Biochimica et Biophysica Acta 1847 (2015) 69–78

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Ultrafast time-resolved vibrational spectroscopies of carotenoids in photosynthesis ☆

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ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 22 August 2014
Accepted 5 September 2014
Available online 16 September 2014

Keywords:
Carotenoid
Photosynthesis
Time-resolved stimulated Raman spectroscopy
Coherent spectroscopy
Four-wave mixing spectroscopy

ABSTRACT

This review discusses the application of time-resolved vibrational spectroscopies to the studies of carotenoids in photosynthesis. The focus is on the ultrafast time regime and the study of photophysics and photochemistry of carotenoids by femtosecond time-resolved stimulated Raman and four-wave mixing spectroscopies. This article is part of a Special Issue entitled: Vibrational spectroscopies and bioenergetic systems.

1. Introduction

Carotenoids are ubiquitous pigments in photosynthesis [1,2]. They absorb blue-green spectral region of sunlight and transfer the captured energy to chlorophylls that have scant absorption in this spectral range. This singlet–singlet type energy transfer defines the overall efficiency of photosynthetic light reaction. Carotenoids also serve as the scavenger of the excess amount of light exposure. In this case, triplet–triplet type energy transfer from chlorophylls to carotenoids does have an important role. In the light-harvesting pigment–protein complexes from purple photosynthetic bacteria, carotenoids have an additional role of structural stabilization of the pigment–protein complexes [3].

In order to fully understand the functions of carotenoids in photosynthesis it is highly desired to accumulate the information of carotenoids in their excited electronic states. A number of spectroscopic studies have already been done to achieve this objective [4]. Among them time-resolved Raman spectroscopy has been making it possible to explore the relationship of the structures of carotenoids and their functions in the electronic excited states [5]. This is particularly owing to the fact that resonance Raman effect makes it possible to enhance only the signals from carotenoids bound to photosynthetic pigment–protein complexes. Classical spontaneous time-resolved Raman spectroscopy in the picosecond to sub-millisecond time regime had clarified the structure–function relationships of the *cis-trans* isomers of carotenoids both in the lowest excited singlet S_1 and triplet T_1 states [5]. This spontaneous method automatically has the limitation for time-resolution due to the uncertainty principle of quantum mechanics applied to pulse laser sources, i.e. time and frequency of light pulse cannot accurately be determined at the same time. Therefore the conventional time-resolved Raman spectroscopy had only been applicable to as fast as picosecond time resolution. Recent advancement of nonlinear spectroscopic techniques, however, did open a new door to make a breakthrough against this limitation. Now we can make a good access to molecular vibration of carotenoids in femtosecond time regime. In this review, vibrational spectroscopies on carotenoids in such an ultrafast time regime are extensively outlined.

2. The outline of ultrafast relaxation processes of carotenoids following photoexcitation

Carotenoids give rise to the characteristic yellow, orange, and red colors because they absorb blue to green region of light. The lowest

Abbreviations: BChl, bacteriochlorophyll; SVD, singular value decomposition; LHCII, light-harvesting complex II; TPE, two-photon excitation; FWM, four-wave mixing; SWM, six-wave mixing; TG, transient grating; Bphe, bacteriopheophytin; CARS, coherent anti-Stokes Raman scattering; ET, energy transfer; IC, internal conversion

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http://dx.doi.org/10.1016/j.bbabio.2014.09.001
0005-2728/

excited singlet states in general pigment molecules are the optically allowed state for one-photon transition from the ground state. These energies, hence, decide the colors of the pigment molecules. However, carotenoids have optically forbidden singlet excited-state whose energy is lower than the optically allowed singlet excited-state. The ground electronic state of the carotenoid has the $^1A_g^-$ symmetry assuming the C_{2h} point symmetry of its polyene backbone. Therefore, the lowest singlet excited state, S_1 and designated as $2^1A_g^-$, is optically forbidden. The lowest optically active state is the $1^1B_u^+$ (S_2) state. The photogenerated $1^1B_u^+$ state converts to the $2^1A_g^-$ state within 100–300 fs and the lifetime of the $2^1A_g^-$ state is several picoseconds [6]. In the light-harvesting pigment–protein complexes in purple photosynthetic bacteria singlet–singlet energy transfer from both of these singlet excited-states to bacteriochlorophyll (BChl) molecules was identified based on the research using sub-picosecond time-resolved fluorescence spectroscopy [7]. Another yet controversial dark state, $1^1B_u^-$ or sometimes designated as S_X or X, has been predicted and discovered between the $1^1B_u^+$ and $2^1A_g^-$ states [8–12].

Another type of intermediate excited state, termed as S* has been found with carotenoids both free in solution and bound to light-harvesting complexes, and the things become even more complicated [13–17]. At the higher-energy side of the $S_1 \rightarrow S_n$ transition, a new transient absorption band was detected by means of pump–probe time-resolved absorption spectroscopy and subsequent spectral analysis using SVD (singular value decomposition) and global fitting. This newly identified absorption band was assigned to the S* state. The lifetime of this particular state was determined to be between 5 and 12 ps depending on both the species of carotenoid and on whether it was in or out of the light-harvesting complexes. The S* state decayed into the triplet state when the carotenoid was bound to the LH2 complex. However, when the carotenoid was free in organic solvent the S* state decayed to the ground state without generating the triplet state. Applying a pump–dump and transient absorption technique for β-carotene, lycopene, and zeaxanthin, Wohlleben et al. re-examined the origin of the S* state with the carotenoid free in solution (S^*_{sol}) [16]. They suggested that the S^*_{sol} state is a vibrationally excited ground state (S^*_{sol} = hot S_0), which is populated by a combination of impulsive Raman scattering of the pump pulse and $S_1 \rightarrow S_0$ internal conversion. They also found the S* state of the protein-bound carotenoid and re-designated it as S^*_T. These ideas have recently been supported by the author's group for spirilloxanthin both free in solution and bound to light-harvesting complexes [17].

Involvement of vibrationally excited states in the relaxation process of carotenoids after photoexcitation was initially detected by time-resolved absorption spectroscopy [18–20], and has also been studied by time-resolved stimulated Raman spectroscopy [21–25]. We will discuss this important issue below in a later section.

The involvement of intramolecular charge-transfer type intermediate states (S_{ICT}) in the relaxation from $S_2 \rightarrow S_1$ is well documented for polar carotenoids such as peridinin and fucoxanthin (see Fig. 1 for chemical structures of these molecules) [26–36]. However, discussion of these charge transfer states is beyond the scope of this review. Readers who are interested in more details about this state should consult the excellent review by Polívka and Sundström [37].

Fig. 1 shows a schematic illustration of the relative energies of the carotenoid excited singlet states discussed above together with the proposed relaxation pathways from the S_2 state as well as the energy-transfer pathways between carotenoid and BChl. Since the relaxation from the S_2 state is very fast, ultrafast vibrational spectroscopies discussed in this review are going to be important to clarify the structure–function relationship of the above singlet excited-states.

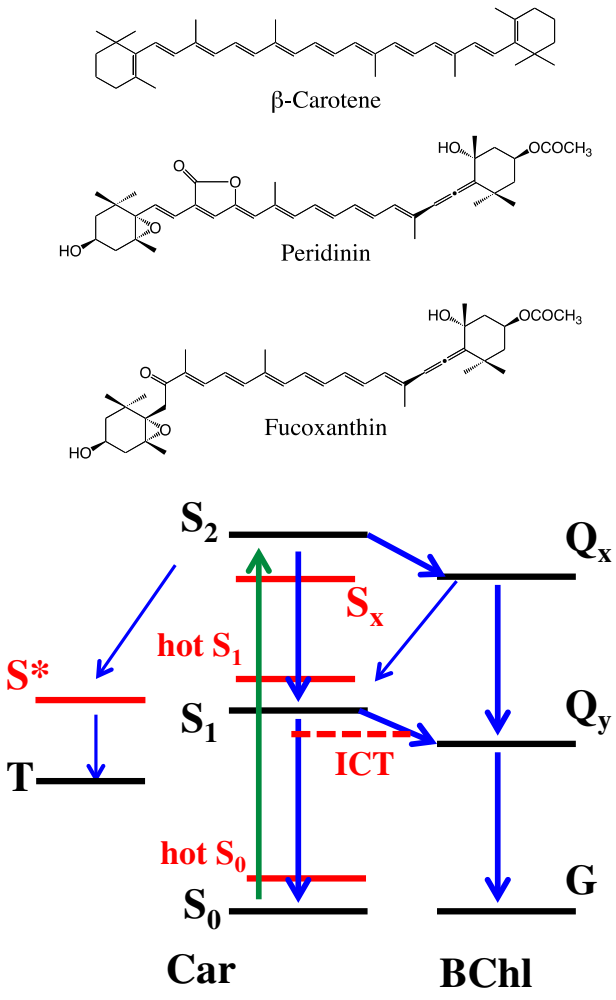

Fig. 1. Chemical structures of β-carotene, peridinin, and fucoxanthin, and a schematic description of energy diagrams together with relaxation and energy-transfer pathways of carotenoids following photoexcitation up to the S_2 state.

3. Time-resolved Raman and two-photon excitation spectroscopies

Fig. 2 shows the steady-state absorption spectrum of all-*trans*-β-carotene (see Fig. 1 for its chemical structure). The absorption peak that corresponds to optically allowed $1^1B_u^+$ (S_2) singlet excited-state appears at 2.55 eV (486 nm). The peaks appear at 2.73 eV (455 nm) and 2.90 eV (428 nm) are side bands due to molecular vibrations. In the case of carotenoids these side bands are mainly due to ν_1 (~1500 cm^{-1}, C=C stretching) and ν_2 (~1200 cm^{-1}, C–C stretching) modes. The lowest excited singlet (S_1) state of carotenoids is lying in energy below the S_2 state. This S_1 state is designated as $2^1A_g^-$, which has the same symmetric character with the ground (S_0) state, and hence the transition from the S_0 state is one-photon forbidden. Therefore the $S_0 \rightarrow S_1$ absorption cannot be detected in the conventional steady-state absorption spectrum. However, carotenoids somewhat break their symmetry in solution, and very weak fluorescence can be observed [38]. The absorption spectrum of the $2^1A_g^-$ (S_1) state shown in Fig. 2 was obtained by the spectral simulation using the parameters determined by fluorescence spectroscopy.

3.1. Time-resolved Raman gain and loss spectroscopies

Raman spectroscopy is a spectroscopic technique to investigate the molecular vibrations using the energy difference between the excitation laser light and the scattered radiation. Time-resolved Raman spectroscopy can be utilized to investigate the time evolution of molecular vibrations, structures, and the structural change of molecules in the excited electronic states. Time-resolved Raman spectroscopy on

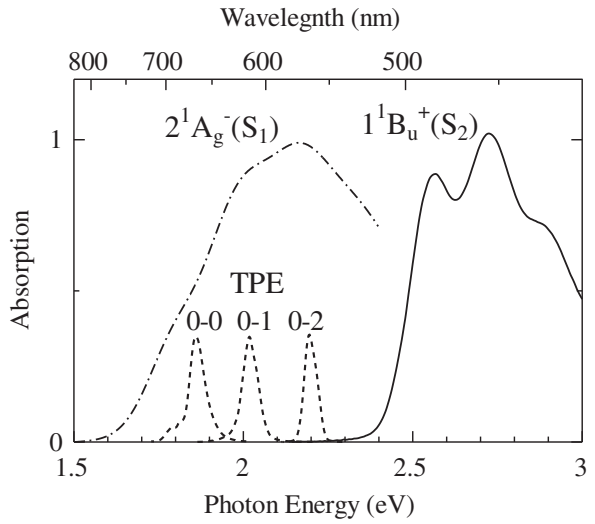

Fig. 2. Steady state absorption spectra of β-carotene in cyclohexane that corresponds to the transition from the ground $1^1A_g^-$ (S_0) state to the $1^1B_u^+$ (S_2) state (solid line) and that to the $2^1A_g^-$ (S_1) state (dotted broken line). The latter spectrum was predicted based on a theoretical calculation. The bands illustrated with broken lines are the spectra of excitation laser light sources that were used for two-photon excitation (TPE). The energies of these spectra were doubled from the original values.

polyene molecules clarified that the ν_1 (C═C stretching) mode in the $2^1A_g^-$ (S_1) state shows unusually high frequency compared to that in the ground state [39]. In general, molecular vibrations in the excited electronic states are expected to have lower frequencies than their counterparts in the ground electronic state because of the expansion of wave functions in the excited electronic states. However, polyene molecules do not obey this simple rule. This anomaly was explained as due to strong vibronic coupling between the ground $1^1A_g^-$ (S_0) and the lowest excited singlet $2^1A_g^-$ (S_1) states [39]. Similarly, in the case of carotenoids picosecond time-resolved Raman spectroscopy had clarified that the ν_1 mode in the $2^1A_g^-$ (S_1) state appears at extraordinary high frequency [40,41]. According to the development of ultrafast laser systems, it becomes easier to observe the ultrafast phenomena with temporal resolution in femtosecond time regime. As its consequence, it has been well expected to reveal the detailed mechanisms that produce anomalous vibrational behavior of the $2^1A_g^-$ state of carotenoids. However, using the conventional method, it is difficult to obtain the satisfactory frequency resolution for Raman spectroscopy due to the uncertainty principle of quantum mechanics. Femtosecond time-resolved Raman spectroscopy can become feasible using the process of stimulated Raman scattering [42–45].

Three laser pulses are used in the femtosecond stimulated Raman spectroscopy. Fig. 3 shows its outline. The first laser pulse (frequency, ω_{ex}) excites the molecule to its excited electronic state (e). The target excited-state for Raman measurement is then populated following the relaxation process. In Fig. 3 it is assumed that the vibrational ground ($l = 0$) and excited ($l = 1$) states in the electronic excited state, respectively, have the population N_0 and N_1. If Raman excitation laser pulse (frequency, ω_R) and probe light pulse (frequency, ω_S) were simultaneously irradiated to the sample at time t, the transmittance difference (ΔT) in the Stokes side induced by the stimulated Raman process can be described as follows [42].

$$\Delta T(\omega_S, t) \propto \frac{N_0(t) - N_1(t)}{[\omega_S - (\omega_R - \omega_{10})]^2 + (\gamma_{10} + \gamma_R)^2} \quad (1)$$

Here, ω_{10} corresponds to the frequency difference between $l = 1$ and $l = 0$ vibrational states. The observed signal has a peak at the

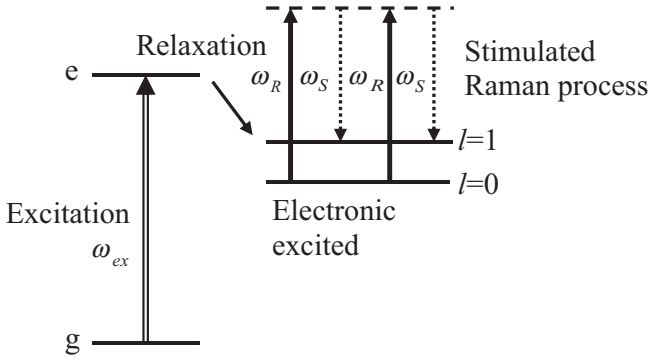

Fig. 3. The outline of time-resolved Raman spectroscopy using the process of stimulated Raman scattering.

position whose frequency is ω_{10} lower than the frequency of Raman excitation pulse (ω_R). Spectral bandwidth becomes the summation of the widths of vibration γ_{10} and the Raman excitation pulse γ_R. Signal intensity is proportional to the difference of the population of vibrational ground and excited states. In this method, both the femtosecond time-resolution and the sufficiently high spectral resolution for Raman measurement are simultaneously satisfied. Moreover, when the population of the excited state vibration exceeds that of the ground state vibration ($N_1 > N_0$), the sign of the signal is reverted, i.e. population change of the vibrational states can be observed.

Fig. 4 shows time-resolved Raman signals of β-carotene [21]. The signal at 1520 cm^{-1} is the ν_1 mode of β-carotene in the ground $1^1A_g^-$ (S_0) state. Following the photoexcitation the signal of the ground-state vibration decreases its intensity, and the ν_1 mode in the lowest excited singlet $2^1A_g^-$ (S_1) state appears at 1800 cm^{-1}. The sign of the 1800 cm^{-1} is negative. This means that the $2^1A_g^-$ (S_1) state is in the vibrational excited state ($l = 1$ level in Fig. 3) for more than several picoseconds after photoexcitation.

Conjugation length dependence of the vibrational relaxation process in the $2^1A_g^-$ (S_1) state was investigated by means of the time-resolved stimulated Raman spectroscopy on the homologues of β-carotene that have the number of conjugated C═C bonds (n) from 7 to 15 ($n = 11$ for β-carotene) [24]. In all the homologues the decrease of the ν_1 mode intensity in the ground $1^1A_g^-$ (S_0) state was observed following photoexcitation. This is due to the fact the ground state population was decreased by photoexcitation. However, ν_1 mode in the $2^1A_g^-$ (S_1) state that appears around 1800 cm^{-1} showed the conjugation length dependence. The negative signals of the ν_1 mode were observed for

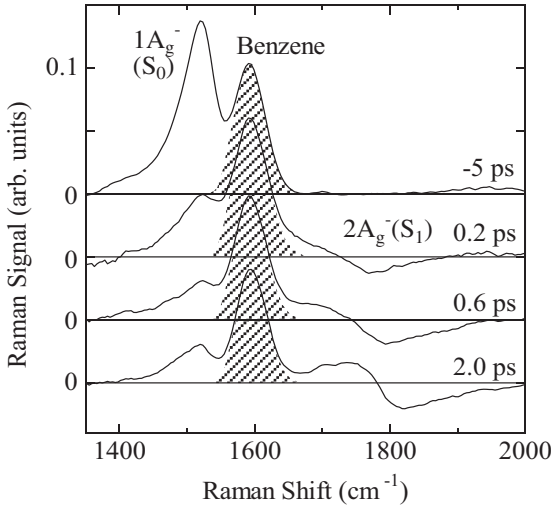

Fig. 4. Time-resolved stimulated Raman spectra of β-carotene in benzene following photoexcitation. Shadowed bands show the Raman lines of the solvent.

the homologues that have $n = 11$ or less, and this ν_1 mode was assigned to the vibrational excited state in the $2^1A_g^-$ (S_1) state. However, the homologues that have $n = 13$ or more did not show such negative signals, and it was concluded that the $2^1A_g^-$ (S_1) state relaxed to the vibrational ground state immediately after photoexcitation in these homologues. These results show that in the shorter conjugated carotenoids vibrational relaxation of the ν_1 mode is slower than several picoseconds in the $2^1A_g^-$ (S_1) state, while in the longer conjugated carotenoids vibrational relaxation takes place within 1 ps after photoexcitation.

3.2. Two-photon excitation spectroscopy

It is desirable to control the population of initial vibrational states following photoexcitation in order to precisely investigate the vibrational relaxation process in the excited electronic state. With regard to the $1^1B_u^+$ (S_2) state of carotenoids, relaxation process has been investigated by selective generation of vibrational ground and excited states using a wavelength-tunable excitation laser light source [46]. In this same study transient absorption spectra of the $2^1A_g^-$ (S_1) state also showed the difference depending on the excitation wavelengths, and hence it was suggested that the vibrations induced to carotenoids affect its relaxation process more than several picoseconds. The experimental methods to induce molecular vibration coherently using ultrafast laser pulses have been well developed, and these methods are applied to carotenoids [47]. However, it is difficult to control the population of the initial vibrational states in the $2^1A_g^-$ (S_1) state, since this electronic state is usually generated through internal conversion from the $1^1B_u^+$ (S_2) state.

In order to solve this issue it is necessary to directly excite the $2^1A_g^-$ (S_1) state. Two-photon excitation is allowed for the transition from the ground to $2^1A_g^-$ (S_1) state. There is a report that showed the direct excitation of the vibrational excited states in the $2^1A_g^-$ (S_1) state using a tunable infrared excitation laser light source [48]. Walla et al. observed the fluorescence from bacteriochlorophyll following two-photon excitation of the $2^1A_g^-$ (S_1) state of carotenoids and subsequent energy transfer in LHCII (light-harvesting pigment–protein complex in higher plants and algae). They found the increase of energy transfer efficiency from carotenoid to bacteriochlorophyll by the selective excitation of the vibrational excited state in the $2^1A_g^-$ (S_1) state.

The measurement of transient absorption spectra using time-resolved absorption spectroscopy is a powerful tool to reveal the vibrational relaxation processes. However, spectroscopic apparatus with sufficiently high accuracy is required for two-photon excitation, since the signals detected using this spectroscopic method is very weak. The higher accuracy of the time-resolved spectroscopy can be achieved increasing the rate of detection of the probe signals so as to synchronize the repetition rate of the laser pulses. Using this technology the accuracy of 10^{-4} to 10^{-5} of the absorbance change (ΔA) is readily obtained within a short period of time of data acquisition [30].

The excitation light sources used for two-photon excitation (TPE) are in resonance to the vibrational ground (1330 nm, TPE 0–0), first excited (1230 nm, TPE 0–1), and second excited (1130 nm, TPE 0–2) states in the $2^1A_g^-$ (S_1) state as shown in Fig. 2. These excitation light sources are not in resonance to the $1^1B_u^+$ (S_2) state, but a nonlinear optical effect between the excitation and probe pulses induces a large transient signal at the delay time of 0.0 ps (time-origin) [49]. However, this nonlinear signal immediately disappears, and the signals detected after 0.5 ps following excitation are assignable to those of the $2^1A_g^-$ (S_1) state directly generated by two-photon excitation. Fig. 5 shows the transient absorption spectra of β-carotene recorded at 5 ps after two-photon excitation [50]. Apparent difference can be seen between the spectra of TPE 0–0 excitation and the other. In the case of TPE 0–0 excitation the maximum absorbance of transient absorption appears at 2.23 eV (556 nm), while the corresponding maxima shift to lower in energy at 2.21 eV (561 nm) for other excitations. This difference can be explained by the difference of the states of ν_1 mode between

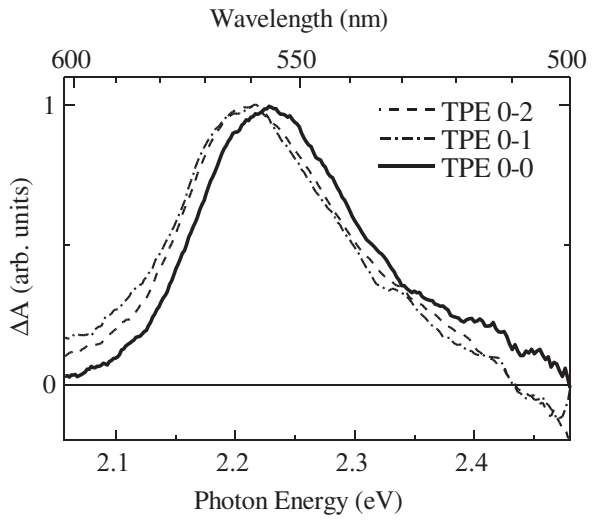

Fig. 5. Transient absorption spectra of β-carotene in cyclohexane recorded at 5 ps after two-photon excitation to the $2^1A_g^-$ (S_1) state.

TPE 0–0 and other excitations. Moreover, the signal due to vibrational relaxation shows an increase until 1 ps after excitation in the case of TPE 0–2 excitation. However, in the cases of TPE 0–0 and TPE 0–1 excitations such a kind of increase of the signal due to vibrational relaxation is not observed. As the consequence, the vibrational relaxation processes in the $2^1A_g^-$ (S_1) state can be summarized as follows.

(1) When the vibrational ground state ($l = 0$) in the $2^1A_g^-$ (S_1) state is excited, transient absorption from the $l = 0$ state is observed.
(2) When the vibrationally excited $l = 1$ state in the $2^1A_g^-$ (S_1) state is excited, the excited electronic state stays in the $l = 1$ level of the ν_1 mode for a while. Therefore, signal change due to vibrational relaxation cannot be observed. The maximum of transient absorption shows the low energy shift reflecting the effect of strong vibronic coupling as mentioned above.
(3) When the vibrationally excited $l = 2$ state in the $2^1A_g^-$ (S_1) state is excited, vibrational relaxation to the $l = 1$ level of the ν_1 mode takes place within 1 ps after excitation. However, the excited state then stays in the $l = 1$ state for a while, the transient absorption spectrum that shifted lower in energy can be observed.

Both the time-resolved Raman and two-photon excitation spectroscopies clarified that vibrationally excited $l = 1$ state of the ν_1 mode in the $2^1A_g^-$ (S_1) state of β-carotene has amazingly a long lifetime more than several picoseconds. This finding suggests that the vibrational excited state in the excited electronic state of carotenoids may have an important role in the light-harvesting process of photosynthesis.

4. Coherent spectroscopy

Coherent spectroscopy has been developed using gaseous samples such as an iodine [51–54]. This is because that the fruitful information on both the electronic and vibrational states had already been obtained for these gaseous samples. In addition, the period of vibrational motions of these samples were long enough than the laser pulses to be used for the measurements, and hence the discrimination of the signals characteristic of coherent spectroscopy was relatively easier for these samples. After that, owing to the development of new techniques as well as laser light sources, coherent spectroscopy has been applied to more complicated system such as liquids, inorganic solids, and proteins etc. Observing the coherent component of the signals makes it possible to obtain the unified information on the dynamics of both vibrational and electronic states, interaction between these states, and the effect of the surrounding environment on the materials. Moreover, controlling the shape and interval as well as the phase of incident laser pulses can

regulate the efficiencies of photochemical reactions, i.e. reaction pathways.

The coherent signals appear due to quantum mechanical interference between the states, and hence reflect the magnitude of the coupling between the states. In what follows, coherent signals that have been observed for carotenoids are surveyed. The readers should consult with the literatures for the details of physical backgrounds to understand the coherent signals [55,56].

4.1. Image of n-wave mixing signal

Fig. 6 shows an example of four-wave mixing (FWM) and six-wave mixing (SWM) signals from β-carotene [47]. In general *n*-wave mixing measurement is performed with an optical configuration illustrated in Fig. 7(a). Namely, laser light is split to three using a beam splitter, and two of them are independently guided to translational stages in order to give time delays among three laser pulses. If these three laser pulses were well focused on a single spot of the sample, FWM signals can be observed. This type of optical configuration, where three laser pulses are irradiated to the sample from three distinct directions, is called the BOXCARS configuration.

Three intense laser spots surrounded with a central triangle in Fig. 6 are the excitation laser lights transmitted through the sample. When three excitation laser pulses simultaneously reach to the sample (zero time delays), FWM and SWM signals are generated in the area surrounding the transmitted excitation laser light. As illustrated in Fig. 7(b), if we define the wave-vectors of excitation and signal lights, respectively, as k_i ($i = 1, 2, 3$) and k_S, FWM signals appear at the direction that satisfies the relation of $k_S = \pm k_1 \pm k_2 \pm k_3$ and $k_S = 2k_i - k_j$ ($j = 1, 2, 3$ and $j \neq i$). SWM signals that are pointed with arrows in Fig. 6 appear following an additional interaction with two more excitation laser pulses (5 times interaction all together), for example as $k_S = -2k_1 + 2k_2 + k_3$. It is to be noted here that FWM and SWM signals are strong enough for carotenoids to be identified with our naked eyes. This means that carotenoids are suitable to investigate their nonlinear optical responses. The information concerning the coherence can be obtained investigating the time evolution of the FWM signals.

In the next section, coherent spectroscopy based on the measurements of FWM signals is described. The wording of four-wave means that one signal wave is obtained with the irradiation of three incident light waves, i.e. four light waves all together are closely related to each other in order to generate FWM signals.

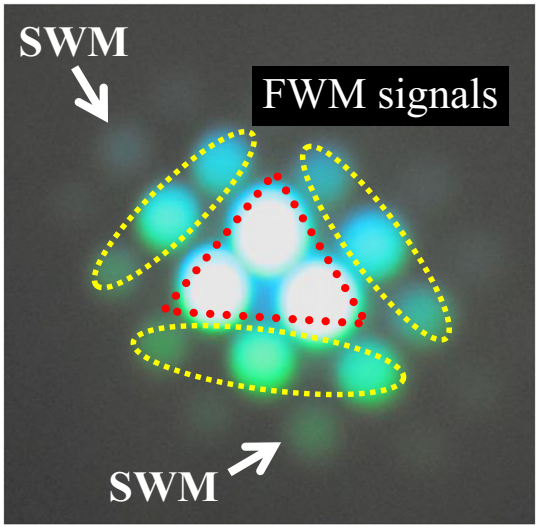

Fig. 6. A photograph of four-wave mixing (FWM) signals. Central three bright spots are excitation laser lights. FWM signals surrounded by dotted ellipsoids can readily be observed with naked eyes. Six-wave mixing (SWM) signals that are generated through higher order nonlinear interaction can also be observed.

4.2. Four-wave mixing (FWM) signal

FWM signals in carotenoids are reported for β-carotene and its homologues, lycopene, astaxanthin, and spheroidene [47,57–68]. As an example, the results of β-carotene are shown here. Fig. 8(a) shows the time evolution of a FWM signal in β-carotene, which is obtained by observing a single spot shown in Fig. 6. Here abscissa shows the time interval T between pulse 2 and pulse 3 (see Fig. 7(c)). The time interval τ between pulse 1 and pulse 2 was set to be zero. The FWM signal that is measured under this condition is frequently called as a transient grating (TG) signal. The intense signal that appears around time origin in Fig. 8(a) is called a coherent spike. Following this spike signal, coherent vibration signal with the very fast period of oscillation in about 20–30 fs can be observed on top of the slowly decaying background with the lifetime about 5 ps.

The origin of this coherent vibration can be clarified if the TG signal in Fig. 8(b) is Fourier transformed. As readily can be understood based on the comparison with the Raman spectrum of β-carotene shown in Fig. 9(b), the peaks obtained by the Fourier transformation of the coherent vibration (Fig. 9(a)) show good coincidence with those of Raman spectrum of β-carotene. Namely, the peaks appeared at $\nu_1 = 1522\ \mathrm{cm}^{-1}$ and $\nu_2 = 1157\ \mathrm{cm}^{-1}$, respectively, are attributed to the totally symmetric vibration of C═C and C−C stretchings, and the peak appeared at $\nu_3 = 1007\ \mathrm{cm}^{-1}$ is attributed to the in-plane rocking vibration of methyl groups. These vibrational modes appeared because all the β-carotene molecules under inspection start to vibrate in phase, i.e. coherently, following the impulsive excitation with ultrashort laser pulses. The stretching vibrations of carotenoids usually appear in the 1000 1500 cm^{-1} frequency domain. These frequencies correspond to 30–20 fs in the time domain, if the frequency to time domain conversion is performed. Therefore, coherent vibration can be induced in carotenoids, if we use the sub-20 fs ultrashort laser pulses for the FWM experiment. Namely, as illustrated in Fig. 10, the first and second laser pulses generate a coherent state in vibrational ground (g and g') and excited (e and e') states, and as its consequence real-time observation of vibrational wave-packets becomes possible.

The most important information that is obtained by the measurement of FWM signals is the coupling between carotenoids and their surrounding environment. This information is reflected to spectral density (see Fig. 9(c)). It is known that there are couplings with slow vibration of 100 fs (~300 cm^{-1}) or less in organic solvents [47,62,64]. Obtaining the spectral density, various optical responses including absorption and fluorescence spectra can be calculated, and hence the precise discussion on the experimental data based on theoretical models becomes feasible [55]. As one of those examples, Fig. 8(c) shows the result of calculation for FWM signal. The experimental result is well reproduced with the theoretical calculation. As shown here spectral density includes meaningful information, however, there are scant reports on carotenoids bound to pigment–protein complexes [69]. On the other hand, many studies have already been performed on the coherent vibrations that directly reflect the effect of coupling with surrounding environment in bacteriochlorophyll [70–77]. To reveal the correlation among these coherent vibrations as well as the role of coherent vibration in excitation energy transfer is going to be important from now on.

Coherent vibrations are also observed in the electronic excited state of a carotenoid. Motzkus et al. were successful to observe the coherent vibration in the S_1 state of β-carotene with 20 fs temporal and 10 cm^{-1} spectral resolutions [58,59,61]. They introduced pre-pump pule that excite β-carotene to its S_2 state for FWM measurement (pump–FWM) to produce the population of the S_1 state via internal conversion from the S_2 state. They concluded that coherence of molecular vibration is not conserved during the process of $S_2 \rightarrow S_1$ internal conversion [58]. They also showed that the lifetime of coherent vibration in the S_1 state is an order of magnitude smaller than that in the ground state for all the vibrational modes.

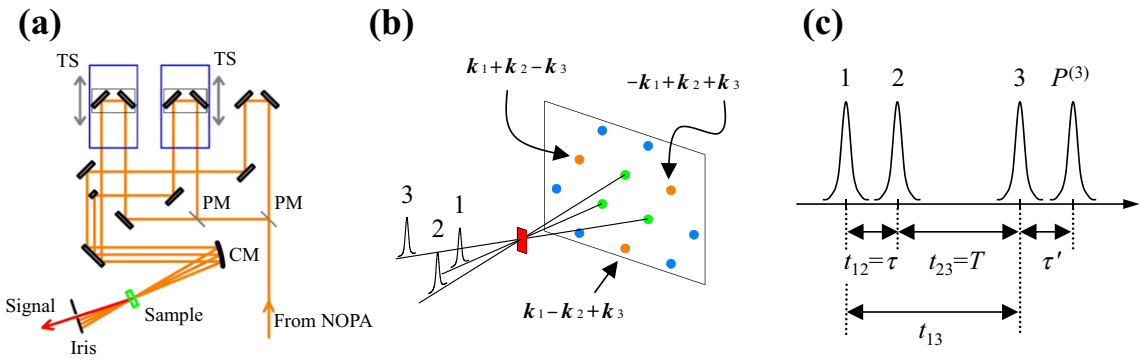

Fig. 7. (a) Optical configuration of the interferometer used for the measurements of four-wave mixing (FWM) signals. The light from a non-collinearly phase-matched optical parametric amplifier (NOPA) is split into three using two pellicle mirrors (PM). Three laser pulses thus produced are then focused to excite the sample using a collimating mirror (CM). Time intervals between each of these three laser pulses were controlled using two translational stages (TS). The FWM signals are generated along the directions that satisfy the phase-match condition. One of these signals is selected using an iris diaphragm. (b) When three laser pulses are irradiated to the sample, the FWM signals can be generated along the directions that satisfy the phase-match conditions. (c) The relation between coherent time τ and population time T. t_{12} (t_{13}) is the time interval between pulses 1 and 2 (1 and 3) when they arrive at the sample.

We so far described the coherence between vibrational states. If we swap the vibrational states shown in Fig. 10 to electronic states, it can be readily imagined that the coherence between electronic states should also appear. However, the observation of the electronic coherence itself is one of the hottest topics in the research field of ultrafast spectroscopy, and hence there are scant reports on the electronic coherence in carotenoids. In what follows, we show some relevant reports. Very long-lived (>660 fs) coherence among electronic excited states was found in Fenna–Matthew–Olson (FMO) BChl complex isolated from a green sulfur bacterium *Chlorobium tepidum* [78]. This observation suggests that the excitation energy-transfer among BChls in FMO complex cannot be simply explained by the classical picture that shows the hopping of excitation energy between the BChl molecules, but it should be interpreted as the superposition of quantum mechanical states. The

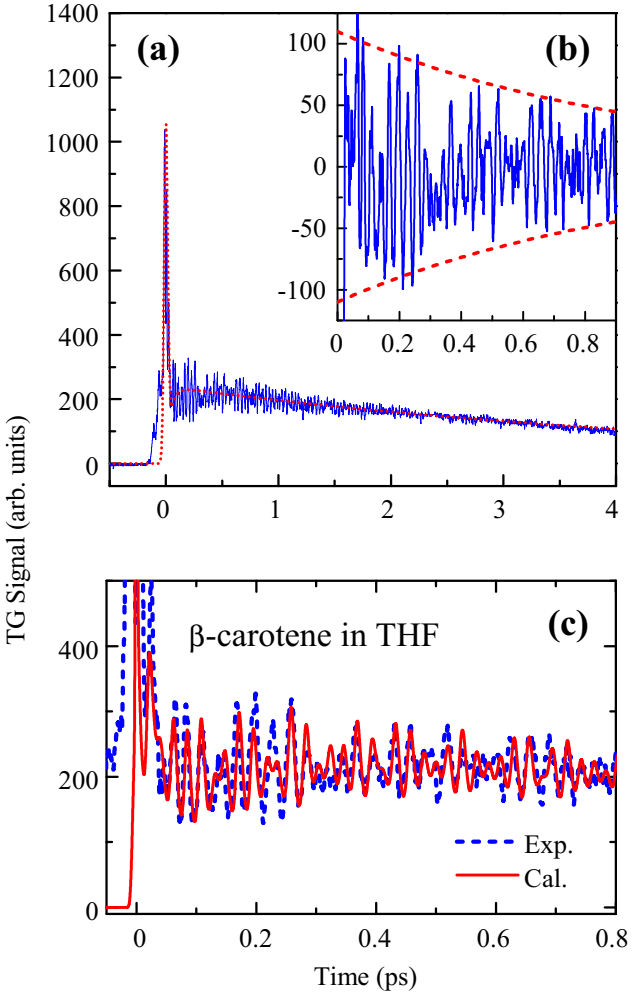

Fig. 8. (a) Experimentally observed time-evolution of the transient-grating (TG) signal. A slowly varying background shown with dotted lines reflects the lifetime of the electronic excited states. If the background is subtracted from the original TG signal, coherent vibration component can be extracted as shown in (b). The decay time of coherent vibration is determined to be around 1 ps. (c) Comparison of the experimentally observed TG signal (broken line) and the result of theoretical calculation (solid line).

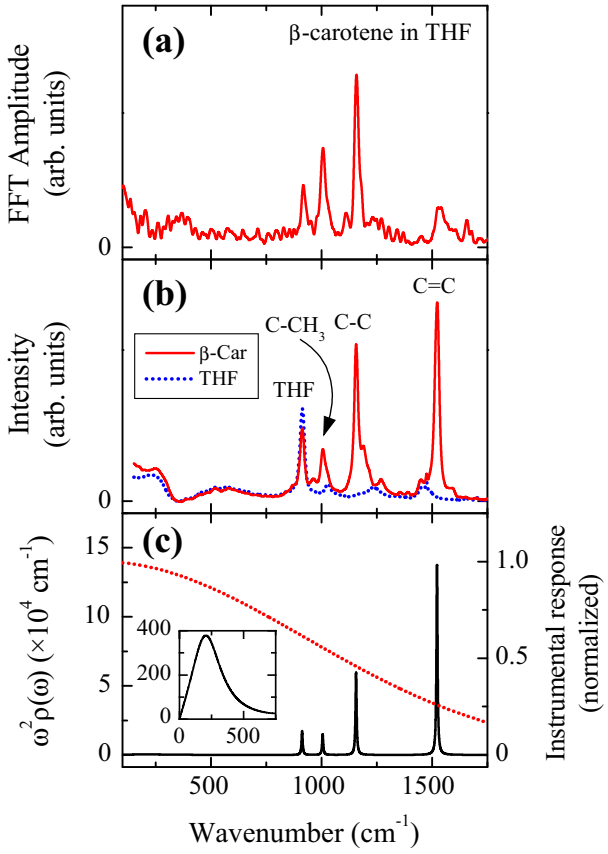

Fig. 9. (a) Fourier transformed spectrum of the coherent signal component shown in Fig. 7(b). (b) Raman spectra of β-carotene and solvent THF (tetrahydrofuran). (c) Spectral density (solid line) and the response function of the detecting system (dotted line). The inset shows the spectral density that reflects the system-bath (β-carotene and solvent THF) interaction in the low-frequency regime.

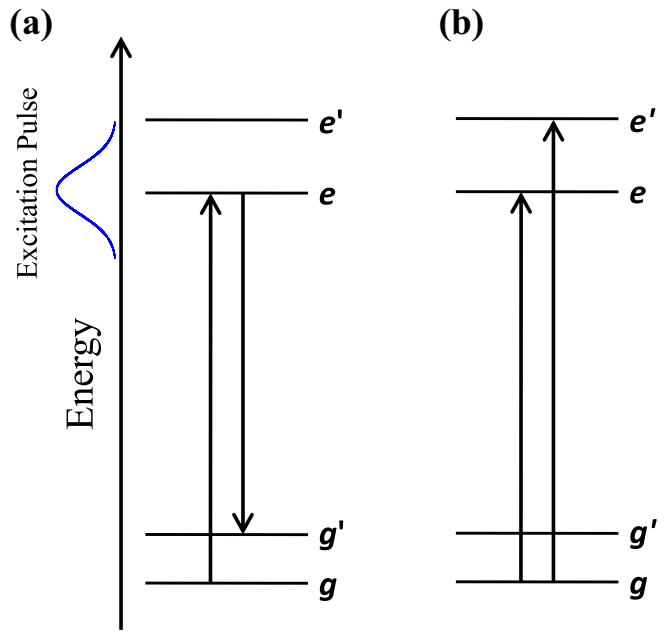

Fig. 10. Coherent vibration (a) in the ground state and (b) in the excited state induced by the exposure of two excitation laser pulses ($-\boldsymbol{k}_1$ and $\boldsymbol{k}_2$). The spectral bandwidth of the excitation pulse should exceed the energy difference of the vibrational levels.

electronic coherence was also found between bacteriopheophytin (Bphe) and accessory BChl molecules in the reaction from a purple photosynthetic bacterium *Rhdobactor sphaeroides* [79]. The remarkable point in this report is that decoherence time (440 fs) is much longer than the excitation energy-transfer time (250 fs) from Bphe to accessory BChl. This result suggests a possibility that electronic coherence may have some relevance in the excitation energy-transfer process. Moreover, coherent energy-transfer was found within the conjugated system in π-conjugated polymer MEH-PPV [80]. It is a big challenge of photosynthesis research to obtain the detailed information on the coherence within and between carotenoids and that between carotenoid and BChl.

4.3. Coherent anti-Stokes Raman Scattering (CARS)

CARS spectroscopy is a similar spectroscopic technique with FWM spectroscopy in a sense that both of them observe the third-order nonlinear optical response by irradiating three independent incident radiations to the sample. Big differences between them are that CARS uses spectrally broad light for the second pulse (called as a Stokes light), and that CARS signals appear at the higher energy side of the excitation light (anti-Stokes side).

Hamaguchi et al. were successful to apply the CARS technique to β-carotene with 1.2 ps time-resolution [81]. Introducing vibrationally hot ground state S_0^* in the process of $S_1 \rightarrow S_0$ internal conversion, they have estimated the relaxation time constants of $S_1 \rightarrow S_0^*$ and $S_0^* \rightarrow S_0$, respectively, to be 7 ps and 11 ps. These results show good agreement with those determined independently using femtosecond time-resolved Raman spectroscopy [21,23]. Siebert et al. applied pump–CARS measurement to β-carotene, in which pre-pump laser pulse was introduced before starting the CARS measurement, and reported the relationship between the relaxation of excitation energy and molecular vibrations [82–84]. According to the series of studies, they concluded that the C═C stretching vibration (1524 cm^{-1}) has the largest contribution as the accepting mode for excitation energy, and the other modes have the similar but less contribution in the order, C – C stretching (1157 cm^{-1}), C – CH_3 rocking (1004 cm^{-1}), C═C – C bending (872 cm^{-1}), and C – H bending (1269 cm^{-1}).

4.4. Pump–probe spectroscopy

The reasons why FWM spectroscopy is used to detect coherent signals are (1) since the FWM signals appear not on the same optical axis of the excitation light sources, background-free signals can be obtained, and (2) changing the time intervals between the three excitation laser pulses makes it possible to select the required response functions. However, it is known that coherent molecular vibrations can be detected without using the FWM technique. One of the most representative ones is to use pump–probe spectroscopy with extremely ultra-short laser pulses (sub-10 fs). With this spectroscopic technique, since three laser pulses interact to each other to generate the signals, the similar information that is obtained by FWM spectroscopy can also be obtained. Cerullo et al. have observed the coherent vibrations in the ground state of β-carotene, okenone, and rhodopin-glucoside in wide spectral regime [85–88]. Most of the previous researches using pump–probe spectroscopy mainly focus their attention on the population dynamics, and hence the number of research that pays attention to the coherence of the system is still very restricted. Beside these, some interesting phenomena have already been found; (1) the coherent vibration changes its phase with the amount of π nearby the isosbestic point that has appeared because of the competition of both bleaching of the ground state absorption and the appearance of $S_n \leftarrow S_1$ transient absorption, and (2) the phase of coherent vibration changes continuously at time due to the effect of frequency chirp of probe light [88].

4.5. Coherent control

Recently, expanding the utility of coherent spectroscopy, a research motivation to control the photophysical and photochemical reaction pathways is activated. Namely, when the ensemble of molecules is moving along some reaction coordinates, it can be guided to the selective reaction pathway suppressing simultaneously unnecessary pathways by the use of ultra-short laser pulses.

As already mentioned above, sub-20 fs laser pulses are required to excite coherently the molecular vibrations of carotenoids. Because of the uncertainty principle in quantum mechanics, such an ultra-short laser pulse is spectrally broad. As its consequence, excitation using the ultra-short laser pulse induces various coherent modes of molecular vibration (see Fig. 10). An optical pulse-shaping device such as a space-light modulator can generate periodic laser light pulses called pulse trains. If the period of this pulse trains, for example, were adjusted to match the period of a specific molecular vibrational mode, then only the specific coherent vibrational mode can be excited and the other coherent modes can completely be suppressed. The C – CH_3 in-plain rocking, C – C stretching, and C═C stretching modes of β-carotene in cyclohexane, respectively, have the frequencies of $\nu_3 = 1004\ cm^{-1}$, $\nu_2 = 1157\ cm^{-1}$, and $\nu_1 = 1524\ cm^{-1}$. If these frequencies are converted to time domain (period), they become 33.2 fs, 28.8 fs, and 21.9 fs, respectively. Hauer et al. were successful to selectively excite coherent vibration corresponding to either of these vibrational modes using thus shaped pulse trains [89]. Especially adopting a resonance excitation condition, the selectively excited coherent vibrational mode was enhanced with its intensity 5.7 times larger than that without using the pulse-shaping technique. Konradi et al. confirmed the validity of the selective mode excitation almost at the same time [90].

Motzkus et al. were successful to quantum control the excitation energy-transfer (ET) efficiency from carotenoid to bacteriochlorophyll in the LH2 complex from a purple photosynthetic bacterium *Rhodopseudomonas acidophila* [91–93]. They regulated the phase at each wavelengths of a femtosecond laser pulse using a space-light modulator based on the application of evolutional algorithms by monitoring the ET efficiency as well as the rate of internal conversion (IC) of carotenoid. It was shown that the 30% of the ratio of IC/ET can be controlled, and that ET and IC states can be switched by changing the phase of π for excitation light pulse. This is indeed a striking

finding because complicated photosynthetic light-harvesting reaction was proved to be controllable. In their first report downward regulation of the ET from carotenoid to bacteriochlorophyll was reported. In this report they mentioned that in principle the upward regulation might become possible. Later on this idea was proved to be true. Owing to the improvement of the evolutional algorithms, they were successful to enhance the efficiencies of ET and IC independently. Periodic pulse trains are used in order to make feasible the coherent control experiment. In carotenoids, it is proposed that to coincide the period of the pulse trains to that of the coherent vibration is a key factor.

5. Conclusion

Since starting the application of femtosecond time-resolved spectroscopy to observe the excited-state dynamics in carotenoids, a number of issues that could not be clarified by means of steady-state spectroscopy have already been solved. However, at the same time, the rapid improvement of the measurement accuracy makes it possible to detect weak signals that have never been observed before. Owing to these observations, the understanding of the excited-state dynamics becomes much more complicated. Pump–probe spectroscopy that has frequently been used among the femtosecond spectroscopies is widely applied to investigate the excited-state dynamics. This is partially because the optical set-up for this spectroscopy is relatively easy. This experimental method is indeed a powerful tool to detect the signals in wide spectral range. However, the materials have ultrafast relaxation processes that sometimes show broad spectral features, and hence various signals overlap to each other. Because of this reason, complementary information obtained by the other spectroscopic methods is required in order to correctly understand the excited-state dynamics that was observed by the pump–probe spectroscopy. Recently many nonlinear spectroscopic techniques, which include FWM measurements (transient grating, transient lens, three-pulse photon echo, photon-echo peak-shift, coherent Raman spectroscopy), have been applied to the investigation of carotenoids [47,57–59,62,82,90,94]. It will be the major challenge in the next generation to comprehensively interpret the experimental data obtained using these sophisticated spectroscopies together with those obtained by the pump–probe spectroscopy. Support from the theoretical studies is indispensable in order to verify the results of ultrafast spectroscopies. Owing to the rapid improvement of the data processing power of computer systems, more accurate theoretical calculations that include the effect of electronic couplings (compensation for the effect of interaction among many electrons) have become possible for the system with the larger number of n of conjugated C═C bonds than the classical works by Tavan and Schulten [8,9]. In the reports of Tavan and Schulten the largest number of n was restricted to 8. However, Kurashige et al. were successful to extend the number to $n = 14$ [95]. Especially in recent years the algorithm of quantum chemical calculations has been well established. As its consequence theoretical calculations that include time-dependent perturbation as well as complicated electronic couplings have been reported [95–97]. The calculation methods of the exact energies of complex molecules in the electronic excited-states have not been established so far. We hope that the exact calculations on the electronic excited-states of carotenoids will be possible in near future.

Acknowledgement

HH thanks Scientific Research on Inovative Areas “All Nippon Artificial Photosynthesis Project for Living Earth (AnApple)” (No. 24107002H) from the Japan Society for the Promotion of Science (JSPS) for finacial support.

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