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Effect of the mutation of carotenoids on the dynamics of energy transfer in light-harvesting complexes (LH2) from Rhodobacter sphaeroides 601 at room temperature*

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Energy transfers in two kinds of peripheral light-harvesting complexes (LH2) of Rhodobacter sphaeroides (RS) 601 are studied by using femtosecond pump–probe spectroscopy with tunable laser wavelength at room temperature. These two complexes are native LH2 (RS601) and green carotenoid mutated LH2 (GM309). The obtained results demonstrate that, compared with spheroidenes with ten conjugated double bonds in native RS601, carotenoid in GM309 containing neurosporenes with nine conjugated double bonds can lead to a reduction in energy transfer rate in the B800-to-B850 band and the disturbance in the energy relaxation processes within the excitonic B850 band.

Keywords: light harvesting complex (LH2), carotenoid mutation, energy transfer, femtosecond pump-probe
PACC: 0660J, 3580B, 8725B, 4280W

1. Introduction

The peripheral light-harvesting complexes (LH2), which serve as pigment antennas to efficiently absorb sunlight and transfer excitation energy to reaction centres, are part of the photosynthetic unit in many species of purple bacteria. The LH2 in Rhodobacter Sphaeroides (RS) is an ideal system to study the physical mechanism of energy transfer, in that its pigment arrangement has been recently demonstrated to be similar to that of Rhodopseudomonas Acidophila.[1] Highly resolved crystal structure (2.0Å) of LH2 revealed that such a complex had a ninefold symmetry.[2] Each repeating unit consisted of single B800 bacteria chlorophyll (BChl α), a pair of B850 BChl α molecules, two carotenoids and two transmembrane alpha helices, α and β.[1,3]

Carotenoid always plays diverse roles in light-harvesting complex, including light harvesting, photoprotection, and structure stabilization.[4] It has become a hot topic in photosynthesis research to understand the function of carotenoid. Meanwhile, understanding such a mechanism depends on the information from a variety of detecting techniques, such as Fourier transform Raman spectroscopy,[5] femtosecond (fs) pump–probe and femtosecond transient absorption.[6−11]

For a number of light harvesting systems, experimental investigations have shown that carotenoid is able to transfer energy directly from both S_1 and S_2 states to the states of BChl-B800 and BChl-B850. When the Q_y band of BChl was excited, the band shift of the carotenoid was observed in several light harvesting complexes.[6,10] And based on the quantum chemical calculations, it was interpreted as a Stark shift due to the change of the local electric field near carotenoid molecules upon photoexcitation of BChl molecules.[6] For the B800-to-B850 energy transfer, it was found that the excitation energy in B800 pigments could be efficiently transferred to B850 in a

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time of about 0.7ps at room temperature and 1.2 ps at 77 K.[12−14] But the calculations that were based on the conventional Förster theory cannot perfectly explain the experimentally measured transfer time.[15,16] It was suggested that some processes in the calculations were underestimated. For example, the additional electronic coupling mediated by the carotenoid caused the B800-B850 couplings to increase by approximately 20%–30% via the mixing of the BChl and carotenoid transition moments.[15]

In the present paper, by using one- or two-colour fs pump–probe technique at tuned wavelengths, we investigate the effect of the mutation of the carotenoids on the ultrafast dynamical processes of energy transfer in LH2. In this experiment, a comparison between the native LH2 (RS601) and carotenoid mutated GM309-LH2 exhibits remarkably different B800-to-B850 energy transfer rates and energy relaxation processes within the excitonic B850 band.

2. Materials and methods

Rhodobacter sphaeroides 601 (RS601) is a wild strain for laboratory use. GM309, a special green mutant strain (GM309) was acquired by ethylmethane sulfonate (EMS) induced mutagenesis from RS601. HPLC-Mass analysis of carotenoids showed that the neurosporene was accumulated in LH2 of GM309.[17,18] In our experiments, the mutation frequency of pigment mutants in surviving cells is 1.6×10^{-3} Hz. In organisms with such a low mutation frequency, it is unlikely to find double mutants of both apoproteins and carotenoid biosynthesis.

Femtosecond one-colour pump–probe measurements were carried out at room temperature by using a Spectra Physics Ti: sapphire fs laser. The output laser pulses from the oscillation stage had a duration of 120 fs and repetition frequency of 82 MHz. When the wavelength of the laser beam was tuned from 770 nm to 855 nm, the average power output from the laser system was kept at 500 mW in order to work at the same power density at different wavelengths, the excitation pump photon density per pulse on the sample was less than 6 $\times10^{13}$photon/cm^2. Two-colour pump–probe measurements were taken by using the output from an amplifier that had a 1-kHz repetition frequency, 140-fs duration and 800-nm centre wavelength. The laser output was split by a beam splitter into two beams: one was used as the pump beam and the other was utilized to generate the supercontinuum from which the probe beam was selected by a monochromator. The description of the detailed experimental setup can be found in Ref.[19].

3. Results and discussions

The absorption spectra of two kinds of LH2 samples are shown in Fig.1. In the near-infrared region, all spectra are dominated by some characteristic peaks due to the Q_y bands of BChl, while the positions of B800 and B850 remain at 800 nm and 848 nm for the two LH2 samples. The calculated spectroscopic properties of the BChl-B850 ring in LH2 indicate that the absorption transition in BChl-B850 spanned over the range from 780 to 880 nm.[20] According to the excitonic model, the major absorption near 850 nm is due to two degenerate delocalized exciton levels ($k = \pm1$), while the transition from the ground state to the lowest exciton level (k=0) is formally forbidden. Because of the energetic and structural disorder effect, the originally degenerated exciton levels may be distorted, which leads to the possible transition to all these exciton levels.[21] Moreover, in the 400–550 nm region, due to the vibrational bands of carotenoid, a typical three-peak absorption feature from RS601 dominates the absorption spectrum. A significant blue shift of about 20 nm was observed at the three absorption peaks of carotenoid from GM309, because there existed a higher energy level of neurosporene with nine conjugated double bonds than that of spheroidene with ten conjugated double bonds.[22,23]

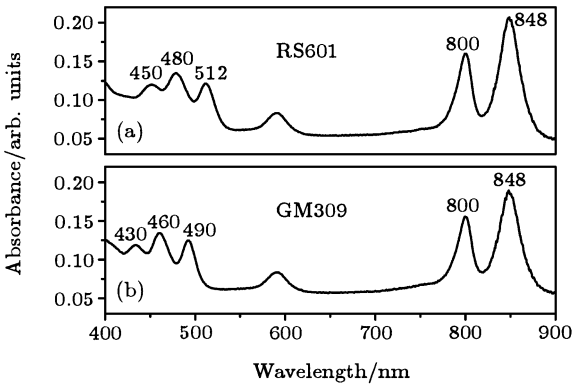


Fig.1. Room-temperature absorption spectra of LH2 from Rhodobacter sphaeroides: (a) wild-type RS601 containing spheroidene; (b) green carotenoid mutated GM309 containing neurosporene.

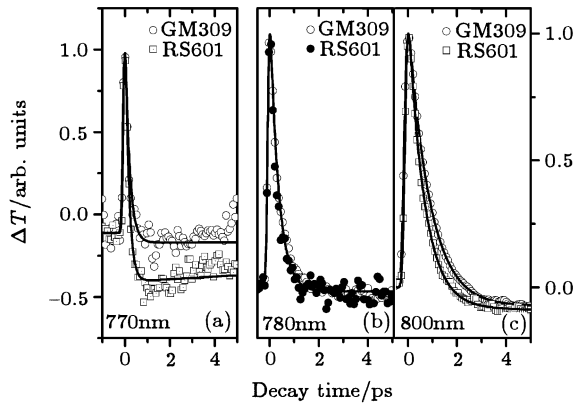


Fig.2. The normalized one-colour fs pump–probe dynamic features of RS601 and GM309 at (a) 770 nm, (b) 780 nm and (c) 800nm. The solid lines in (c) represent the fitting results.

The one-colour fs pump–probe results of RS601 and GM309 at 770 nm, 780 nm and 800 nm are given in Figs.2(a), (b) and (c), respectively. Under the excitation of 800 nm (see Fig.2(c)), the dynamics exhibits an ultrafast decay of the photobleaching (PB) and an additional photoabsorption (PA) attributed to the excited state absorption of B850 band.[24] The decay time of this PB signal, which mainly reflects the energy transfer rate from B800 to B850, increases dramatically in GM309 (870 fs) in comparison with that in RS601 (650 fs). As the excited wavelength is tuned to the blue side of B800 absorption band, both of the LH2 complexes also exhibit a dominant transient PB signal at 780 nm (see Fig.2(b)). The ultrafast decay of the PB process with a time constant of 0.35 ps could also be interpreted as the B800-to-B850 energy transfer.[25] The dynamical processes of the two LH2 complexes show a similar evolution because a higher excitonic level of the B850 is mainly located at about 780 nm, and the strong coupling between B800 and B850 always plays a dominant role in dynamics at this wavelength and speeds up the energy transfer process.[25,26] When the wavelength is tuned to 770 nm (see Fig.2(a)), the contribution of the coupling between B800 and B850 obviously decreases, and the difference in dynamics between these two LH2 complexes recurs (170fs for RS601 and 230fs for GM309). When the wavelength is tuned to the red side of the B800 absorption band, the decay processes in the kinetics of two LH2 complexes, under the excitation of 810 nm, also show a clear difference between their time constants: about 620 fs for GM309 and 560 fs for RS601 (see Fig.3). It has been postulated that the discrepancy between the measured and calculated energy transfer rates from B800 to B850 may partly come from the mediated coupling via the carotenoid.[14,16] It was reported recently that the singlet-singlet energy transfer rate was increased by through-band exchange interaction.[27–29] In particular, Kilså highlighted an expected increase in electronic energy transfer rate for the decreased energy splitting between the singlet excited states of both the donor and the bridge.[27] In our experiment, we find that the energy transfer rate between B800 and B850 may decrease as the neurosporene in GM309 induces a higher band gap between the donor of B800 and the bridge of carotenoid than that in RS601. We also find that the intensity of fluorescence emission from B850 of GM309 is 40 percent of the intensity of RS601 upon photoexcitation of B800 pigments, which reflects that the energy transfer from B800 to B850 is partly blocked by the mutation of carotenoid and the nonradiative relaxation process may play a crucial role in the mutated LH2 complexes.

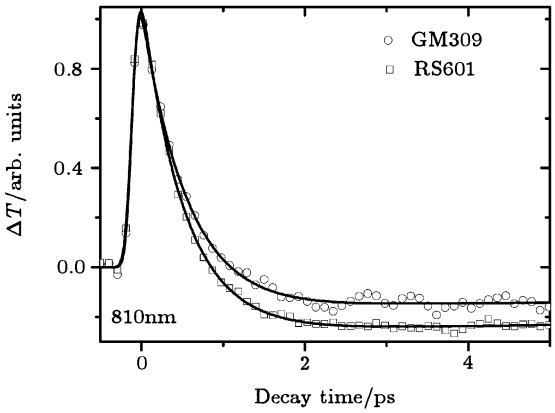


Fig.3. The normalized room-temperature one-colour fs pump–probe dynamic features of the RS601 and GM309 at 810 nm. The solid lines represent the fitting results.

When the laser wavelength is tuned from 820 nm to 860 nm, B850 bands are mainly excited.[30] The experimental results clearly demonstrate the dynamical process of the excited B850 pigments with distinct excitation wavelength dependence (see Fig.4). The dominant PB or PA signal depends on the absorption cross-sections of both the ground state and the excited state. Upon excitation of 825-nm pulses (see Fig.4(a)), the RS601 and GM309 display ultrafast PA signals with a little difference existing in between, which is mainly dominated by the fast energy equilibrium within B850 pigments and the coherence effect between the pump and probe pulses. When the excitation wavelength is tuned from the blue to the red side of B850 band, a distinct difference in dynamic features

between RS601 and GM309 is observed. The dynamic features measured at 845 nm and 855 nm are shown in Figs.4(b) and (c), respectively. Fitting results of 845 show 0.17 ps (A_1=47%), 1.7 ps (A_2=35%) and $>$ 100 ps (A_3=18%) for RS601 and 0.18 ps (A_1=45%), 1.7 ps (A_2=44%) and $>$ 100 ps (A_3=11%) for GM309. For the excitation of 855 nm, the fittings of the decay processes give rise to three time-components with 0.2 ps (A_1=47%), 1.8 ps (A_2=22%) and $>$ 100 ps (A_3=31%) for RS601 as well as 0.2 ps (A_1=40%), 2 ps (A_2=46%) and $>$ 100 ps (A_3=14%) for GM309, respectively. The last time constant that corresponds to the long lifetime of the lowest exciton state of B850 ($\tau \sim$1 ns[31]) cannot be accurately fitted because of the relatively short time window. The time-resolved fluorescences of these two samples are measured, and the fluorescence lifetime of B850 in GM309 (1026 $\pm$ 50 ps) is very similar to that of native RS601 (1060$\pm$50 ps) (the data are not shown here). Comparing the dynamic features of these two LH2 complexes, three decay components show nearly the same temporal evolution with different amplitudes.

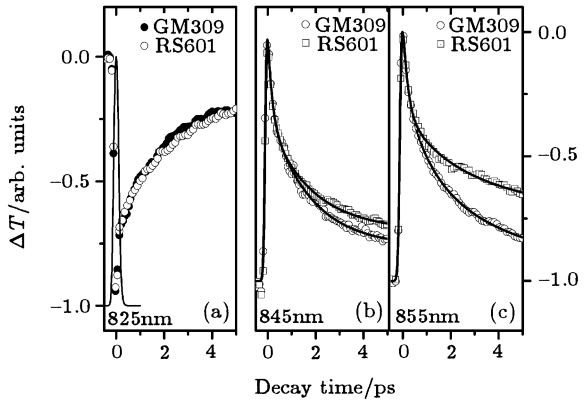


Fig.4. The normalized room-temperature one-colour fs pump–probe dynamic features of the RS601 and GM309 within the B850 absorption band: (a) 825 nm and the cross-correlation trace between the excitation pulse and the probe pulse. (b) 845 nm and (c) 855 nm. The solid lines in (c) represent the fitting results.

The power dependence of the fs pump–probe dynamic feature at 850 nm is shown in Fig.5. In order to investigate whether the excitation annihilation affects the measured dynamic feature, the excitation intensity is attenuated from 100% to 1% using neutral density filters. The lowest excitation intensity (0.4 mW) traces have a negligible amount of annihilation contribution, and in the early stage they contain only a very small fast evolution component. However, dynamic features in these two complexes still show remarkably different relaxation processes (see Fig.5(b)). In general, it is suggested that although the nature of the carotenoid bound to the LH2 complex in Rhodobacter sphaeroides changes as spheroidene is replaced by neurosporene, there appears no significant influence on the Q_y electronic transition of the bound BChl molecules as seen from the absorption spectra in Fig.1. But the influence may significantly be exerted on the structure of the B850 binding site,[5,18] which may also bring on the variation in the spectral relaxation within the excitonic B850 band. Considering the multi-relaxation processes within the excitonic state of B850, it is possible that the excitation energy in B850 band of GM309 will be redistributed in these relaxation processes, but a detailed understanding of the dynamic features within B850 band of these LH2 complexes still needs further experimental investigation.

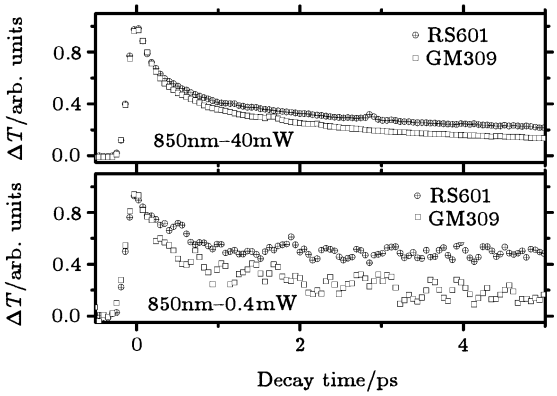


Fig.5. The power dependence of the pump–probe dynamic features of RS601 at 850 nm, where the average pump power is reduced from (a) 40 mW to (b) 0.4 mW.

Two-colour fs pump–probe measurements are also carried out with a probe beam at 860 nm under the excitation of 800 nm (shown in Fig.6). Since the high excitation intensity may lead to singlet–singlet annihilation within LH2 complexes (see the solid square curve in Figs.6(a) and (b)), great care is required to excite the sample by using the excitation intensity as low as possible. The excitation of B800 pigments results in the transient dynamics from B850 with a fast rise of photobleaching and a slow decay process. The fast rise component (see Fig.6(c)) mainly reflects the energy transfer from B800-to-B850 and the cross-correlation between the pump and the probe beams. A slow rising time constant in GM309 also reflects the decreased energy transfer rate of B800-to-B850 in comparison with that in RS601. By using the rate equations for the LH2 system, we find that the first

half of rise edge is mainly determined by the cross-correlation between the pump beam and the probe beam, while the remaining part reflects the energy transfer process of B800-to-B850. The fitting results of the energy transfer rate for the two samples are in good agreement with those obtained in one-colour pump–probe measurement at 800 nm. In addition, the slow decay processes in these two samples, representing the energy relaxation in B850 band,[32,33] also show dramatic variation. The decay trace of RS601 exhibits a single long-lived decay process as shown in Fig.6(a) (the open square curve), while for GM309 (see the open square curve in Fig.6(b)), a fast decay kinetic feature exists in its early decay stage except for the long-lived process. The different decay kinetic features in these two samples also imply the variations of energy relaxation process within B850 band with the structure changing at the B850 binding site.

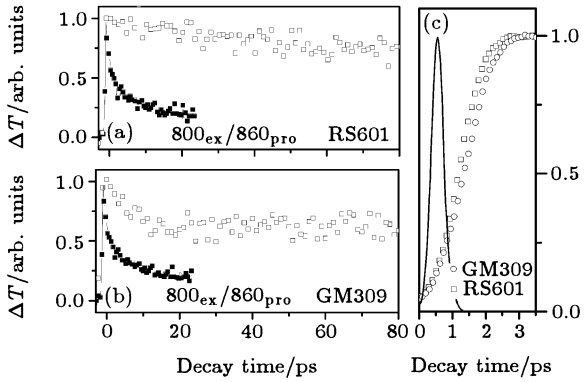


Fig.6. The normalized room-temperature two-colour fs pump–probe dynamic features of RS601 and GM309 at a probe wavelength of 860 nm with the excitation of B800 at 800 nm: (a) RS601 and (b) GM309. Symbols represent the results obtained at high (solid square) and low (open square) excitation intensities. (c) The details of the rise edges of two samples and the cross-correlation trace between the excitation pulse and the probe pulse.

4. Conclusions

By using the femtosecond pump–probe technique, we have investigated the ultrafast dynamic behaviours of two kinds of Rhodobacter sphaeroides LH2, native RS601 and the green carotenoid mutated GM309 containing the neurosporene. The mutation of carotenoid has a great effect on the optical properties of the photosensitive pigments and their fs dynamics. In the B800 absorption band, the energy transfer rate between B800 and B850 in GM309 is generally reduced in that the neurosporene with nine conjugated double bonds results in a higher band gap between the donor of B800 and the bridge of carotenoid than in RS601. Since a higher excitonic level of the B850 is mainly located at about 780 nm, an exceptive phenomenon that the strong coupling between the higher-lying excitonic levels of B850 and excited state of B800 leads to an increase in B800-to-B850 energy transfer rate of these two samples with the same time constant is also observed at the excitation of 780 nm. When excitation wavelength is tuned from 820 nm to 855nm, the dynamic feature is mainly attributed to the excited B850 pigments, and two LH2 complexes show an obvious differences in their decay dynamics. It is likely that the structure of the B850 binding site changes significantly as a result of containing different carotenoids, and this influence may noticeably modify the relaxation process in the excitonic B850 band. At last, in the two-colour pump–probe measurements, GM309 also shows a reduction in B800-to-850 energy transfer rate and a different relaxation process compared with those in RS601, which further confirms the observed significant effects of the mutation of carotenoids on the ultrafast dynamics of the LH2 complexes.

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