for lycopene in solution and in tomato fruit by Gill et al. and were attributed by these authors to environmental effects [30]. Spirilloxanthin has however been described as relatively unstable under the all-trans form in solution [31]. The observed shift of the ν_1 band could be due to a cis isomerisation of spirilloxanthin in the extract solution. If such is the case, the very limited spectral variations observed would most probably implicate that the isomerisation affects bonds located near the ends of the polyene chain and that the molecule retains an inversion center.

Nature of the cis forms present in reaction centers

A precise identification of the conformations assumed by carotenoids bound to reaction centers is presently prevented by the lack of a series of well characterised isomers of a C_{40} carotenoid. For the same reason, it is not possible to ascertain whether all the carotenoids studied here assume the same conformation or not. The close similarity between their resonance Raman spectra, together with the high sensitivity of resonance Raman spectra of visual pigments to their isomerisation states [23] indeed suggest their assuming identical conformations in reaction centers. The few differences observed may originate in the different numbers of conjugated double bonds in the carotenoids we have studied. However, the splitting of the ν_2 band of spheroidene and of neurosporene could perhaps indicate that a specific conformation is imposed by reaction centers of *Rps. spheroides*. Elements for a characterization of the cis forms present in reaction centers are found in electronic absorption and in resonance Raman spectra. Most of them are common to all carotenoids and will be discussed without particularising the species.

The native cis forms are unstable and are quickly isomerised towards the all-trans form or a form close to it (spirilloxanthin). This indicates that the native cis forms are sterically hindered. An apparent inconsistency arises with the fact that the $^1B \leftarrow {}^1A$ band has well-resolved vibrational sublevels in all these compounds (Fig. 5) [24,32]. It seems likely that the broadening of levels resulting from steric hindrance may be compensated in part by the uniformisation of the environment and rigidification of the molecule when bound to its host polypeptide of the reaction center. The ratio of the extinction coefficient of the $^1C \leftarrow {}^1A$ band to that of the $^1B \leftarrow {}^1A$ band, not higher than 1 : 10, is lower than expected for a central monocis form, and suggests non central or di-cis forms [24]. The components of the $^1B \leftarrow {}^1A$ bands of spirilloxanthin and of spheroidene are blue shifted from their positions in chromatophores by 5—10 nm. These values suggest mono- or, more probably, di-cis forms [24]. Resonance Raman spectra of the native cis forms each exhibit the same differences with respect to the spectrum of 15,15′-*cis*-β-carotene. Among these are the absence of structure of the band at 1240 cm^{-1}, and higher intensities of the bands at 955—960 cm^{-1} and at 490 cm^{-1}. As mentioned earlier, 15,15′-*cis*-β-carotene yields a resonances Raman band at 784 cm^{-1}, which is probably characteristic, as in infrared spectra of an unmethylated, cis double bond. This band may correlate with much weaker bands only, in spectra of carotenoid bound to reaction centers, at 785 cm^{-1} for spirilloxanthin and spheroidene, and

at 775 cm^{-1} for chloroxanthin (Fig. 3). This suggests that these carotenoids possess methylated cis double bond(s). Since in the present compounds, no steric hindrance can result from a cis conformation only involving the methylated double bonds [24], a further implication should be that the native conformations are at least di-cis and involve at least one unmethylated cis double bond.

Taken together, these data suggest that spheroidene, neurosporene, chloroxanthin, 1,2-dihydrolycopene and spirilloxanthin bound to their respective reaction centers may all assume the same conformation, which could be a hindered, di-*cis* form involving one methylated double bond and one unmethylated.

Conclusion

Carotenoids attached to the reaction centers of different species of photosynthetic bacteria of the Athiorhodaceae family assume closely similar, if not identical conformational states. This implicates close structural similarities between these reaction centers, including *Rps. viridis*, despite their containing chemically different pigments. Such a specific conformation may have two types of functions. First, a conformation of the polyene chain may have the simple result of bringing the carotenoid closer to different sites of the reaction center where it should play a role. Second, a specific cis conformation may confer a specific property to the carotenoid, e.g. adjusting an energy level to a proper value. These two types of functions may result in more efficient photoprotection of the pigments of the reaction center [2,3,33].

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