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Strategies to enhance the excitation energy-transfer efficiency in the light-harvesting system using the intra-molecular charge transfer character of carotenoids

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Abstract

Fucoxanthin is a carotenoid that is mainly found in the light-harvesting complexes from blown algae and diatoms. Due to the presence of a carbonyl group attached to the polyene chain in polar environments excitation produces an excited intra-molecular charge transfer. This intra-molecular charge transfer state plays a key role in the highly efficient (~95%) energy-transfer from fucoxanthin to chlorophyll *a* in the light-harvesting complexes from brown algae. In purple bacterial light-harvesting systems the efficiency of excitation energy-transfer from carotenoids to bacteriochlorophylls depends on the extent of conjugation of the carotenoids. In this study we were successful, for the first time, to incorporate fucoxanthin into the light-harvesting complex 1 from the purple photosynthetic bacterium, *Rhodospirillum rubrum* G9+ (a carotenoidless strain). Femtosecond pump-probe spectroscopy was applied to this reconstituted light-harvesting complex in order to determine the efficiency of excitation energy-transfer from fucoxanthin to bacteriochlorophyll *a* when they are bound to the light-harvesting 1 apo-proteins.

Introduction

Carotenoids are bound to both peripheral light-harvesting (LH) 2 and LH1 'core' pigment-protein complexes in purple photosynthetic bacteria. In these complexes carotenoids serve as an additional light-harvesting pigments that capture the energy in the blue-green spectral region of the solar spectrum and transfer this energy on to bacteriochlorophylls [1]. Singlet-singlet excitation energy-transfer (EET) is involved in this process. The efficiency of EET from carotenoid to bacteriochlorophyll in the LH complexes depends on the number of conjugated C=C double bonds (n) present within the carotenoid [2]. In native LH2 complexes EET ranges from 53 to 92% [3], whereas in the case of the LH1 complexes reconstituted with exogenous carotenoids EET ranges from 36 to 78% [4]. Carotenoids with n = 9 or 10 C=C bonds show nearly 90% efficiency of EET to bacteriochlorophyll *a*. However the EET efficiency suddenly drops down to less than 56% if n becomes larger than 11. In native LH1 complexes from the purple photosynthetic bacterium *Rhodospirillum* (*Rsp.*) *rubrum* strain S1, with spirilloxanthin (n = 13) as the major carotenoid, EET shows only 27% carotenoid to bacteriochlorophyll *a* efficiency [5]. These findings can be interpreted as follows.

Carotenoids show strong absorption bands in 400 – 500 nm spectral region. The origin of these absorption bands is assigned to the $S_0 \rightarrow S_2$ transition. Carotenoids have another singlet-excited state below the S_2 state in energy. This state is called as S_1 and cannot be accessed through a one photon transition from the S_0 state [6-8]. Therefore, the S_1 state is a dark state and can only be accessed from the internal conversion process from the S_2 state (as long as the carotenoid is excited by one photon). Through detailed femtosecond time-resolved absorption spectroscopic measurements carotenoid to bacteriochlorophyll EET processes have been investigated in both LH2 and LH1 complexes [2, 9]. It has been suggested that carotenoids with n = 9 or 10 C=C bonds utilize both the S_2 and S_1 states to realize the highly efficient EET from carotenoid to bacteriochlorophyll, while carotenoids with $n \geq 11$ cannot utilize the EET channel from the S_1 state and hence the overall singlet EET efficiency shows sudden drop-off. These finding clearly show the importance of the involvement of the optically dark S_1 state for the highly efficient EET from carotenoid to bacteriochlorophyll.

Highly efficient EET from carotenoid to chlorophyll *a* has been reported for the marine brown alga *Cladosiphon okamuranus* TOKIDA (Okinawa Mozuku) [10]. In this alga fucoxanthin (chemical structure is shown in Fig. 1) is bound to the light-harvesting fucoxanthin-chlorophyll *a*/*c* protein (FCP) and shows nearly 100% EET efficiency to chlorophyll *a*. The origin of this highly efficient EET is due to the involvement of an intra-molecular charge-transfer (ICT) state that is coupled to the S_1 state of fucoxanthin [11]. It has been suggested that the ICT character of fucoxanthin in FCP extends the absorption band to longer wavelengths and enhances its electronic interaction with chlorophyll *a* molecules [11], leading to efficient energy transfer from fucoxanthin to chlorophyll *a*. It was also suggested that the coupling of S_1 and ICT enhances the S_1/ICT − S_0 transition dipole moment of fucoxanthin so as to enable fucoxanthin to efficiently transfer excitation energy to chlorophyll *a* [11]. The involvement of an ICT state in carotenoid to chlorophyll EET suggests a strategy to enhance the efficiency of photosynthetic light-harvesting by utilizing carotenoids with such an ICT state.

In order to make the practical usage of artificial light-harvesting antenna systems feasible it should be remembered that the light-harvesting system should not only be efficient but also have a sufficient cross section. In this regard, carotenoids can be important components in the construction of bio-inspired artificial antenna systems. One of possible approaches when constructing an artificial photosynthesis mimic is to use synthetic chromophores that are related to the natural pigments but are more stable and are bound covalently. Dyads and triads systems containing carotenoids have proved to be a powerful tool towards achieving this objective [12-24]. Reconstitution of photosynthetic pigment protein complexes has been proved to be another powerful technique with which not only to study function of carotenoids but also towards constructing bio-mimic artificial light-harvesting antenna [25]. In his regard using LH1 complex is advantageous since LH1 complex can be fully reconstituted *in vitro* from its constituent parts, *i.e.*, LH1-α and LH1-β polypeptides and the pigments (bacteriochlorophyll *a* and carotenoid) [4, 5, 26-36].

The aim of the present study was to test the possibility for the incorporation of the algal carotenoid fucoxanthin into the LH1 system from purple photosynthetic bacteria. To achieve this, reconstitution experiments have been performed using LH1 complexes from the purple

photosynthetic bacterium *Rsp. rubrum* strain G9+ (a carotenoidless mutant) [5, 37]. Femtosecond time-resolved absorption spectroscopy has been applied to the reconstituted LH1 complex in order to investigate whether an ICT state participates in the carotenoid to bacteriochlorophyll singlet-singlet EET processes in this case.

Results and discussion

The LH1 complexes from *Rsp. rubrum* G9+ easily dissociate their ring structures to B820 heterodimer and B780 monomer subunits on increasing the detergent (β-OG) concentration as illustrated in Fig. 1. These processes are completely reversible. The subunit type complexes are reassembled to form LH1 ring type complexes when the β-OG concentration is decreased. The addition of carotenoid molecules to LH1 complexes is facilitated when they are added to the B820 heterodimer subunits prior to reassembly of the native LH1 complexes. We have used this method to incorporate fucoxanthin into the LH1 system from *Rsp. rubrum*. This method proved to be successful. It has long been believed that only carotenoids without end-ring structures can only be incorporated into the LH1 complexes from purple photosynthetic bacteria. This is because carotenoids that have end-ring structures are rarely found in purple photosynthetic bacteria (a notable exception is okenone in *Marichromatium purparatum* [38]), and are believed not to be able to fit into the biding site of carotenoids in the LH1 complexes due to the steric hindrance.

Fig. 2 shows absorption spectra of B820 complexes (without carotenoids) and the LH1 complexes in which fucoxanthin was incorporated. The Q_y absorption band of the B820 complexes (820 nm) shows a shift to 874 nm when the B820 complexes are reassembled into the LH1 complex. At the same time, the absorption bands that is due to fucoxanthin increases in the spectral region of 420 – 520 nm. The absorption bands of fucoxanthin in the LH1 complexes show a slight red-shift compared to those in methanol (see Fig. 2). This is a good indication that fucoxanthin is bound to the LH1 complexes and exhibits a red-shift in its absorption band because of the pigment-protein interaction. A similar observation is found for fucoxanthin bound to FCP from a marine alga and diatoms [11, 39-41]. It is also to be noted here that the absorption maximum of the reassembled LH1 complex without fucoxanthin showed its maximum at 866 nm. This means that the pigment-protein interaction of fucoxanthin also induces the red-shift of the Q_y absorption of the LH1 complex. This observation can be another support of the binding of fucoxanthin to the reconstituted LH1 complex. However, whether fucoxanthin is bound within LH1 in a similar position as a native spirilloxanthin molecule needs to be more carefully investigated.

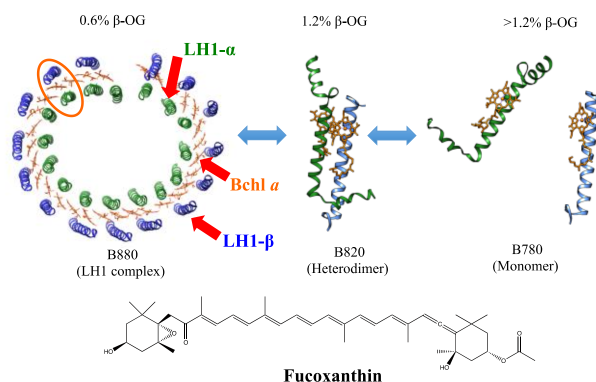

Fig. 1 Schematic illustration of dissociation/reassembly of the LH1 complexes from a purple photosynthetic bacterium *Rsp. rubrum* G9+ together with chemical structure of fucoxanthin, a carotenoid from a brown alga, to be incorporated into the reconstituted LH1 complex. The LH1 (B880) ring-type complex (upper left) is

composed of heterodimer (B820) subunit (upper middle) that is marked with an orange circle in B880 complex. The B820 heterodimer subunit is constituted with a pair of α- and β-polypeptides and 2 bacteriochlorophyll *a* molecules. This can be dissociated to B780 monomer subunit (upper right) by increasing the detergent (β-OB) concentration (*vise versa*).

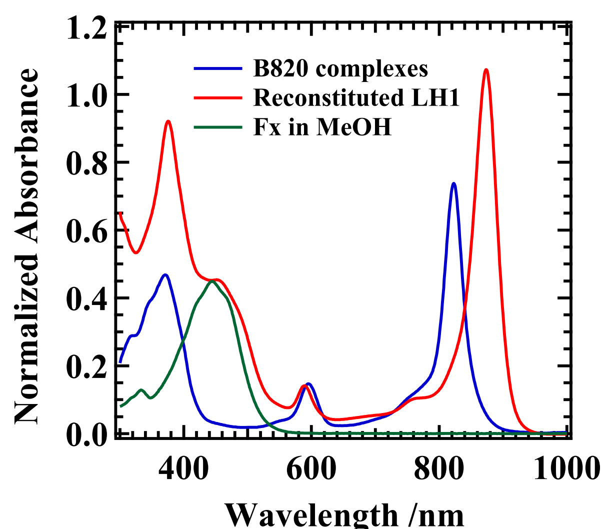

Fig. 2 Absorption spectra of B820 heterodimer complex (blue solid-line) and the reconstituted LH1 complex with fucoxanthin (red solid-line) in 50 mM phosphate buffer (pH 7.0) with 10 mM sodium ascorbate. The former solution contains 1.2% β-OG, while the latter 0.6%. Absorption spectrum of fucoxanthin in 50 mM phosphate buffer solution with 0.6% β-OG and 10 mM sodium ascorbate is also shown for comparison. All the spectra were recorded at 4 °C.

The quantum efficiency of the fluorescence from the S_2 state of carotenoids is as low as 10^{-5} [42, 43]. The EET from carotenoid to bacteriochlorophyll takes place from both the optically allowed S_2 and the forbidden S_1 states of carotenoid. The lifetimes of the S_2 and S_1 states of carotenoid are typically in the order of 100 fs and 10 ps, respectively. Of course these lifetimes depend on the length of conjugation of the polyene backbone of carotenoid. Therefore, EET from carotenoid to bacteriochlorophyll can only take place when the carotenoid is bound in close proximity to bacteriochlorophyll in the light-harvesting pigment protein complexes. In order to confirm that fucoxanthin is truly bound in close proximity to bacteriochlorophyll *a* fluorescence measurements were performed.

Fig. 3 shows the fluorescence emission and excitation spectra of the LH1 complexes with and without fucoxanthin. The fluorescence band around 895 nm is due to the emission from the Q_y band of bacteriochlorophyll *a* in the LH1 complexes. The intensity of the florescence emission exciting in the region where fucoxanthin absorbs is enhanced in the presence of fucoxanthin. The involvement of EET from fucoxanthin can be clearly seen in the fluorescence excitation spectra. These data clearly show EET from the reconstituted fucoxanthin to the bacteriochlorophyll and, therefore, fucoxanthin must be bound in close proximity to the bacteriochlorophylls within the reconstituted LH1 complexes. The efficiency of singlet EET from fucoxanthin to bacteriochlorophyll *a* was determined to be 28.1% based on the comparison of the area under the peak of fractional absorbance and the fluorescence excitation spectra in the spectral range of fucoxanthin (420 – 520 nm) as given in Fig. 4.

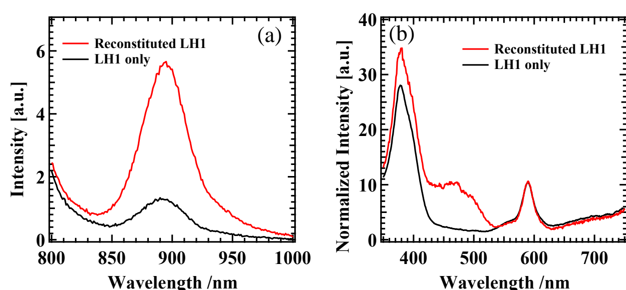

Fig. 3 (a) Fluorescence emission and (b) fluorescence excitation spectra recorded at 920 nm of emission for the reconstituted LH1 complex with fucoxanthin (red solid-line) and native LH1 complex (black solid-line) from *Rsp. rubrum* G9+ in 50 mM phosphate buffer (pH 7.0) containing 0.6% β-OG and 10 mM sodium ascorbate.

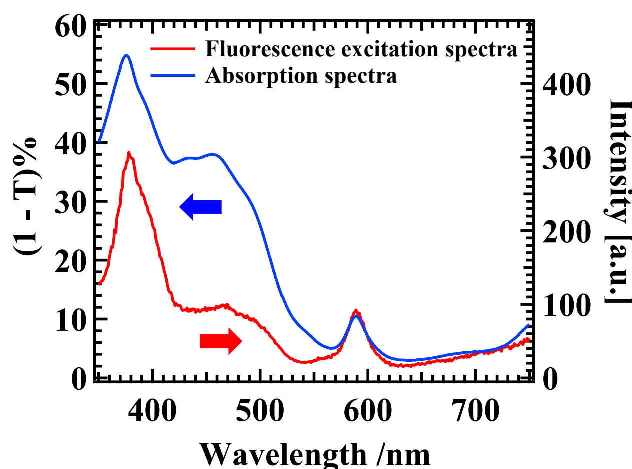

Fig. 4 Comparison of the absorption (fractional absorbance; 1 – T%, blue solid-line) and fluorescence excitation (recorded at 920 nm of emission, red solid-line) spectra of the reconstituted LH1 complex with fucoxanthin in 50 mM phosphate buffer (pH 7.0) containing 0.6% β-OG and 10 mM sodium ascorbate at 4 °C.

In order to investigate whether the ICT state is involved in the singlet EET processes from fucoxanthin to bacteriochlorophyll *a* femtosecond time-resolved absorption spectral measurements were performed. To compare the kinetics of fucoxanthin following photoexcitation with or without EET, femtosecond time-resolved absorption measurements were performed for both the buffer solution of fucoxanthin in β-OG micelle and that of the reconstituted LH1 complexes with fucoxanthin. Fig. 5 shows the set of time-resolved absorption spectra. Both fucoxanthin in detergent micelles and that incorporated into the LH1 complexes show a typical S_1/ICT $\rightarrow$ S_n transient absorption feature that has already been described for fucoxanthin in organic solvents [44-51]. This transient absorption can be ascribed to the vibrational hot S_1/ICT state and it appears immediately after excitation (see 57.6 and 307 fs spectra in Fig. 5(a) and 103 and 316 fs spectra in Fig. 5(b)). These transient absorption bands shift to the slightly blue-shifted transient absorption at 1.22 ps after excitation, reflecting vibrational relaxation processes in the S_1/ICT state. The transient absorption spectra observed in Fig. 5(a) and 5(b) show a typical "S_1-like" transient absorption feature peaking around 550 nm together with a structured transient absorption in the 580 – 700 nm spectral region that can be assigned to the ICT state of fucoxanthin. Nevertheless, the kinetic traces observed at 550 nm show quite interesting differences between these two samples (see Fig. 6). Fucoxanthin in detergent micelles shows a monotonic exponential decay in the time delay regime shown in Fig. 6. On the contrary, fucoxanthin incorporated to the LH1 complexes apparently has both a fast and a slow decay component. In order

to get much deeper insight on this issue we have performed global and target analyses on the whole data set of the time-resolved absorption spectra using a Glotaran program [52, 53]. In order to discuss the S_2 state kinetics special care must be made to record the data set in the very early stages after photoexcitation. Since we have mainly focused on the relaxation processes of the S_1/ICT state in this study, we will discuss this important issue separately in a future study.

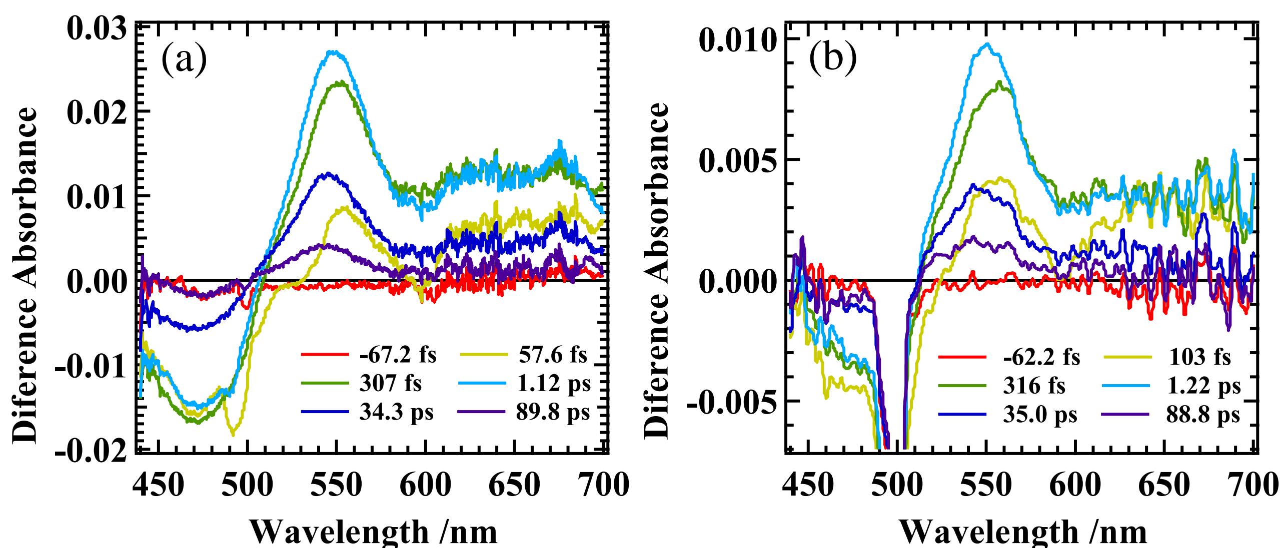

Fig. 5 Femtosecond time-resolved absorption spectra of (a) fucoxanthin-β-OG micelle and (b) reconstituted LH1 complex with fucoxanthin in 50 mM phosphate buffer (pH 7.0) containing 0.6% β-OG and 10 mM sodium ascorbate at room temperature following excitation up to the S_2 state of fucoxanthin with 500 nm laser pulse.

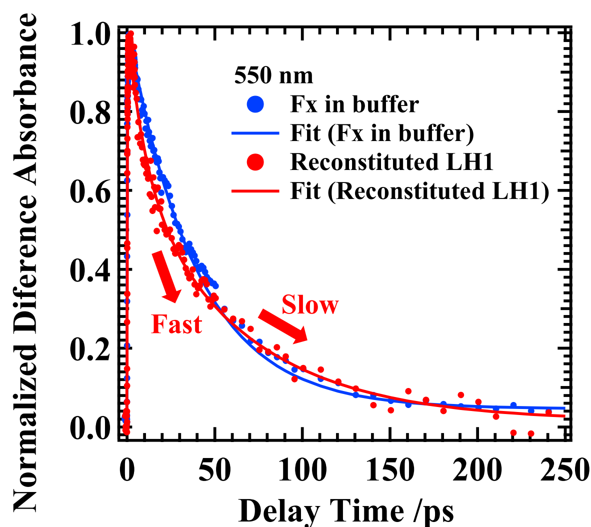

Fig. 6 Kinetic traces of the femtosecond transient absorption of fucoxanthin recorded at room temperature in β-OG micelle (blue closed-circle) and that incorporated to the reconstituted LH1 complex (red solid-circle) recorded at 550 nm. The results of the fitting for kinetics traces (blue and red solid-lines) based on the results of global and target analyses are also shown for the purpose of guide of eyes.

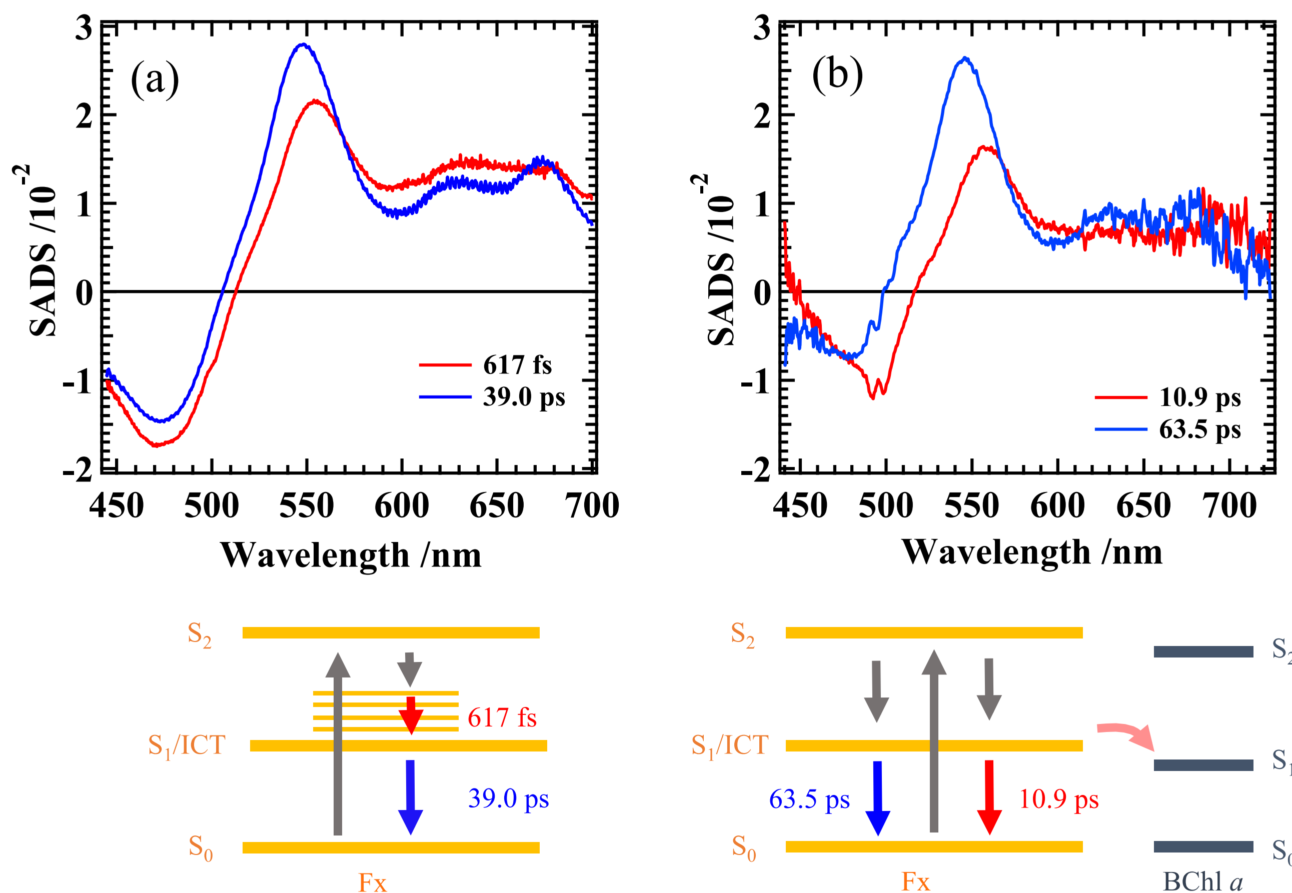

Fig. 7 The results of global and target analyses performed against the whole data set of femtosecond time-resolved absorption spectra of (a) fucoxanthin in β-OG micelle and (b) that incorporated to the reconstituted LH1 complex. The sequential (left) and parallel (right) models to analyze these data sets are illustrated at the bottom of this figure.

The results of the global and target analyses are displayed in Fig. 7. The data from the fucoxanthin-detergent micelles were successfully analyzed based on a sequential model. The species associated difference absorption spectra (SADS) show two components with lifetimes of 617 ± 20 fs and 39.0 ± 0.04 ps. This result is consistent with that of previous studies of fucoxanthin in organic solvents [46-51]. Therefore, the former component can be assigned to vibrational relaxation in the S_1/ICT state of fucoxanthin and the latter component can be assigned to the internal conversion of the S_1/ICT state of fucoxanthin. Indeed the spectral band shape of the 617 fs component is similar but slightly red-shifted to that of the 39.0 ps component reflecting the vibrationally hot nature of the initial S_1/ICT state. It should be noted here that both the 617 fs and 39.0 ps show the structured absorption in the spectral region of 580 – 700 nm together with strong absorption bound around 550 nm. This is a typical characteristic of the S_1/ICT state of fucoxanthin. It is interesting to note that 39.0 ps lifetime is just in-between the reported S_1/ICT lifetime of fucoxanthin in methanol (23.7 ± 0.2 ps) and that in cyclohexane (62.0 ± 1.0 ps) [46].

In contrast the results with the reconstituted LH1 complexes with fucoxanthin could only successfully be analyzed assuming a parallel model. The SADS of the reconstituted LH1 also show two components, the spectral band-shape of which are quite similar to that of the S_1/ICT state, but the lifetimes of these are very different from those of fucoxanthin in detergent micelles. The lifetime of the fast component

is 10.9 ± 0.15 ps, while that of the slow component is 63.5 ± 0.85 ps, as illustrated in Fig. 6 and 7. The 10.9 ps lifetime is too long as to be assigned to vibrational relaxation in the S_1/ICT state. Therefore, it is suggested that there may be two pools of fucoxanthin that are incorporated into the reconstituted LH1 complexes. One is bound in close proximity to bacteriochlorophyll *a* so that it can transfer excitation energy to bacteriochlorophyll. As a consequence the lifetime of the S_1/ICT state of this pool becomes short. The other pool is not correctly bound close to bacteriochlorophyll *a* so that it cannot transfer excitation energy to the bacteriochlorophyll *a*. This second pool of fucoxanthin may just be located in the detergent micelle. However, the lifetime of the slow component (63.5 ps) is much longer than that of the fucoxanthin in detergent micelles (39.0 ps). As far as we know, 63.5 ps is the longest lifetime of the S_1/ICT state of fucoxanthin in polar environment. So at this time it is not clear exactly where this second pool of fucoxanthin is bound.

The efficiency of EET (Φ_{EET}) can be calculated according to the following formula,

$$\Phi_{EET} = \frac{k_{EET}}{k_{IC} + k_{EET}} \times 100\ (\%) \qquad (1).$$

If we suppose that 63.5 ps component corresponds to the internal conversion process of fucoxanthin incorporated into the LH1 complex (k_{IC} = 1/(63.5 ps)), then the rate of EET can be calculated with the lifetime of 10.9 ps component; $k_{EET} + k_{IC}$ = 1/(10.9 ps)). Based on this idea Φ_{EET} can be determined to be 83.0%. If we adopt the 39.0 ps component as the internal conversion process of fucoxanthin in the LH1 complexes, the Φ_{EET} can be calculated to be 72.0%. This analysis suggests that the EET efficiency for fucoxanthin to bacteriochlorophyll *a* in LH1 may be higher than that initially determined from the fluorescence excitation spectra because of the presence of a significant pool of unconnected fucoxanthin. This pool would contribute to the absorbance spectrum but not to the fluorescence emission spectrum, thereby, apparently lowering the efficiency of EET. The time-resolved data allow the contribution of the inactive fucoxanthin pool to be removed giving a better estimate of the efficiency of fucoxanthin to bacteriochlorophyll *a* EET. However, in order to arrive at a final conclusion we need to clarify the exact origin of the 63.5 ps component and this work is continuing.

Experimental

Cell Growth and Preparation of Chromatophores

Cells of *Rsp. rubrum* strain G9+ (a carotenoidless mutant) were photosynthetically grown under anaerobic conditions with C-succinate medium modified from Cohen-Bazire *et al*. at 29 °C [54]. The cells were harvested by centrifugation (18 800g × 10 min at 4 °C), resuspended in 20 mM MES buffer (pH 7.0), and stored in freezer (–30 °C) until use. Chromatophores of *Rsp. rubrum* G9+ were prepared as described previously [55].

Isolation and Purification of B820 Heterodimers from the Native LH1 Complex from *Rsp. rubrum* G9+

The native LH1 complex was isolated from the freshly prepared chromatophores of *Rsp. rubrum* G9+ according to the reported protocols [56, 57]. The B820 heterodimers were isolated from the native LH1 complex as described previously [32, 58, 59] following solubilization with 1.2% octyl-β-glucopyranoside (β-OG) in 50 mM phosphate buffer (pH 7.0) with the presence of 10 mM sodium ascorbate. The B820 heterodimers were further purified by means of sucrose density gradient (0.6 – 0.8 M) ultracentrifugation (165 000g × 16 hrs. at 4°C) and stored in the freezer (–30 °C) until required.

Isolation and Purification of Fucoxanthin

Fucoxanthin was extracted and purified from the brown alga *Cladosiphon okamuranus TOKIDA* (Okinawa-Mozuku) as reported previously [46]. The purity of the fucoxanthin was confirmed to be >95% by ^{1}H-NMR spectroscopy. For the spectroscopic experiments a solution

of fucoxanthin in acetone (OD_{443} = 1 – 2) was dropped, under a vigorous stream of N_2 gas, onto the 50 mM phosphate buffer (pH 7.0) solution containing 1.2% β-OG. Fucoxanthin was thus solubilized in the buffer solution in detergent micelles.

Reconstitution of fucoxanthin into the LH1 complex

The reconstitution of carotenoid into the LH1 complex was performed using the reported protocol [32] with a slight modification. The acetone solution of fucoxanthin (OD_{443} = 2) was very slowly dropped, under vigorous flow of N_2 gas, into a 50 mM phosphate buffer (pH 7.0) solution of B820 (OD_{820} = 0.4 – 0.6) containing 1.2% β-OG at 0 °C. Fucoxanthin was added until its absorbance band became three times as large as the Q_x absorption band of bacteriochlorophyll *a*. The solution was then diluted with the same volume of 50 mM phosphate buffer (pH 7.0) with 10 mM sodium ascorbate so that the final concentration of β-OG becomes 0.6%. This last procedure (dilution of the detergent concentration) induces the reassembly of the B820 heterodimers to form the LH1 ring structure [57]. During this process fucoxanthin is incorporated into the LH1 complex.

Steady-state spectroscopic measurements

The absorption spectra of the sample solutions before and after the fs time-resolved absorption measurements were recorded on a JASCO V-670 UV-vis spectrophotometer at 4 °C using the same 2 mm optical path-length cell that was used for the time-resolved absorption measurements. Fluorescence emission and excitation spectra of the native and reconstituted LH1 complexes were recorded with a JASCO FP-6600 spectrofluorometer using a 1 cm optical path-length quartz cuvette at 4 °C [5]. For these spectral measurements the absorbance of the LH1 complexes at the peak of the Q_y transition of bacteriochlorophyll *a* was adjusted to OD_{880} = 0.1. The fluorescence-excitation spectra were recorded at the emission from B880 band detected at 920 nm. The singlet-singlet EET efficiency from carotenoid to bacteriochlorophyll *a* in the LH1 complexes was determined by comparing the fluorescence excitation spectra with the fractional absorption (1 – T%) spectra normalizing at the peak of the Q_x transition of bacteriochlorophyll *a*.

Femtosecond time-resolved absorption measurements

Details of fs pump-probe spectroscopy is described elsewhere [46, 60]. The excitation pulse at 500 nm used to excite the S_0 – S_2 absorption band of fucoxanthin was generated by an optical parametric amplifier (Spectra Physics, OPA-800CF). The excitation intensity was set to 34 - 68 nJ/pulse and the beam was focused on to the sample with a diameter of 200 μm. The instrumental response function of the system, determined by cross-correlation between excitation and probe pulses, was about 130 fs. The cross-correlation function was used to determine the precise zero time delay at each probe wavelength. The uncertainty in the zero time delay was less than 20 fs after chirp compensation.

The sample was placed in a static quartz cuvette that has an optical path-length of 2 mm. The optical density of the sample was set to be 0.5 at the excitation wavelength 500 nm. The sample solution was continuously stirred with a small magnetic stirring bar during the measurements. The integrity of the sample was checked by comparison of the steady-state absorption spectra of the sample before and after the measurements. All the time-resolved measurements were performed at room temperature.

Conclusions

In this study we were successful, for the first time, to incorporate fucoxanthin, an algal carotenoid, into the LH1 system from the purple photosynthetic bacterium *Rsp. rubrum* G9+, a carotenoidless mutant strain. The presence of EET from fucoxanthin to bacteriochlorophyll *a* was confirmed by the measurement of fluorescence excitation spectra. The overall efficiency of singlet EET from fucoxanthin to bacteriochlorophyll *a* was determined to be 28.1 % from the fluorescence excitation data. The detailed kinetics of the singlet EET processes from fucoxanthin to bacteriochlorophyll *a* were investigated using femtosecond time-resolved absorption spectroscopy. The results suggest that there are two types of fucoxanthin that are

bound to the LH1 complexes. One shows the EET to bacteriochlorophyll *a* and the other does now show EET. The efficiency of EET from the former type of fucoxanthin to bacteriochlorophyll *a* was estimated to be as high as 83%. This highly efficient EET is able to be achieved by the utilization of the S_1/ICT character of fucoxanthin. The utilization of the ICT character of carotenoids is a possible strategy to enhance the singlet EET efficiency from carotenoid to bacteriochlorophyll. This idea might be applicable in the construction of highly efficient light-harvesting antennas necessary for artificial photosynthesis.

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