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The $2^1A_g^-$ state of a carotenoid bound to spinach chloroplast as revealed by picosecond transient Raman spectroscopy

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The S_1 Raman spectrum of a carotenoid bound to the photosynthetic membrane (spinach chloroplasts) is reported for the first time. The spectrum is ascribed mainly to all-*trans*-β-carotene (the specific component of the reaction centers and the core antenna complexes). The C = C stretching line at 1753 cm^{-1} evidences; (1) that the S_1 state of the carotenoid detected is actually the $2^1A_g^-$ state, and (2) that the vibronic coupling between the $2^1A_g^-$ (S_1) state and the $^1A_g^-$ (S_0) state is weakened by the binding of the carotenoid to the apo-complex (peptides and chlorophylls).

Carotenoids in the S_1 and T_1 states are related to the functions of light-harvesting and photo-protection [1]. In both singlet energy transfer from a carotenoid to a chlorophyll (light-harvesting function) and triplet energy transfer from a chlorophyll to a carotenoid (photo-protective function), the molecular structures and the molecular dynamics of the excited states involved are of primary importance.

In spinach chloroplasts, lutein, β-carotene, violaxanthin and neoxanthin are present; β-carotene is specifically bound to the reaction centers and the core antennas (PS I and PS II), while the xanthophylls are bound to the light-harvesting complexes (LHC I and LHC II) [2]. Transient absorption spectroscopy was applied to spinach chloroplasts, PS I and II particles and LHC; (1) the lifetime of T_1 carotenoids (in the order of 10^0 μs), (2) energy transfer from T_1 chlorophylls, and (3) interactions of T_1 carotenoids with S_1 and T_1 chlorophylls were reported [3–5]. Very recently, transient absorption spectroscopy with femtosecond time-resolution was applied to the thylakoid membranes of algae [6]; energy transfer from S_1 carotenoids to chlorophylls ($\approx 10^{-1}$ ps) was detected.

Since the optically allowed $^1B_u^+$ state is too high to facilitate energy transfer from a carotenoid to a chlorophyll via the nearest Q_x level of the chlorophyll, the involvement of the optically forbidden $2^1A_g^-$ state below the $^1B_u^+$ state has been suggested. Thrash et al. [7,8] presented the first experimental evidence for this by recording the excitation profile of the resonance Raman scattering of β-carotene in cyclohexane. Very recently, picosecond (ps) transient Raman spectroscopy, a powerful tool to elucidate the molecular structures and the molecular vibrations in the S_1 state, has been applied to carotenoids in solutions, and additional evidence for the $2^1A_g^-$ state as the S_1 state has been presented [9–12].

In the present investigation, we have applied ps Raman spectroscopy to carotenoids in spinach chloroplasts in order to obtain evidence for the $2^1A_g^-$ state of carotenoids bound to the real photosynthetic membrane, and to investigate possible modification of the particular state due to intermolecular interactions with the apo-complex consisting of peptides and chlorophylls. We compared the transient Raman spectrum of S_1 and T_1 carotenoid(s) bound to spinach chloroplasts with the S_1 and T_1 spectra of the carotenoid extract (a mixture), all-*trans*-lutein, *and* all-*trans*-β-carotene in solutions. (For the S_0, S_1 and T_1 Raman spectra of all-*trans*-β-carotene, those reported in Ref. 12 are reproduced and used for comparison.) Results indicated that the S_1 carotenoid detected in the chloroplast membrane is β-carotene, and that the vibronic coupling between the $2^1A_g^-$ (S_1) state and the $^1A_g^-$ (S_0) state through a C = C stretching vibration is weakened upon binding of the carotenoid to the apo-complex.

Chloroplasts were prepared by the method of Satoh et al. [13] from spinach of commercial source; modifica-

Abbreviations: LHC, light-harvesting complex; PS, photosystem; THF, tetrahydrofuran.

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tions were that ascorbate and EDTA were not used in the preparation (to reduce the fluorescence background). The chloroplasts were suspended in a buffer solution, 400 mM sucrose/10 mM NaCl/5 mM $MgCl_2$/50 mM Tris-HCl (pH 7.5) and stored at $-20\,^{\circ}C$; it was diluted to $A_{532} = 5$ for Raman measurements.

Carotenoid components were extracted from spinach leaves (hereafter denoted as 'the carotenoid extract'). The methanol/acetone (3 : 1) extract of spinach leaves was saponified with 6% potassium hydroxide at room temperature for 3 h. The carotenoid component was extracted from the solution with benzene. The composition of the carotenoid components was determined using HPLC [14] to be lutein 41%, β-carotene 27%, violaxanthin 23% and neoxanthin 9%; the values of ε used at the detection wavelength of 440 nm were 124000 for lutein [15], 110000 for β-carotene [16], 128000 for violaxanthin [17] and 134000 for neoxanthin [18]. Analysis of the carotenoid components extracted from the above chloroplasts showed lutein 45%, β-carotene 27%, violaxanthin 26% and neoxanthin 2%. All-*trans*-lutein was isolated from the carotenoid extract by HPLC (column, 4 mm i.d. × 250 mm packed with LiChrosorb Si 60 5 μm; eluent, 30% ethylacetate in benzene), and then recrystallized from tetrahydrofuran (THF) plus *n*-hexane.

For the measurements of S_1 Raman spectra, $\approx 1 \cdot 10^{-3}$ M lutein (a similar estimated amount for the carotenoid extract) in THF solution was used. While for the measurement of T_1 Raman spectra, $\approx 5 \cdot 10^{-5}$ M lutein (the carotenoid extract) and $5 \cdot 10^{-3}$ M anthracene in THF solution were used. Raman spectra of S_1 carotenoids were recorded by using the 532 nm mode-locked (ML, 76 MHz), Q-switched (QS, 800 Hz) pulse (duration ≈ 100 ps) train (< 50 pulses in QS envelope) from a Nd : YAG laser (Quantronix 416). In order to extract the contribution of the S_1 state, a difference spectrum of high power (50 mW) minus low power (5 mW) was taken. Raman spectra of S_0 carotenoids were recorded with low power. Details are described elsewhere [9,11,12]. Raman spectra of T_1 carotenoids were recorded by exciting anthracene (sensitizer) with the 337 nm pulses (10 Hz) of a N_2 laser (NDC JL-1000L), and by probing with the 532 nm pulses (10 Hz) from a Nd : YAG laser (Quantel YG480). Details are described in Ref. 19. All the Raman spectra were recorded at room temperature ($\approx 25\,^{\circ}C$).

Firstly, we compare the S_0, T_1 and S_1 Raman spectra of carotenoids bound to the chloroplasts and in solution. Fig. 1 shows the S_0 Raman spectra of; (1) carotenoids bound to the chloroplasts, (2) the carotenoid extract, (3) all-*trans*-lutein and (4) all-*trans*-β-carotene (2–4 in THF solutions). In the chloroplasts as well as in the carotenoid extract, lutein is the major component (45–40%), and β-carotene ($\approx 25\%$) and violaxanthin ($\approx 25\%$) are the second major components. (Neoxanthin

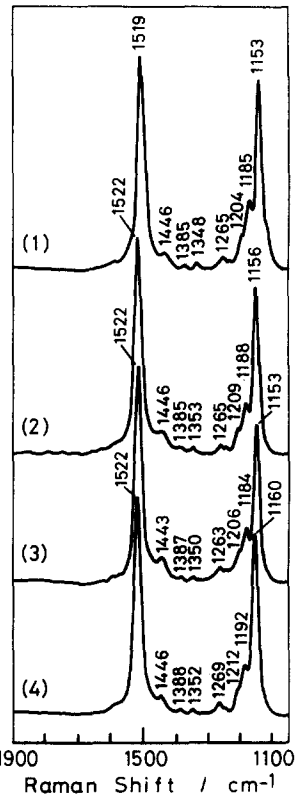

Fig. 1. The Raman spectra of carotenoids in the S_0 state. (1) Carotenoids bound to the chloroplasts, and the THF solutions of (2) the carotenoid extract, (3) all-*trans*-lutein and (4) all-*trans*-β-carotene. (Spectrum 4 is reproduced from Ref. 12, with permission) Probing at 532 nm (low power).

is excluded from the following discussion because of its small amount. Violaxanthin was found to be too unstable to be isolated by our present technique; instead, the carotenoid extract was used for comparison.) Since the λ_{max} of the $^1B_u^+ \leftarrow {}^1A_g^-$ absorption appears at 480 nm for the chloroplast suspension, and those for the carotenoid extract, lutein and β-carotene (THF solutions) appear at 450, 452, and 460 nm, respectively, the probing wavelength of 532 nm constitutes similar pre-resonance conditions; β-carotene is under the best resonance condition among the carotenoids in solution. All the Raman spectra are essentially the same; the spectral pattern in the 1300–1100 cm^{-1} region, i.e., a strong line around 1160 cm^{-1} (the C_{14}–C_{15} stretching) [20], indicates that all the carotenoids are in the all-*trans* configuration. The spectral similarity is ascribed to the same polyene backbone of the present carotenoids.

Fig. 2 shows the Raman spectrum of the excited-state carotenoids in the chloroplasts. In the present technique of transient Raman measurements [12], all ML pulses in the QS envelope were used for pumping and probing; the pump and probe pulses are in complete overlap both spacially and temporary. There are 30–50 ML pulses (duration ≈ 100 ps, interval 13 ns) in the QS

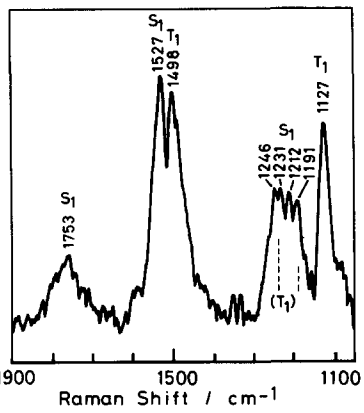

Fig. 2. The Raman spectra of the excited (S_1 and T_1) states of carotenoid(s) bound to the chloroplasts. A difference spectrum of high power minus low power was taken by using the 532 nm pulses.

envelope (interval 1.25 ms). Here, an S_1 carotenoid (lifetime in the range of $\approx 10^0$–10^1 ps) is expected to be pumped and probed within each ML pulse, while a T_1 carotenoid (lifetime, $\approx 10^0$–10^1 μs) is pumped and probed by all the ML pulses (0–0.65 μs after excitation). Thus, in the case of β-carotene, for example, the T_1 Raman lines were detected with comparative intensity to those of the S_1 state, even though the quantum yield of intersystem crossing is as low as 0.001. In the case of carotenoids in the chloroplasts, the S_1 state is produced by direct excitation at 532 nm, while the T_1 state can be produced either by intersystem crossing from the S_1 state or by energy transfer from T_1 chlorophylls (within 12 ns after excitation of chlorophylls [4]). Therefore, both the S_1 and T_1 state are expected to be detected in the present ps Raman spectroscopy using the pulse train. Actually, the Raman lines in Fig. 2 can be explained in terms of a mixtures of S_1 and T_1 carotenoid(s) (see below).

Fig. 3 shows the T_1 Raman spectra of; (1) the carotenoid extract, (2) lutein and (3) β-carotene. (Hereafter, all-*trans*-lutein and all-*trans*-β-carotene will be denoted simply as lutein and β-carotene.)

β-Carotene in THF solution shows the $T_n \leftarrow T_1$ absorption at 520 nm [21]. Assuming the order in the wavelength of the $T_n \leftarrow T_1$ absorption, i.e., the carotenoid extract < lutein < β-carotene, which is the same as that in the $S_2 \leftarrow S_0$ ($^1B_u^+ \leftarrow {}^1A_g^-$) absorption (450 < 452 < 460 nm), T_1 β-carotene is expected to be under the best resonance condition when probed at 532 nm. However, all the carotenoids can be considered to be under similar pre-resonance conditions. All the Raman spectra are very similar to one another; the frequencies and relative intensities of the Raman lines around 1495 cm^{-1} (the 'C = C' stretching), 1238 cm^{-1} (the 'C–H' in-plane bending) and 1130 cm^{-1} (the 'C–C' stretching) [19] are almost the same. (A pair of double (single) quotation marks will be used hereafter to denote a bond in the T_1 (S_1) state.) The spectral similarity has been ascribed to the same 'triplet-excited region' localized at the central part of the polyene chain [19]. The most distinct difference is found for the Raman line around 1190–1180 cm^{-1}; it can be used for the identification of T_1 carotenoids.

Fig. 4 shows the S_1 Raman spectra of; (1) the carotenoid extract, (2) lutein and (3) β-carotene. β-Carotene in THF solution shows the $S_n \leftarrow S_1$ absorption at 563 nm [12]. Assuming the order in the wavelength of the $S_n \leftarrow S_1$ absorption, the carotenoid extract < lutein < β-carotene, which is the same as that in the $S_2 \leftarrow S_0$ absorption, S_1 β-carotene is expected to be under the worst resonance condition when probed at 532 nm. Assuming a difference between the longest and the shortest wavelengths of the $S_n \leftarrow S_1$ absorptions among the carotenoids which is similar to that in the $S_2 \leftarrow S_0$ absorption (10 nm, see above), however, all the carotenoids are considered to be under similar resonance conditions. In all the spectra, the 'C = C' stretching line at 1793 cm^{-1}, the extremely high frequency of which has been ascribed to the vibronic coupling between the $2^1A_g^-(S_1)$ state and the $^1A_g^-(S_0)$ state in the case of β-carotene [9], is seen with the same frequency. The

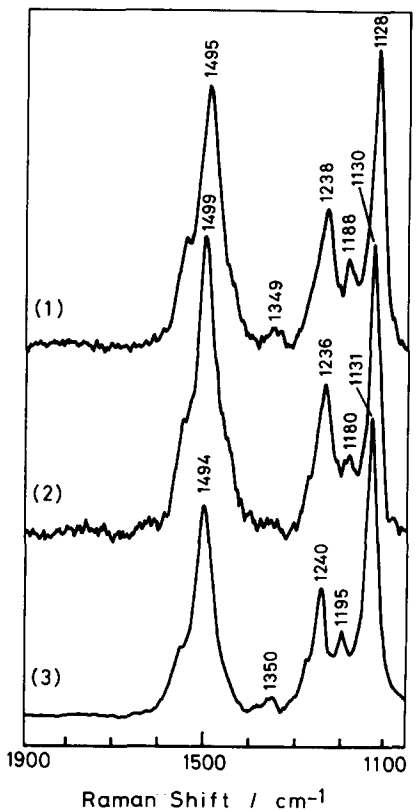

Fig. 3. The Raman spectra of carotenoids in the T_1 state. (1) The carotenoid extract, (2) all-*trans*-lutein and (3) all-*trans*-β-carotene. (Spectra 3 is from Ref. 12). The T_1 state of each carotenoid (THF solution) was produced by triplet energy transfer from anthracene (pumped at 337 nm), and was probed at 532 nm.

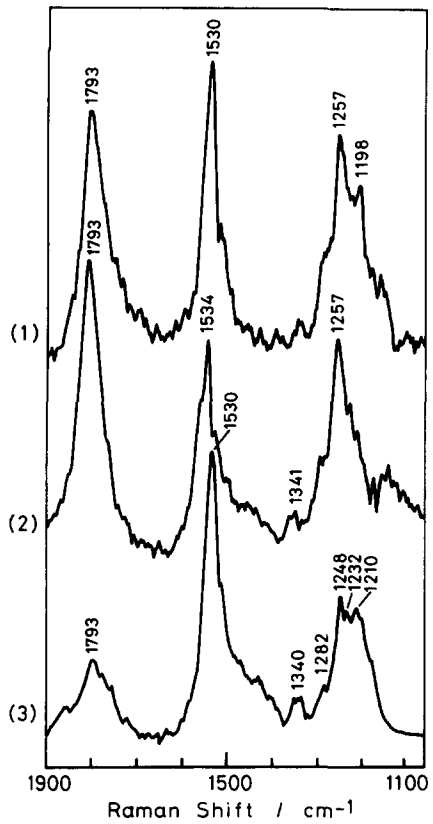

Fig. 4. The Raman spectra of carotenoids in the S_1 state. (1) The carotenoid extract, (2) all-*trans*-lutein and (3) all-*trans*-β-carotene (THF solutions). Difference spectra of high power minus low power were taken by using the 532 nm pulses. (Spectra 3 is from Ref. 12, in which the contribution of the coexistent T_1 state has been subtracted.)

other 'C = C' stretching line without vibronic coupling [12] is also seen with similar frequencies (1534–1530 cm^{-1}). (See Refs. 9 and 12 for detailed discussion on the 'C = C' stretching with and without the vibronic coupling.) However, the spectral patterns in the 1300–1100 cm^{-1} region are completely different from one another. S_1 lutein is characterized by a strong line at 1257 cm^{-1}, while S_1 β-carotene is characterized by Raman lines with comparable intensity at 1248, 1232 and 1210 cm^{-1}. The S_1 Raman spectrum of the carotenoid extract is regarded approximately as an overlap of the S_1 Raman spectra of lutein and β-carotene. The peak at 1198 cm^{-1} is absent either from the spectrum of β-carotene or from that of lutein; it is ascribable to a Raman line of S_1 violaxanthin. The above comparison shows that the S_1 Raman lines in this region depend on the end groups and that they can be used for identification of S_1 carotenoids. (S_1 β-apo-8′-carotenal [11] and S_1 spheroidene (Kuki, Hashimoto and Koyama, unpublished data) also gives rise to completely different spectral patterns in this region.) The effect of excitation seems to extend over the entire carotenoid molecule.

The Raman lines of the excited state carotenoids bound to the chloroplast membranes (Fig. 2) are now assigned by comparison with the above T_1 and S_1 Raman spectra of free carotenoids. The Raman lines at 1498 and 1127 cm^{-1} are definitely assigned to T_1 carotenoids from their frequencies. Weaker T_1 Raman lines are expected to be buried in the profile in the 1300–1150 cm^{-1} region. Since T_1 Raman spectra of the component carotenoids are similar to one another, and since the key Raman line in the region of 1195–1180 cm^{-1} is buried in the profile, it is difficult to identify which carotenoid bound to the chloroplasts is in the T_1 state.

The Raman lines at 1753 and 1527 cm^{-1} are assigned to S_1 carotenoids; the former line (the vibronically coupled 'C = C' stretching) is shifted by 40 cm^{-1} to the lower frequency when compared with the corresponding line of free carotenoids (see below for discussion on this observation). As for a group of Raman lines in the 1300–1150 cm^{-1} region, comparison of the relative intensities of the T_1 and S_1 Raman lines of free carotenoids shows that the contribution of the S_1 state to the profile is higher than that of the T_1 state. It should be noted that the strong line of S_1 lutein at 1257 cm^{-1} is missing from the profile and that the frequencies of the Raman lines in the profile, i.e., at 1246, 1231 and 1212 cm^{-1}, correspond very well to those of S_1 β-carotene, i.e., at 1248, 1232 and 1210 cm^{-1}. The observation strongly suggests that the S_1 carotenoid detected in the chloroplasts is not lutein but mainly β-carotene. The Raman line at 1191 cm^{-1} is left to be assigned, and the possibility of the coexistence of other S_1 carotenoids can not be excluded. (The contribution of T_1 carotenoids in the 1300–1150 cm^{-1} region of the spectrum for the chloroplasts is not obvious. Most probably, the Raman lines of the carotenoid extract, lutein and β-carotene at 1248 1236 and 1240 cm^{-1}, respectively, should give rise to a broad profile around 1238 cm^{-1}, while those at 1188, 1180 and 1195 cm^{-1} another broad profile around 1188 cm^{-1}. Both profiles expected are indicated by broken lines in the figure; they may have caused the S_1 Raman lines at 1231 and 1191 cm^{-1} to be pushed to the higher intensities. In the above assignment of S_1 β-carotene as the major component of the S_1 carotenoid, we compared frequencies rather than relative intensities, because the latter is more sensitive to the effect of minor components.)

Secondly, we discuss the above inhomogeneous population of the S_1 carotenoids in the chloroplasts: In solution, S_1 lutein could be detected as easily as S_1 β-carotene as shown in Fig. 4, a fact which indicates that the resonance conditions and the lifetimes are similar to one another in solutions. Therefore, the difference found in the chloroplasts should be ascribed to different lifetimes of the bound S_1 carotenoids. Since lutein is mainly bound to the light-harvesting complexes (LHC II) [22,23] and is responsible for efficient energy transfer to chlorophyll, an extremely short S_1 lifetime

($\approx 10^{-1}$ ps), as has been shown in the case of algae [6], is expected. If this is the case, the bound lutein must be too short-lived to be detected by the present 100 ps pulses. On the other hand, the fact that the S_1 β-carotene bound to the chloroplasts has been detected as easily as in solution indicates that energy transfer to chlorophyll is not so efficient in the case of this carotenoid, and that the lifetime is in the order of 10^1 ps. (The lifetime of β-carotene in *n*-hexane solution is 14 ps [9]).

The temporal inhomogeneity in the S_1 population for the carotenoids bound to the photosynthetic systems may be related to their functions. Lutein in LHC is assumed to be responsible for the primary energy transfer to chlorophyll. In the subsequent processes of energy transfer to the reaction centers, chlorophylls, not carotenoids, are supposed to be involved [24,25]. On the other hand, β-carotene is an intrinsic component of the PS I and PS II reaction centers and of the core antenna complexes. A speculative explanation is that β-carotene functions as a scavenger of excess light energy around the reaction center; it can be excited to the S_1 state, intersystem cross to the T_1 state, and then dissipate the triplet energy. Annihilation of S_1 and T_1 chlorophylls by T_1 carotenoids has been reported [4,5].

Finally, we discuss the vibronically coupled 'C = C' stretching line of the carotenoid bound to the chloroplasts as evidence (1) for weakened vibronic coupling upon binding of the carotenoid to the apo-complex and (2) for the $2^1A_g^-$ state as the S_1 state. It has been shown by Maeda and co-workers [26] that the frequency of the particular mode shifts to the higher frequency when the vibronic coupling is strengthened. Therefore, the shift of the frequency from 1793 to 1753 cm^{-1} indicates a weakening of the vibronic coupling upon binding of the carotenoid. The strength of the vibronic coupling is proportional to

$$\frac{\langle \psi_1^0 : \frac{\partial \hat{\mathscr{H}}}{\partial Q} : \psi_0^0 \rangle}{E_1 - E_0}$$

where $\hat{\mathscr{H}}$, the Hamiltonian operator; Q, the stretching coordinate; E_1 and E_0 (ψ_1^0 and ψ_0^0), the energies (wave functions) of the $2^1A_g^-$ (S_1) and $^1A_g^-$ (S_0) states [27]. Therefore, the weakened vibronic coupling indicates either a decrease in the exchange integral perturbed by the nuclear motion (a change in wave function) or an increase in the gap of the state energies. If the latter is the case, the state ordering of $^1B_u^+$ (S_2), $2^1A_g^-$ (S_1) and $^1A_g^-$ (S_0) can be a critical problem, because a decrease in the gap between the $^1B_u^+$ (S_2) and $^1A_g^-$ (S_0) state upon binding of carotenoids to the apo-complex has been well documented (detected as the shift of the $^1B_u^+ \leftarrow {}^1A_g^-$ absorption). However, the presence of the vibronically coupled 'C = C' stretching line provides direct and strong evidence for the $2^1A_g^-$ state as the S_1 state, at least in the case of β-carotene bound to the thylakoid membranes. It should be noted also that the weakened vibronic coupling is advantageous for β-carotene to suppress relaxation to the S_0 state [9] and to facilitate the formation of the T_1 state through intersystem crossing.

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References

1 Siefermann-Harms, D. (1987) Physiol. Plant. 69, 561–568.
2 Siefermann-Harms, D. (1985) Biochim. Biophys. Acta 811, 325–355.
3 Mathis, P., Butler, W.L. and Satoh, K. (1979) Photochem. Photobiol. 30, 603–614.
4 Kramer, H. and Mathis, P. (1980) Biochim. Biophys. Acta 593, 319–329.
5 Shepanski, J.F., Williams, D.J. and Kalisky, Y. (1984) Biochim. Biophys. Acta 766, 116–125.
6 Trautman, J.K., Shreve, A., Albrecht, A.C. and Owens, T.G. (1989) Physiol. Plant. 76, A79.
7 Thrash, R.J., Fang, H.L.-B. and Leroi, G.E. (1977) J. Chem. Phys. 67, 5930–5933.
8 Thrash, R.J., Fang, H.L.-B. and Leroi, G.E. (1979) Photochem. Photobiol. 29, 1049–1050.
9 Hashimoto, H. and Koyama, Y. (1989) Chem. Phys. Lett. 154, 321–325.
10 Noguchi, T., Kolaczkowski, S., Arbour, C., Aramaki, S., Atkinson, G.H., Hayashi, H. and Tasumi, M. (1989) Photochem. Photobiol. 50, 603–609.
11 Hashimoto, H. and Koyama, Y. (1989) Chem. Phys. Lett. 162, 523–527.
12 Hashimoto, H. and Koyama, Y. (1989) Chem. Phys. Lett. 163, 251–256.
13 Satoh, K. (1982) in Methods in Chloroplast Molecular Biology (Edelman, M., Hallick, R.B. and Chua, N.-H., eds.), pp. 845–856, Elsevier, Amsterdam.
14 Kohata, K. and Watanabe, M. (1988) J. Phycol. 24, 58–66.
15 Takagi, S., Shiroishi, M. and Takagi, T. (1981) Agric. Biol. Chem. 45, 1159–1165.
16 Zechmeister, L. and Polár, A. (1943) J. Am. Chem. Soc. 65, 1522–1528.
17 Bartlett, L., Klyne, W., Mose, W.P., Scopes, P.M., Galasko, G., Mallams, A.K., Weedon, B.C.L., Szabolcs, J. and Tóth, G. (1969) J. Chem. Soc. (c), 2527–2545.
18 Cholonoky, L., Györgyfty, K., Rónai, A., Szabolcs, J., Tóth, G., Galasko, G., Mallams, A.K., Waight, E.S. and Weedon, B.C. (1969) J. Chem. Soc. (c), 1256–1263.
19 Hashimoto, H. and Koyama, Y. (1988) J. Phys. Chem. 92, 2101–2108.
20 Koyama, Y., Takatsuka, I., Nakata, M. and Tasumi, M. (1988) J. Raman Spectrosc. 19, 37–49.
21 Hashimoto, H., Koyama, Y., Ichimura, K. and Kobayashi, T. (1989) Chem. Phys. Lett. 162, 517–522.
22 Plumley, F.G. and Schmidt, G.W. (1987) Proc. Natl. Acad. Sci. USA 84, 146–150.
23 Peter, G.F. and Thornber, J.P. (1988) in Photosynthetic Light-Harvesting Systems. Organization and Function (Scheer, H. and Schneider, S., eds.), pp. 175–186, Walter de Gruyter, Berlin.

24 Van Grondelle, R. and Sundström, V. (1988) in Photosynthetic Light-Harvesting Systems. Organization and Function (Scheer, H. and Schneider, S., eds.), pp. 403–438, Walter de Gruyter, Berlin.
25 Mimuro, M. (1988) in Photosynthetic Light-Harvesting Systems. Organization and Function (Scheer, H. and Schneider, S., eds.), pp. 589–600, Walter de Gruyter, Berlin.
26 Kasama, A., Taya, M., Kamisuki, T., Adachi, Y. and Maeda, S. (1987) in Time-Resolved Vibrational Spectroscopy (Atkinson, G.H., ed.), pp. 286–319, Gordon & Breach, New York.
27 Auerbach, R.A., Christensen, R.L., Granville, M.F. and Kohler, B.E. (1981) J. Chem. Phys. 74, 4–9.