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Femtosecond stimulated Raman spectroscopy of the dark S_1 excited state of carotenoid in photosynthetic light harvesting complex*

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Vibrational dynamics of the excited state in the light-harvesting complex (LH1) have been investigated by femtosecond stimulated Raman spectroscopy (FSRS). The native and reconstituted LH1 complexes have same dynamics. The ν_1 (C=C stretching) vibrational mode of spirilloxanthin in LH1 shows ultrafast high-frequency shift in the S_1 excited state with a time constant of 0.3 ps. It is assigned to the vibrational relaxation of the S_1 state following the internal conversion from the photoexcited S_2 state.

Key words: vibrational dynamics, carotenoid, spirilloxanthin, light-harvesting, femtosecond stimulated Raman spectroscopy, reconstituted LH1

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INTRODUCTION

Carotenoids (Cars) play important roles in photosynthesis. In the antenna complexes, they act as light-harvesting and photo-protecting pigments (Frank & Cogdell, 1996; Polívka & Sundström, 2004). Light energy absorbed by carotenoids is transferred to bacteriochlorophyll (BChl) with high efficiency. In the photo-protective function, carotenoids efficiently quench the BChl triplet state to prevent sensitized generation of reactive singlet oxygen. The S_0 ground state of all-*trans*-carotenoids has A_g^- symmetry assuming that their linear polyene backbone has C_{2h} point group symmetry. The lowest singlet excited state, S_1 (the $2A_g^-$ state), is optically forbidden. The S_2 ($1B_u^+$) state is the lowest optically allowed state. The energy transfer to BChl has been reported to occur from both the S_2 and S_1 states. Recently, the S* state has been reported to have considerable importance in the light-harvesting function (Cong *et al.*, 2008; Nakamura *et al.*, 2011). It is the precursor on the reaction pathway toward triplet formation and plays a critical role in efficient excitation energy deactivation in the LH1 complex.

The vibrational dynamics of the S_1 state in carotenoids have been attracted much interest. The tunable photoexcitation of the S_2 state has revealed that the excess vibrational energy induced in the S_2 state remains even after the internal conversion to the S_1 state and affects the relaxation kinetics (Kosumi *et al.*, 2005). The efficient energy transfer from the vibrational hot level of the S_1 state to BChl has been reported in the light-harvesting complexes (Wehling & Walla, 2005). Therefore, further investigations of the vibrational dynamics of the S_1 state are needed.

Femtosecond stimulated Raman spectroscopy (FSRS) has been recognized as a powerful method for studying vibrational dynamics in ultrafast phenomena. Combination of narrowband Raman pump pulse and ultrashort probe pulse has enabled femtosecond temporal resolution and a few tens wavenumber of spectral resolution (Yoshizawa & Kurosawa, 2000; Yoshizawa *et al.*, 2001). The progresses in this decade have achieved the tunable Raman pump pulse which is useful for selective measurement of the transient state (Shim & Mathies, 2008).

In this work, we used native LH1 complex from *Rhodospirillum rubrum* S1 and reconstituted LH1 with purified spirilloxanthin that is hereafter called LH1(Spx). LH1(Spx) is used to remove the effect of other kinds of carotenoids involved in the native LH1. The vibrational dynamics of the S_1 state of carotenoid in the LH1 complexes have been investigated by the FSRS.

MATERIALS AND METHODS

Sample preparation. The native LH1 complex from wild-type *Rhodospirillum rubrum* S1 contains spirilloxanthin as a major carotenoid (Evans *et al.*, 1988). In this study, the reconstituted LH1(Spx) was prepared as described elsewhere (Nakagawa *et al.*, 2008). The solution of LH1(Spx) was dispersed in a poly-vinyl alcohol film (PVA, Kuraray Co., Ltd., PVA-217) on a glass plate (Nakamura *et al.*, 2011). The solution of the native LH1 was measured using a 1 mm flow cell. During the measurements, the film sample was translated linearly and the solution was circulated to avoid degradation and the accumulation of any potential photoproducts.

Laser spectroscopy. The femtosecond dispersed time-resolved absorption spectroscopy setup shown in Fig. 1 is based on an amplified mode-locked Ti:Sapphire laser system operating at 1 kHz (Kosumi *et al.*, 2010). A fraction of the amplified pulses was used to drive two independent optical parametric amplifiers (OPA). The excitation pulse resonant to the $S_0 \rightarrow S_2$ transition of

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Abbreviations: BChl, bacteriochlorophyll; Car, carotenoid; FSRS, femtosecond stimulated Raman spectroscopy; LH1, light-harvesting complex 1; OPA, optical parametric amplifier; PVA, poly-vinyl alcohol; Spx, spirilloxanthin.

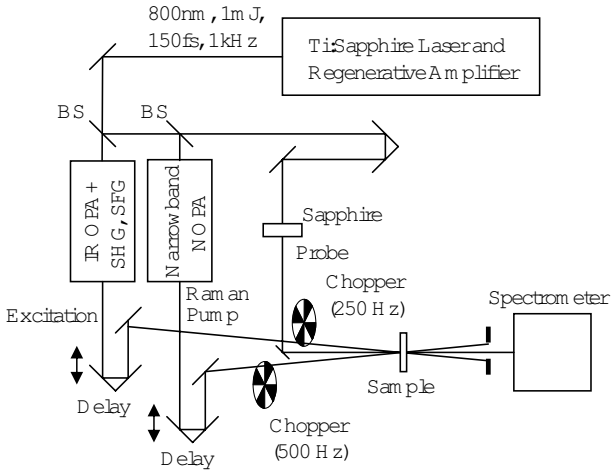

Figure 1. Schematic diagrams of femtosecond stimulated Raman spectroscopy.

spirilloxanthin (500 nm, 100 fs) and the Raman pump pulse resonant to the $S_1 \rightarrow S_m$ transition (620 nm, 20 cm^{-1}) were obtained. The supercontinuum generated by a sapphire plate was used as a broadband probe pulse. The polarizations of all the beams were set to be parallel to one another. A probe pulse after the sample was dispersed onto a linear image sensor with a spectrometer. The excitation and Raman pump beams were modulated at 250 and 500 Hz, respectively, by a mechanical chopper, which was frequency locked to the laser pulse train.

RESULTS AND DISCUSSION

Stationary absorption and Raman spectra

The stationary absorption spectrum of the LH1(Spx) film is shown in Fig. 2. The characteristic vibronic profile of the $S_0 \rightarrow S_2$ transition of carotenoid (spirilloxanthin) appears in the region of 470–550 nm. The Q_x and Q_y bands of BChl are observed at 590 nm and 880 nm, respectively. The wavelengths of the absorption peaks are identical in the native LH1 solution.

Figure 3 shows the stationary spontaneous Raman spectra of the native LH1 solution and the LH1(Spx) film. The major observed signals are assigned to the Car S_0 ground state, because the Raman pump pulse (532 nm) is resonant to the Car $S_0 \rightarrow S_2$ transition. The signals in the native LH1 solution and the LH1(Spx) film have almost the same structures. This suggests that spirilloxanthin is in the same environment both in the native LH1 and the reconstituted LH1. The three major Raman peaks at 1559 cm^{-1} (ν_1), 1149 cm^{-1} (ν_2), and 1001 cm^{-1} (ν_3) are assigned to the C=C symmetric stretching, the C-C symmetric stretching, and the methyl-in-plane rocking mode, respectively (Saito & Tasumi, 1983).

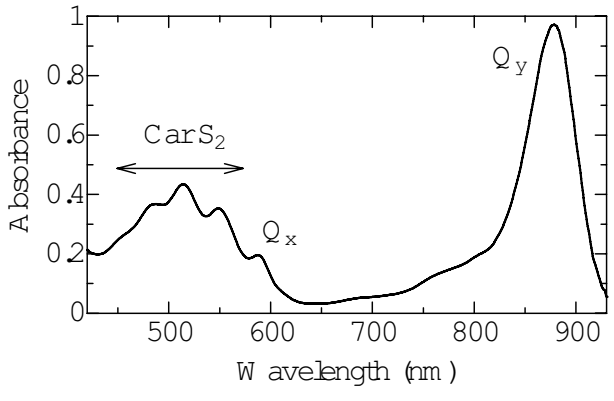

Figure 2. Stationary absorption spectrum of the LH1(Spx) film.

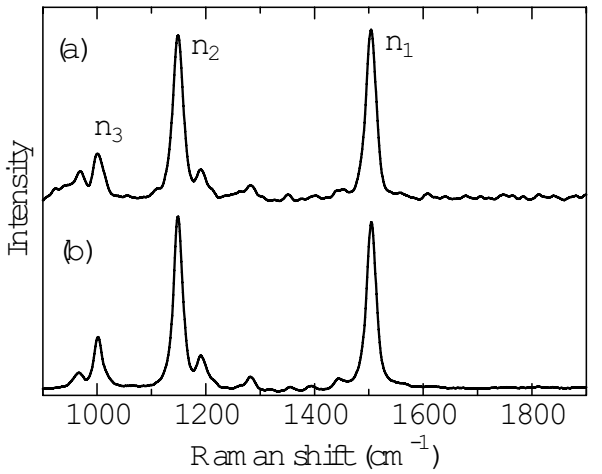

Figure 3. Stationary Raman spectra of (a) the native LH1 solution and (b) the LH1(Spx) film induced by the 532 nm Raman pump.

Transient absorption spectra

The transient absorbance change following the Car S_2 excitation in the LH1(Spx) film is shown in Fig. 4. The native LH1 solution has almost same transient spectra. The observed signals are explained using a previously proposed model (Nakamura *et al.*, 2011). The broad transient absorption due to the Car S_2 state is observed at 0.1 ps in the infrared region. The relaxation to the S_1 state and the energy transfer to the BChl Q_x state occur simultaneously with a time constant of 70 fs (Kosumi *et al.*, 2011). The well-known Car $S_1 \rightarrow S_m$ absorption (620 nm) and the bleaching of the BChl Q_y absorption (890 nm) are clearly observed at 1.0 ps. The relaxation from the hot S_1 state to the S_1 state is observed as temporal spectral change of the $S_1 \rightarrow S_m$ absorption. Then, the S_1 state relaxes to the S_0 ground state with a lifetime of 1.6 ps. At 10 ps, the signal due to the Car S_1 state decreases, but the peak at 580 nm remains. It is assigned to the Car S* state that has a lifetime of 5.7 ps.

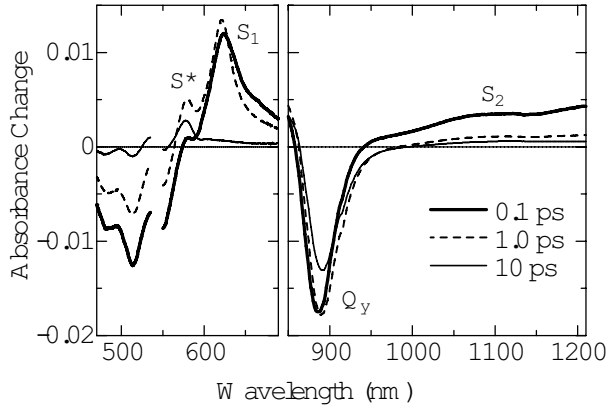

Figure 4. Difference absorbance changes of the LH1(spx) film after the 500 nm excitation.

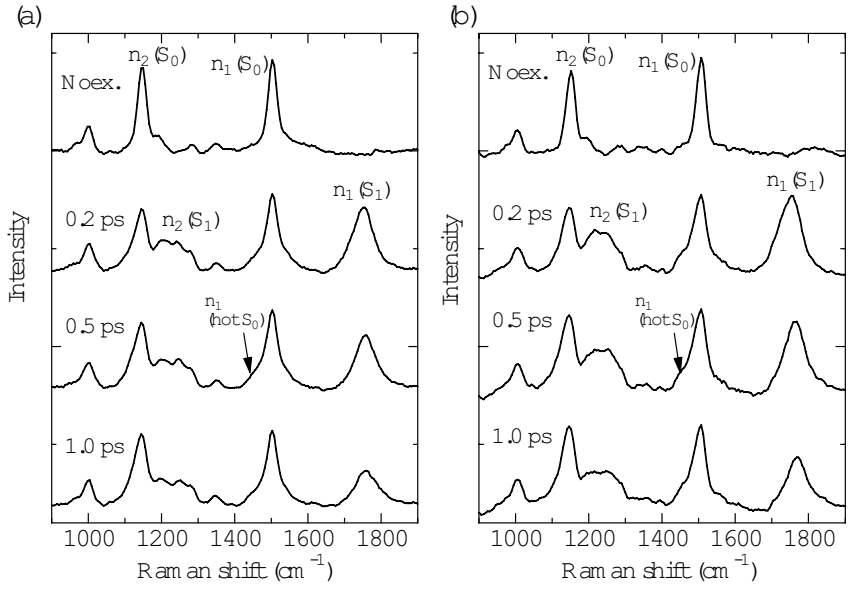

Figure 5. Time-resolved stimulated Raman spectra of (a) the native LH1 solution and (b) the LH1(Spx) film. Noex: without the 500 nm excitation, 0.1, 0.5, 1.0 ps: delay times after the 500 nm excitation.

Femtosecond stimulated Raman spectra

The time-resolved stimulated Raman signals on the Stokes side are shown in Fig. 5. The Raman pump pulse was tuned at 620 nm to be resonant to the Car $S_1 \rightarrow S_n$ transition. The signals observed in the native LH1 solution and the LH1(Spx) film show essentially the same spectral pattern. The signals without the 500 nm excitation pulse (top curves) are assigned to the S_0 ground state. The ν_1, ν_2, and ν_3 modes are observed consistently with the stationary Raman measurement. After the excitation, the ν_1 and ν_2 modes of the S_0 state decrease instantaneously and broad new signals appear around 1750 and 1250 cm^{-1}. They are assigned to the ν_1 and ν_2 modes in the S_1 state, respectively, as well as the signals observed in β-carotene (Yoshizawa *et al.*, 2001). The high-frequency shift of the S_1 excited state is explained in terms of the vibronic coupling through the vibrational mode with A_g symmetry, because they have same A_g symmetry (Hashimoto & Koyama, 1989; Noguchi *et al.*, 1989). On the other hand, the ν_3 mode does not show clear change. Since the ν_3 mode is the methyl-in-plane rocking mode, it has smaller coupling with the S_1 state than the ν_1 and ν_2 modes. The signals assigned to the S_1 state decrease with the 1.6 ps lifetime of the S_1 state. The broad foot observed at the ν_1 mode of the S_0 state decays with a lifetime of 5.4 ps. It is assigned to the hot S_0 state generated by the internal conversion from the S_1 state.

The remarkable feature observed in the FSRS signal is transient peak shift of the ν_1 mode of the S_1 state. The ν_1 signal just after the excitation has a peak at 1740 cm^{-1}, then it shifts to 1767 cm^{-1} with a time constant of 0.3 ps. The transient absorption shown in Fig. 4 shows temporal spectral change with the same time constant. The S_1 transient absorption has broad spectrum at 0.1 ps, then the peak shifts to shorter wavelength and the spectrum becomes narrower. These changes are explained in terms of the vibrational relaxation in the S_1 state (Kosumi *et al.*, 2005). The hot S_1 state is generated by the internal conversion from the initially excited S_2 state, then it relaxes to the vibrational ground level of the S_1 state. However, the ν_2 mode of the S_1 state does not show the temporal spectral change. This means that the hot S_1 state of the ν_1 mode is generated because it is the coupled mode of the internal conversion from the S_2 state to the S_1 state similar to that from the S_1 state to the S_0 state.

The vibrational features of spirilloxanthin in the native LH1 and the reconstituted LH1(Spx) have been investigated by the stationary and time-resolved Raman spectroscopies. The vibrational modes of the S_0 ground state observed by the ordinary spontaneous Raman spectroscopy have the same frequencies. The environment of the reconstituted spirilloxanthin is identical to that of the native LH1 complex as previously revealed by the Stark spectroscopy (Nakagawa *et al.*, 2008). The dynamics following the excitation of the Car S_2 state are also the same in the native LH1 and LH1(Spx). The S_1 state in the LH1 complex is in the vibrational excited level just after the formation from the S_2 state. The relatively slow vibrational relaxation of the ν_1 mode is expected to be a universal feature of carotenoids even when they are bound to the LH complexes. Our findings provide the importance of the vibrational dynamics in the energy transfer mechanisms of the LH complexes.

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