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CONFIGURATION OF THE CAROTENOID IN THE REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA

COMPARISON OF THE RESONANCE RAMAN SPECTRUM OF THE REACTION CENTER OF *RHODOPSEUDOMONAS SPHAEROIDES* G1C WITH THOSE OF *cis-trans* ISOMERS OF β-CAROTENE

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The resonance Raman spectrum of the reaction center of *Rhodopseudomonas sphaeroides* G1C as well as those of the *cis-trans* isomers of β-carotene (all-*trans*, 9-*cis*, 13-*cis*, 15-*cis* and 9-*cis*,13-*cis*- (or 9-*cis*,13′-*cis*)) have been recorded at liquid N_2 temperature by use of the 457.9, 488.0 and 514.5 nm excitation lines. Comparison of the spectra indicated that the carotenoid in the reaction center takes the 15-*cis* configuration.

Introduction

The carotenoids in the membranes of photosynthetic bacteria exist as an intrinsic and specific component of reaction centers and light-harvesting pigment-protein complexes; in the case of *Rhodopesudomonas sphaeroides* there are three different kinds of complexes, i.e., a reaction center, a B875 complex and a B800-850 complex. The compositions of the pigments are; carotenoid (Car)/bacteriochlorophyll (BChl)/bacteriopheophytin (BPh) = 1:4:2 for the reaction center [1], Car/BChl = 1:1 for the B875 complex [2] and Car/BChl = 1:3 for the B800-850 complex [3]. The functions of the carotenoid in the complexes have been revealed to be (1) absorption of light energy in the 400–500 nm region and its transfer to bacteriochlorophyll and (2) protection of the organism (bacteriochlorophyll) against the photodynamic effect by means of quenching triplet bacteriochlorophyll (^{3}BChl) and singlet oxygen ($^1O_2$) [1,4,5]. Binding of the intrinsic carotenoid to the reaction center or to the B800-850 complex of the carotenoidless mutant R26 reproduced the electronic absorption and CD spectra as well as the above-mentioned biological functions of the wild type [4,6].

The configuration of the carotenoid molecule in the pigment-protein complexes should be closely related to its biological functions. Boucher et al. [4] bound spirilloxanthin to the reaction center isolated from the carotenoidless mutant of *Rhodospirillum rubrum* G9 and measured the difference absorption and CD spectra of the complex after binding minus that before binding of the carotenoid. They identified strong peaks in the region of 350–400 nm and concluded that the carotenoid should take a central mono-*cis* configuration twisted in a protohelical shape. Pioneering works using resonance Raman spectroscopy have been carried out by Lutz and co-workers [7,8]: After having suggested a *cis* configuration of

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spheroidene in the reaction center of *Rps. sphaeroides* of the wild type [7], they measured the Raman spectra of the chromatophores and the reaction centers of various photosynthetic bacteria (*Rps. sphaeroides* 2.4.1, Ga and G1C, *R. rubrum* S1 and *Rps. viridis*) [8]. They established that the carotenoids in the photosynthetic bacteria take the all-*trans* configuration in the chromatophores, in which the light-harvesting complexes predominate. They showed also that the carotenoids take a *cis* configuration in the reaction centers, which they claimed to be common among the organisms examined. They predicted, based on their spectral interpretation, a di-*cis* configuration with one methylated and one unmethylated *cis* double bond. Agalidis et al. [9] bound spheroidene to the reaction center of *Rps. sphaeroides* R26 and showed that binding causes isomerization from the all-*trans* to the *cis* configuration, which is very similar to the carotenoid in the reaction center of the wild type. However, the detailed configuration of the carotenoid in the reaction centers remains to be determined.

Resonance Raman spectroscopy is probably one of the most powerful tools to elucidate the carotenoid configuration; an excitation line with a wavelength around 450–500 nm enhances the vibrational spectrum of the carotenoid only, which is sensitive to its configurational difference. However, Raman data on a set of the *cis-trans* isomers of a C_{40} carotenoid of which the structure is known have not been available except for the all-*trans*- and 15-*cis*-β-carotene, and this has prevented this technique from proving its real usefulness. Very recently, two of the present authors (Saiki, K. and Tsukida, K.) have determined, by means of ^{1}H- and ^{13}C-NMR, the configuration of two additional different isomers of β-carotene, namely, neo U and neo B, to be 9-*cis* and 13-*cis* [20]. A carotenoid with a ring structure at both ends generally gives stable isomers suitable for Raman measurement. In addition, those vibrational modes which couple strongly with the π-π^* (^{1}A-^{1}B) electronic transition of the conjugated polyene chain alone are expected to appear in the spectrum; in other words, the spectrum should depend weakly on the structure at the ends (or the kind of carotenoid) but strongly on the configuration of the conjugated polyene chain. These reasons justify the present approach, i.e., to compare the resonance Raman spectra of β-carotene isomers to the spectrum of neursporene, which is amajor component (96%) of the carotenoids in *Rps. sphaeroides* mutant G1C [10].

Experimental Procedure

Preparation of reaction centers. Cells of *Rps. sphaeroides* G1C strain (a gift from Dr. A.R. Crofts) were grown in the medium described by Cohen-Bazire et al. [11]. Reaction centers were prepared from chromatophores by a modification of the method of Jolchine and Reiss-Husson [12] using lauryldimethylamine oxide (LDAO): 0.4% (w/v, final concentration) LDAO was added to the chromatophore suspension ($A_{850} = 50$) in 10 mM Tris-HCl (pH 8.0) and then the mixture was gently stirred for 60 min at 0°C. The mixture was centrifuged at $144000 \times g$ for 60 min. Ammonium sulfate precipitation was done at a concentration of 0.3 g/ml. The solution was subjected to molecular-sieve chromatography on Sepharose 6B and then on Ultrogel AcA22 columns using the same buffer containing 0.1% LDAO. The chromatography gave four fractions: The second fraction was identified as the light-harvesting B800-850 complex based on its electronic absorption spectrum. The third fraction gave the spectrum of the reaction center. The ratio A_{280}/A_{800} was equal to 1.44. The profile of its SDS-polyacrylamide gel electrophoresis gave three bands of the L, M and H subunits. The reaction center fraction was collected, concentrated by ultrafiltration (Minimodule NM-3, Asahi Kasei), and stored in a frozen state at −50°C before Raman measurements.

Isolation of β-carotene isomers. 15-*cis*-β-Carotene was a gift from F. Hoffmann-La Roche & Co. all-*trans*-β-Carotene was obtained from a commercial source and recrystallized from benzene and methanol. Photoisomerization of all-*trans*-β-carotene through I_2 catalysis and isolation of isomers by lime column chromatography were carried out according to the method of Polgár and Zechmeister [13]: 10 mg of β-carotene were dissolved in 85 ml of *n*-hexane and 0.2 ml of 1% I_2 in *n*-hexane was added. The solution was illuminated by a 15 W fluorescent lamp for 1 h, concentrated, and then applied to a column; the column (4.5 × 50 cm) had

been packed with 300 g of lime (Kishida Chemicals, reagent grade, through 200 mesh) under vacuum and washed with the developing solvent (1% acetone in *n*-hexane). After developing under vacuum the solvent was drained. The lime chromatogram was pushed out of the column and cut according to the fractions. Each isomer was extracted with acetone, *n*-hexane was added to the acetone solution, and then the mixture was washed with water. The *n*-hexane solution of each isomer was dried over Na_2SO_4 and stored in a freezer (−20°C, in the dark). Isomers were identified by their electronic absorption spectra. The shifts of the absorption peak of the longest wavelength (due to the electronic ^1A-^1B and vibrational 0-0 transition) from that of the all-*trans* isomer were −5, −10 and −13 nm for neo U, neo B and neo A, respectively (Ref. 13: −5, −10.5 and −13 nm).

Raman measurement. A reaction center suspension in 0.1% LDAO and 10 mM Tris-HCl (pH 8.0) or an isomer of β-carotene dissolved in *n*-hexane was placed in a capillary (1.7 mm diameter) and dipped into liquid N_2 in a Dewar vessel; the bottom of the capillary was irradiated with the beam of an NEC GLG-2023 Ar^+ laser. The 457.9, 488.0 or 514.5 nm line with power less than 80 mW was used for excitation. The 90° scattering was collected and recorded on a Kawaguchi Electric Works RL-62 Raman spectrometer (optical slit width 6 cm^{-1}). The frequencies of the Raman lines were calibrated using the natural emission lines of the laser.

Results

Electronic absorption spectra

The reaction center and the light-harvesting complex. Fig. 1 shows the electronic absorption spectra of (A) the light-harvesting complex and (B) the reaction center of *Rps. sphaeroides* G1C. The absorption peaks of bacteriochlorophyll of each pigment-protein complex for the G1C mutant coincide, within experimental accuracy, with those for the wild type [2]. The band at 851 nm of the light-harvesting complex of the present preparation is symmetric and it does not indicate any contribution from the 873 nm band of the B875 complex [2]. Thus, this fraction is likely to be the B800-850 complex. The absorption of pheophytin

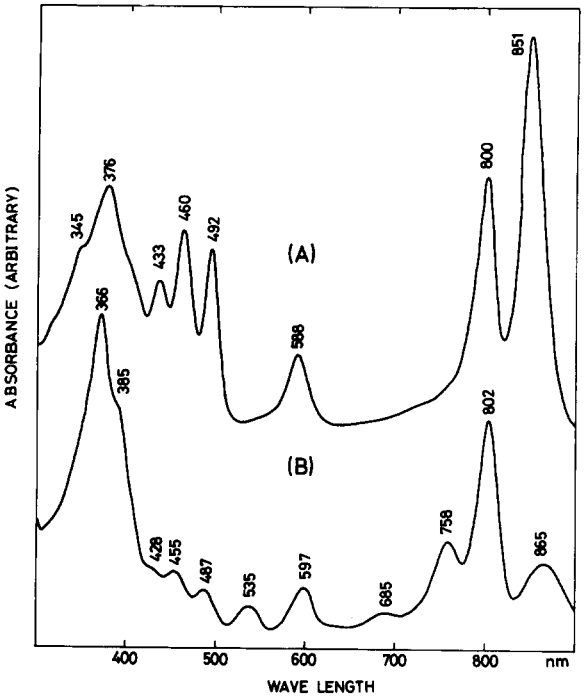


Fig. 1. Electronic absorption spectra of (A) the light-harvesting complex (B800-850) and (B) the reaction center of *Rps. sphaeroides* G1C. (At room temperature.)

in the reaction centers of the G1C mutant and of the wild type agree with each other. However, the absorptions of the carotenoid, neurosporene, of the G1C mutant appear in a wavelength region shorter than that of the absorptions of spheroidene of the wild type, probably due to difference in the length of the conjugated system. It should be noted, in relation to the carotenoid configuration, that the absorptions (the ^1A-^1B electronic transition with the 0-0, 0-1 and 0-2 vibrational transitions) of the carotenoid bound to the reaction center are blue shifted when compared to those bound to the B800-850 complex both for the G1C mutant (492 → 487, 460 → 455 and 433 → 428 nm; Fig. 1) and for the wild type (509 → 500, 475 → 467 and 448 → 440 nm [2]).

β-Carotene isomers. Fig. 2 shows the electornic absorption spectra of the neo U, neo B and neo A isomers together with those of the all-*trans* and synthetic 15-*cis* isomers of β-carotene in *n*-hexane solution. The amount of the neo A isomer obtained was too small to determine the value of the

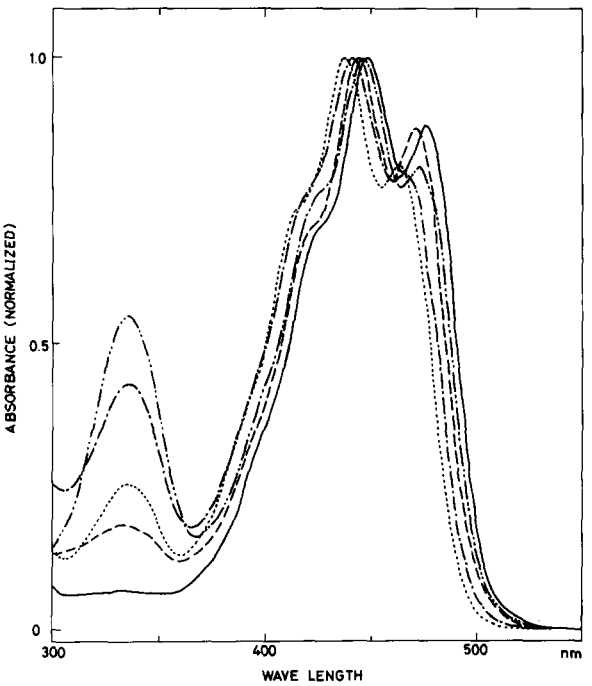


Fig. 2. Electronic absorption spectra of *cis-trans* isomers of β-carotene. (————) all-*trans*; (— — —) 9-*cis* (neo U); (·–·–·) 13-*cis* (neo B); (–···–···–) 15-*cis*; and (·····) 9-*cis*, 13-*cis* (or 9-*cis*,13′-*cis*) (neo A). Each isomer was dissolved in *n*-hexane. λ_{max} of each spectrum is normalized to 1.0. (At room temperature).

molar extinction coefficient. Therefore, we normalized the λ_{max} value of each isomer to be 1.0, so that the intensity of the ^1A-^1C transition relative to that of the ^1A-^1B transition could be compared. Table I lists the wavelengths of the vibrational structures (0-0 and 0-1) of the ^1A-^1B transition as well as the wavelengths of the ^1A-^1C transition (*cis* peak). Shifts from the all-*trans* isomer are also indicated in parentheses.

Fig. 3 shows the molecular configurations of some isomers of β-carotene which are relevant to the present report. Lunde and Zechmeister [14] assigned the configuration of neo U and neo B to be 9-*cis* and 9-*cis*,13′-*cis* based on the ultraviolet, visible and infrared absorption spectra. However, Tsukida et al. [20] recently determined the configuration of neo U and neo B to be 9-*cis* and 13-*cis*, respectively, by means of ^1H- and ^{13}C-NMR. As for the neo A isomer, the spectra suggested that the configuration should be either 9-*cis*,13-*cis* or 9-*cis*,13′-*cis*. However, the spectra gave additional reasonance lines which were not assignable. Its structural assignment is not conclusive at the present stage. Hereafter, we denote the isomers of β-carotene by using their configuration, 9-*cis*, 13-*cis* or 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*), instead of Zechmeister's notation, neo U, neo B or neo A.

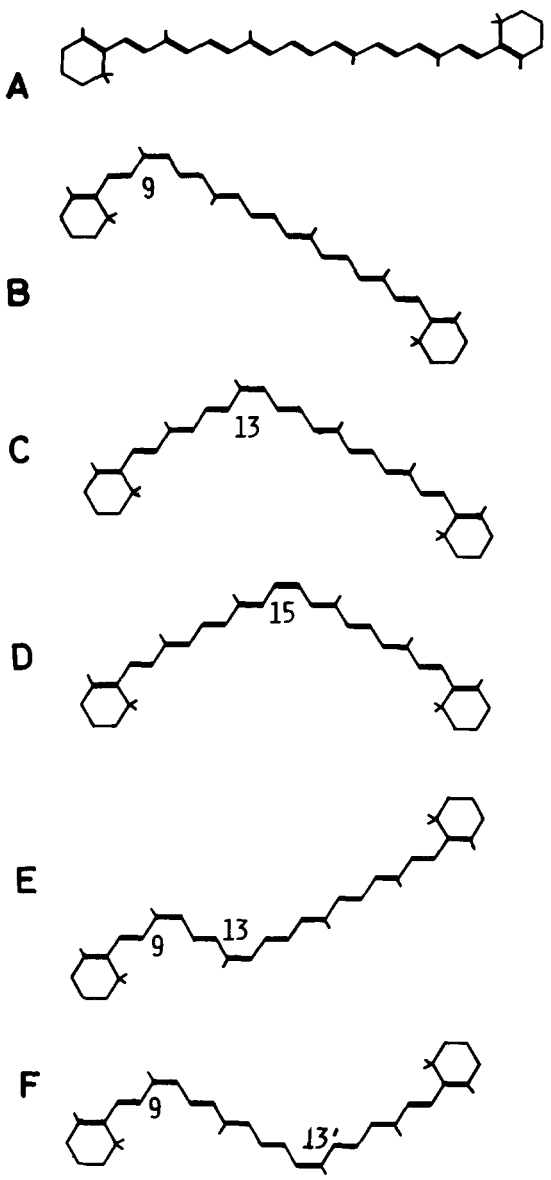


Fig. 3. Molecular configurations of *cis-trans* isomers of β-carotene. (A) all-*trans*, (B) 9-*cis*, (C) 13-*cis*, (D) 15-*cis*, (E) 9-*cis*,13-*cis* and (F) 9-*cis*,13′-*cis*.

Based on the above configurational assignments the absorption spectra of β-carotene isomers are characterized as follows: (1) The intensity of the ^1A-^1C transition (*cis* peak) relative to the ^1A-^1B transition is highest for the 15-*cis* isomer and decreases in the following order: 13-*cis*, 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) and 9-*cis* (see Fig. 2). The *cis* peak was not observed for the all-*trans* isomer. Since it is known that the transition moment of ^1A-^1B is parallel to the molecular axis and that of ^1A-^1C is perpendicular to it, the order of the intensity is understandable from the configuration of the isomers (see Fig. 3). The ^1A-^1C transition is forbidden for the all-*trans* isomer. (2) *trans* to *cis* isomerization causes a blue shift of the ^1A-^1B transition as was established by Zechmeister [15], but his rule of 'a 5 nm shift for mono-*cis* and a 10 nm shift for di-*cis*' does not strictly hold for β-carotene (see Table I).

Resonance Raman spectra

Resonance Raman bands of a polyene chain. The dependene of the intensity of the Raman bands on the excitation wavelength ('excitation profile') has been analyzed for all-*trans*-β-carotene by Inagaki et al. [16]. The results indicated that the relative intensities of resonance Raman lines depend rather strongly on the laser line used for excitation. Therefore, we recorded a set of Raman spectra for each sample using the 457.9, 448.0 and 514.5 nm lines of an Ar^+ laser. In addition, normal coordinate analysis of the all-*trans*, infinite polyene chain by Inagaki et al. [17] showed that the following vibrational modes are expected to appear in the 1700–900 cm^{-1} region of the resonance Raman spectrum of a polyene chain. Mode 1, C=C stretching in the 1540–1530 cm^{-1} region; mode 2, C–C stretching coupled with C–H in-plane deformation in the 1260–1120 cm^{-1} region; and mode 3, C–H in-plane deformation coupled with C–C stretching in the 1120–1030 cm^{-1} region. Mode 1 is expected to reflect the configuration of a polyene chain through the potential-energy matrix, i.e., the C=C stretching force constant. The degree of delocalization of the π-electrons in the conjugated system should decrease when a configurational change from *trans* to *cis* takes place. The C=C stretching frequency (all in-phase) should be highest for the di-*cis* isomers, intermediate for the mono-*cis* isomers and lowest for the all-*trans* isomer. On the other hand, mode 2 is expected to reflect the configuration through the kinetic-energy matrix, i.e., the geometry of the polyene chain. The way of mixing of the vibrations among the C–C stretching units as well as coupling of the vibrational transitions with the ^1A-^1B electronic transition should affect the frequencies and intensities of the resonance Raman lines. For this reason we shall confine ourselves to discussing, as the key bands, the mode 1 and mode 2 Raman bands which are strong and common in the set of the spectra with different excitation.

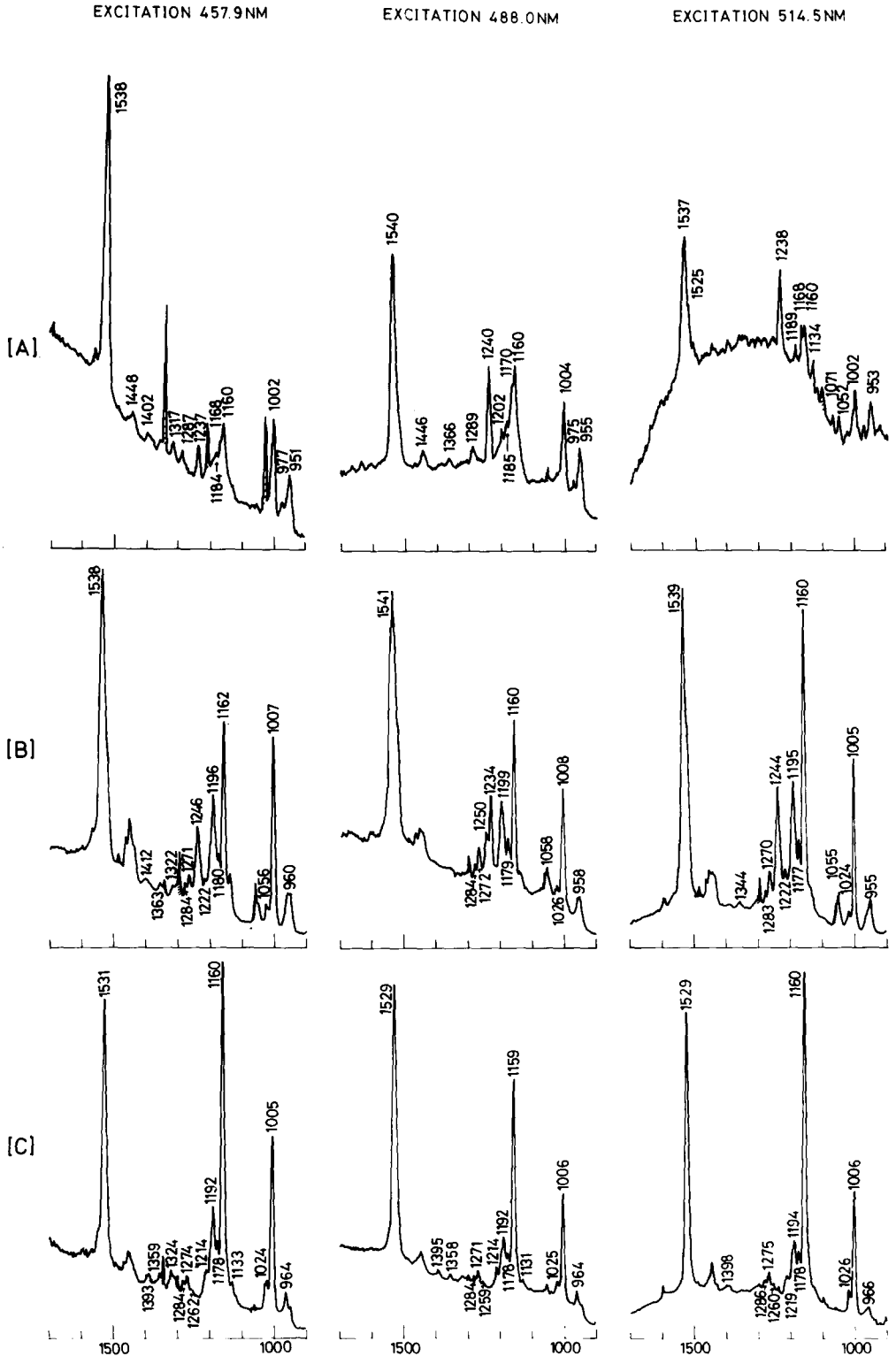
The reaction center and β-carotene isomers. Fig. 4 compares the Raman spectrum of the reaction center of *Rps. sphaeroides* G1C (A) with those of the isomers of β-carotene, namely, 15-*cis* (B), all-*trans* (C), 9-*cis* (D), 13-*cis* (E) and 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) (F). Hatched lines in the spectra are due to the natural emission lines of the Ar^+ laser or due to the solvent. Lutz et al. [8] characterized the Raman spectrum of the reaction center of the wild type, when compared with the spectrum of

TABLE I

ELECTRONIC ABSORPTIONS (nm) OF *cis-trans* ISOMERS OF β-CAROTENE

Shifts from the absorptions of the all-*trans* isomer are given in parentheses.

Transitions		all-*trans*	15-*cis*	9-*cis* (neo U)	13-*cis* (neo B)	9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) (neo A)
Electronic	Vibrational					
^1A-^1B	0-0	476	473 (3)	471 (5)	466 (10)	463 (13)
	0-1	448	446 (2)	444 (4)	441 (7)	437 (11)
^1A-^1C		–	335	333	336	335


EXCITATION 457.9NM
EXCITATION 488.0NM
EXCITATION 514.5NM
[A]
[B]
[C]
1538
1448
1402
1317
1287
1237
1184
1168
1160
1002
977
951
1540
1446
1366
1289
1240
1202
1185
1170
1160
1004
975
955
1537
1525
1238
1189
1168
1160
1134
1071
1052
1002
953
1538
1412
1363
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1284
1271
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1007
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1359
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1214
1192
1178
1160
1133
1024
1005
964
1529
1395
1358
1271
1284
1259
1214
1192
1178
1159
1131
1025
1006
964
1529
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1026
1006
966
1500
1000
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1000
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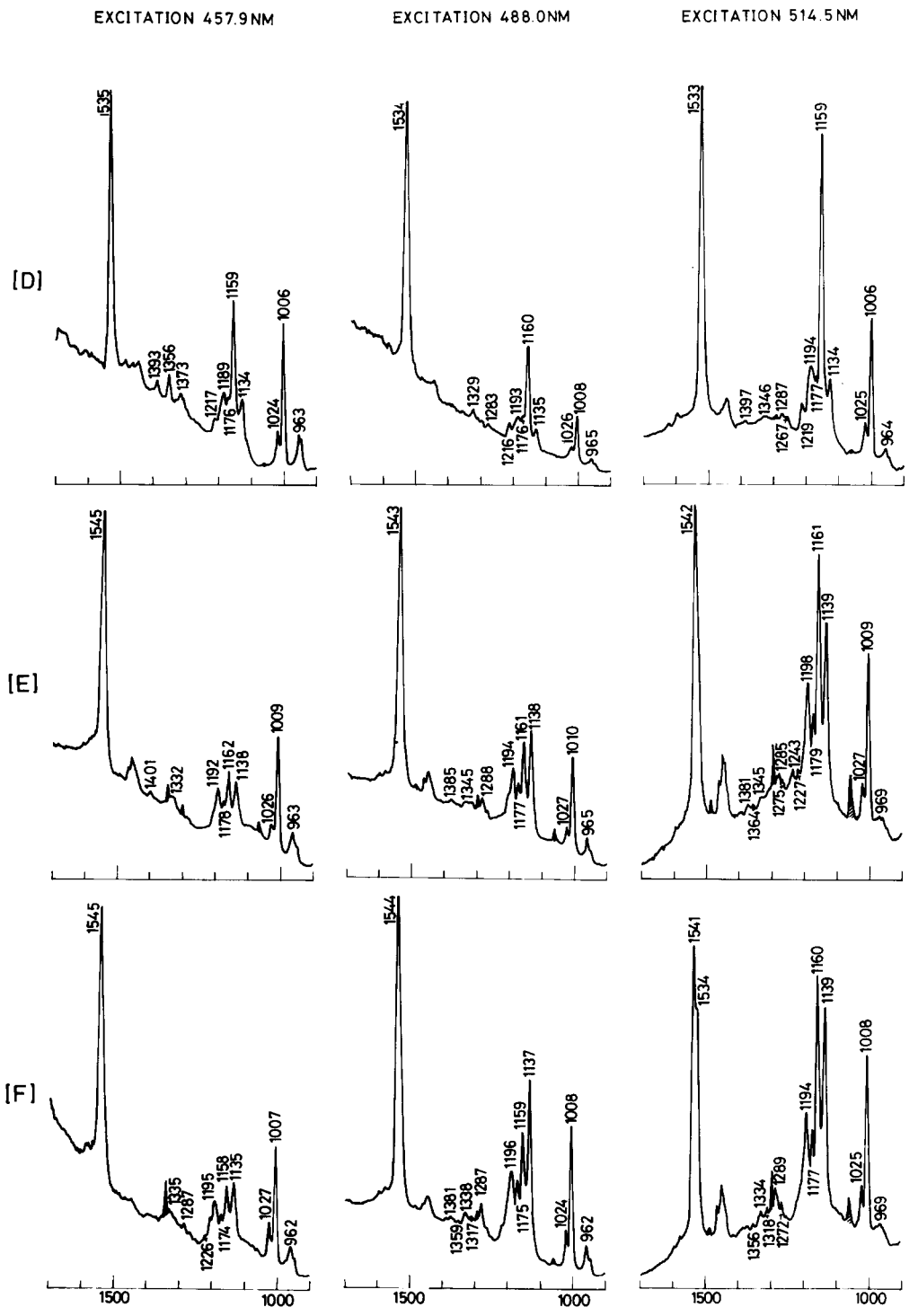

Fig. 4. Raman spectra of (A) the reaction center of *Rps. sphaeroides* G1C and (B–F) *cis-trans* isomers of β-carotene (457.9, 488.0 and 514.5 nm excitation); (B) 15-*cis*, (C) all-*trans*, (D) 9-*cis*, (E) 13-*cis* and (F) 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*). An aqueous solution of the reaction center (0.1% LDAO, 10 mM Tris-HCl, pH 8.0) or an *n*-hexane solution of each isomer of β-carotene in a capillary was dipped into liquid N_2. Optical slit width 6 cm^{-1}. Hatched lines in the spectra are due to the natural emission of the Ar^+ laser or due to the solvent *n*-hexane.

chromatophores, by (1) a 10 cm^{-1} shift of the C=C stretching (mode 1) band to the higher frequency and (2) the appearance of a new band around 1240 cm^{-1}. Among the characteristics pointed out by Lutz et al., the above two are most prominent and commonly observed for all of the different organisms which Lutz et al. [8] examined. In the present experiment, we also observed (1) a 10 cm^{-1} shift of the C=C stretching band and (2) the appearance of a new band around 1238 cm^{-1}. (See Fig. 4A; the Raman spectrum of the B800-850 light-harvesting complex is not shown.) The set of spectra show that the relative intensity of the new band is dependent on the excitation wavelength; when the wavelength of excitation increases, the and increased in intensity.

Comparison of the Raman spectra of β-carotene isomers (Fig. 4B–F) reveals that the frequency of the model 1 Raman band is dependent on the configuration; viz., 1530 cm^{-1} for all-*trans*, 1534 cm^{-1} for 9-*cis*, 1540 cm^{-1} for 15-*cis*, 1543 cm^{-1} for 13-*cis* and 1544 cm^{-1} for 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*). (The frequencies with different excitations are averaged.) Rimai et al. [18] found a linear relationship between the frequency of the C=C stretching vibration and the frequency (or wavelength) of the ^1A-^1B electronic transition for polyenes with different chain lengths. In the present case, the order of increasing frequency of the C=C stretching vibration, when compared to the all-*trans* isomer, i.e. 4 cm^{-1} for 9-*cis*, 13 cm^{-1} for 13-*cis* and 15 cm^{-1} for 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) correlates well with the order of increasing blue shift of the ^1A-^1B transition (0-0 component), i.e., 5 nm for 9-*cis*, 10 nm for 13-*cis* and 13 nm for 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*). The correlation supports the idea that the shift of the C=C stretching band is not due to change in the molecular structure but due to change in the C=C stretching force constant. However, irregularity was found for the 15-*cis* isomer; an increase in the frequency of 10 cm^{-1} and a blue shift of only 3 nm. This is probably due to strong interaction of the vibrational as well as the electronic energy levels between the two halves of the polyene chain because of the symmetry of the 15-*cis* isomer.

Comparison of the spectra of the isomers (Fig. 4B–F) reveals also that there are three distinct spectral patterns for the region 1300–1100 cm^{-1}, where configuration-sensitive mode 2 bands are expected to occur. The spectra of the isomers in this region (488.0 nm excitation) are reproduced in Fig. 5. The all-*trans* and 9-*cis* isomers (Fig. 5C and D) give a spectral pattern which is characterized by a strong band around 1159 cm^{-1} with weak features (pattern 1). The 13-*cis* and 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) isomers (Fig. 5E and F) give another spectral pattern characterized by two strong peaks around 1160 and 1138 cm^{-1} with a medium peak around 1195 cm^{-1} (pattern 2). The 15-*cis* isomer (Fig. 5B) gives a third spectral pattern characterized by presence of a new medium band(s) at around 1240 cm^{-1} (pattern 3).

Comparison of the spectra; configuration of the carotenoid in the reaction center

If we apply the classification of the Raman spectral patterns found for the isomers of β-carotene (Figs. 4 and 5) to the spectrum of the reaction center, it can definitely be classified as pattern 3. In the classification, it should be noted that the band around 1197 cm^{-1} of 15-*cis*-β-carotene is missing in the spectrum of neurosporene in the reaction center. The spectra recorded by Lutz et al. [8] show that a medium band appears around 1195 cm^{-1} for spheroidene and spirilloxanthin but it does not appear for neurosporene and 1,2-dihydrolycopene. The situation is the same both for the reaction centers and for the chromatophores. Therefore, the presence or absence of the band depends not on the configuration but on the kind of the carotenoid.

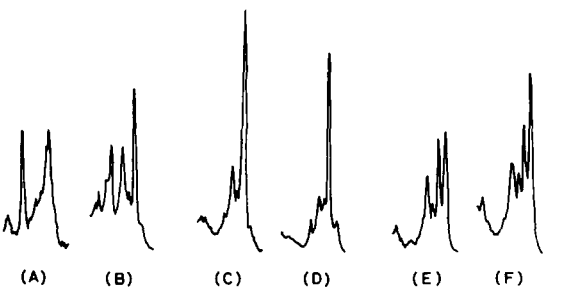

Fig. 5. Raman lines related to the C–C stretching coupled with the C–H in-plane deformation vibrations which give different spectral patterns. Raman spectra of 488.0 nm excitation (Fig. 4) in the region of 1300–1100 cm^{-1} are reproduced; (A) the reaction center of *Rps. sphaeroides* G1C and (B) 15-*cis*, (C) all-*trans*-, (D) 9-*cis*-, (E) 13-*cis*- and (F) 9-*cis*,13-*cis*- (or 9-*cis*,13′-*cis*-) β-carotene.

The Raman bands and the configuration of the polyene chain are correlated as follows. (1) Introducting a 9-*cis* configuration does not produce any large change in the Raman spectrum. Spectra of the all-*trans* and 9-*cis* isomers as well as those of the 13-*cis* and 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*) isomers are similar to each other. (2) A 13-*cis* configuration (13-*cis* or 9-*cis*,13-*cis* (or 9-*cis*,13′-*cis*)) is characterized by the appearance of a strong band around 1138 cm^{-1}. No such strong band is found in the spectrum of the 15-*cis*, all-*trans* or 9-*cis* isomers, although a very weak band is found at 1131 cm^{-1} for the all-*trans* isomer and a weak band at 1134 cm^{-1} for the 9-*cis* isomer. (3) The 15-*cis* configuration is characterized by a medium feature (one or two bands) around 1240 cm^{-1}. No corresponding band is found in this region for the remaining isomers. The above key bands lead us to the conclusion that the carotenoid which is bound to the reaction center takes a 15-*cis* configuration.

It is shown that the C=C stretching frequency of β-carotene is dependent on the configuration. The frequency for the 15-*cis* isomer (1540 cm^{-1}) is 10 cm^{-1} higher than that for the all-*trans* isomer (1530 cm^{-1}). The frequency for neurosporene or spheroidene in the reaction centers is also 10 cm^{-1} higher than that for the carotenoids in the chromatophores. The agreement suggests the 15-*cis* configuration in the reaction center. In the case of a di-*cis* isomer the frequency is expected to be higher. It is shown also that the wavelength of the ^1A-^1B (0-0) transition shifts to the blue when an all-*trans* to *cis* isomerization takes place; the maximum shift was 10 nm for mono-*cis* isomers of β-carotene. The wavelength of absorption of neurosporene (spheroidene) in the reaction centers is 5 nm (9 nm) shorter than that in the chromatophores. The values are within the range of mono-*cis*, and suggest a mono-*cis* configuration, although the electrostatic fields applied to the carotenoid in the reaction center and in the light-harvesting complex are not necessarily the same.

Discussion

Boucher et al. [4] proposed a central mono-*cis* configuration twisted in a protohelical shape for spirilloxanthin bound to the reaction center of *R. rubrum*. Lutz et al. [8] predicted that the configuration should be di-*cis* with one methylated and one unmethylated *cis* double-bond. We have proposed the 15-*cis* configuration, instead.

Boucher's model of a highly twisted configuration is considered to be unlikely on the basis of the Raman data. The spectrum of the reaction center is very similar to the spectrum of the 15-*cis* isomer of β-carotene (Fig. 4A and B) except for a band around 1197 cm^{-1}. If there were such a large twisting, electron delocalization should be divided into two portions and there should be a large shift of the C=C stretching band to a very high frequency.

With respect to the question of mono-*cis* or di-*cis*, there have been additional observations to support the mono-*cis* configuration. Englert and Vecchi [19] separated three mono-*cis* and six di-*cis* isomers of astaxanthin diacetate by means of liquid chromatography, determined the configurations by using ^{1}H-NMR, and recorded the absorption spectra. The shift of λ_{max} (the 0-0 component of the ^1A-^1B transition) from that of the all-*trans* isomer was 8, 9 and 8 nm for the 9-*cis*,13-*cis* and 15-*cis* isomers and 13, 16, 16, 17, 19 and 19 nm for the 9-*cis*,9′-*cis*, 9-*cis*,13′-*cis*, 9-*cis*,15-*cis*, 9-*cis*,13-*cis*, 13-*cis*,13′-*cis* and 13-*cis*,15-*cis* isomers, respectively. The shift of neurosporene or spheroidene mentioned above falls in the range of a mono-*cis* isomer in this case also.

Another key to the question is the intensity of the *cis* peak. Englert and Vecchi [19] found also that the *cis* peak was strong for the 15-*cis* isomer, medium for the 13-*cis* isomer, weak for the 9-*cis* and 9-*cis*,15-*cis* isomers, and very weak for the 9-*cis*,13-*cis* and 9-*cis*,13′-*cis* isomers. No *cis* peak was detected for the 9-*cis*,9′-*cis*, 13-*cis*,13′-*cis* and 13-*cis*,15-*cis* isomers. Therefore, if the *cis* peak is strong, the configuration must be 15-*cis* and if it is weak, the configuration can be 9-*cis*,15-*cis*. Boucher et al. [4] bound spirilloxanthin to the reaction center of *R. rubrum* G9 and measured the difference absorption and CD spectra of the complex after binding minus that before binding of the carotenoid. They observed strong (382 nm) and very strong (398 nm) peaks, the distance from the 0-0 component of the ^1A-^1B transition being approx. 150 and 135 nm. Strong absorptions were found also for spheroidene attached to the above

reaction center at about 155 and 135 nm to the blue of the ^1A-^1B (0-0) transition. The observation led them to conclude a central mono-*cis* configuration.

Agalidis et al. [9] bound spheroidene to the reaction center of *Rps. sphaeroides* R26 and measured the difference absorption spectrum also. They found weak (348 nm) and medium (369 nm) absorptions which are 154 and 133 nm to the blue of the ^1A-^1B (0-0) transition. However, they attributed the stronger absorption at 369 nm to a hyperchromic effect and a slight red shift of the bacteriochlorophyll Soret band. In the case of β-carotene, the *cis* peak appears at 138 nm to the blue of the ^1A-^1B (0-0) transition. Therefore, it is much more reasonable to regard the medium 369 nm band as the *cis* peak, which suggests a mono-*cis* configuration. However, the final conclusion should be postponed until Raman data on a set of di-*cis* isomers of a C_{40} carotenoid become available.

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References

1 Cogdell, R.J., Parson, W.W. and Kerr, M.A. (1976) Biochim. Biophys. Acta 430, 83–93
2 Broglie, R.M., Hunter, C.N., Delepelaire, P., Niederman, R.A., Chua, N.-H. and Clayton, R.K. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 87–91
3 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409–416
4 Boucher, F., Van der Rest, M. and Gingras, G. (1977) Biochim. Biophys. Acta 461, 339–357
5 Krinsky, N.I. (1979) Pure Appl. Chem. 51, 649–660
6 Davidson, E. and Cogdell, R.J. (1981) Biochim. Biophys. Acta 635, 295–303
7 Lutz, M., Kleo, J. and Reiss-Husson, F. (1976) Biochem. Biophys. Res. Commun. 69, 711–717
8 Lutz, M., Agalidis, I., Hervo, G., Cogdell, R.J. and Reiss-Husson, F. (1978) Biochim. Biophys. Acta 503, 287–303
9 Agalidis, I., Lutz, M. and Reiss-Husson, F. (1980) Biochim. Biophys. Acta 589, 264–274
10 Holmes, N.G. and Crofts, A.R. (1977) Biochim. Biophys. Acta 459, 492–505
11 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell. Comp. Physiol. 49, 25–68
12 Jolchine, G. and Reiss-Husson, F. (1974) FEBS Lett. 40, 5–8
13 Polgár, A. and Zechmeister, L. (1942) J. Am. Chem. Soc. 64, 1856–1861
14 Lunde, K. and Zechmeister, L. (1955) J. Am. Chem. Soc. 77, 1647–1653
15 Zechmeister, L. (1962) *cis-trans* Isomeric Carotenoids, Vitamins A and Arylpolyenes, Academic Press, New York pp. 26–30
16 Inagaki, F., Tasumi, M. and Miyazawa, T. (1974) J. Mol. Spectrosc. 50, 286–303
17 Inagaki, F., Tasumi, M. and Miyazawa, T. (1975) J. Raman Spectrosc. 3, 335–343
18 Rimai, L., Heyde, M.E. and Gill, D. (1973) J. Am. Chem. Soc. 95, 4493–4501
19 Englert, G. and Vecchi, M. (1980) Helv. Chim. Acta 63, 1711–1718
20 Tsukida, K., Saiki, K. and Sugiura, M. (1981) J. Nutr. Sci. Vitamin. 27, 551–561