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Review

Ultrafast time-resolved vibrational spectroscopies of carotenoids in photosynthesis ☆

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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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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.

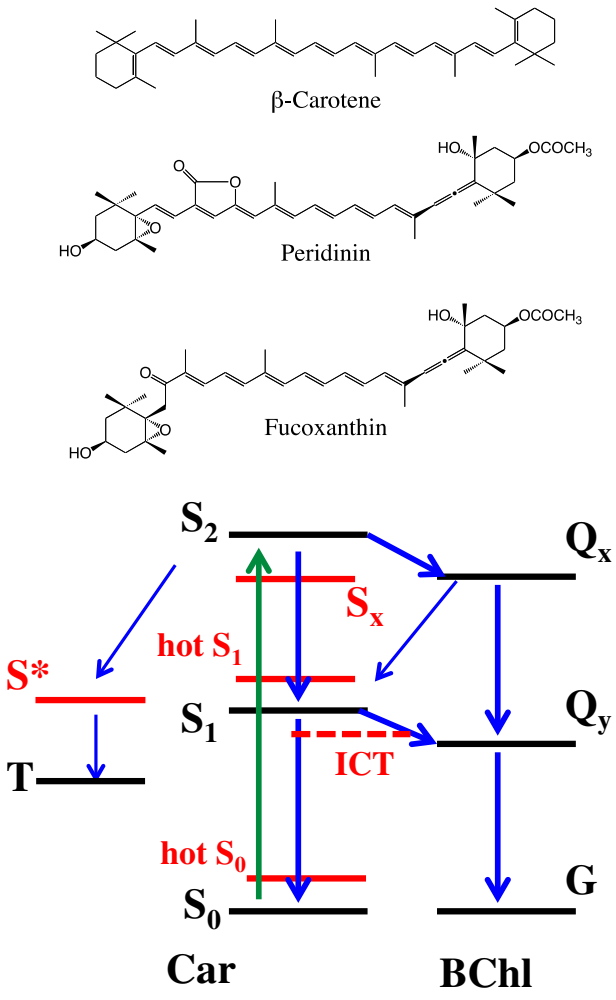

Fig. 1. Chemical structures of β-carotene, peridinin, and fucoxanthin, and a schematic description of energy diagrams together with relaxation and energy-transfer pathways of carotenoids following photoexcitation up to the S_2 state.

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