Femtosecond Dynamics of Energy Transfer in Native B800-B850 and B800-Released LH2 Complexes of Rhodobacter Sphaeroides *

LIU Wei-Min(刘伟民)[1,2], ZHU Rong-Yi(朱荣毅)[1,2], XIA Chen-An(夏辰安)[1], LIU Yuan(刘源)[3], XU Chun-He(徐春和)[3], QIAN Shi-Xiong(钱士雄)[1**]

[1]*Department of Physics, Fudan University, Shanghai 200433*
[2]*National Key Laboratory of Surface Science, Fudan University, Shanghai 200433*
[3]*Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

(Received 29 August 2003)

Two kinds of antenna complexes LH2 of Rhodobacter sphaeroides, wild type RS601 and the removal of B800 pigments (B800-released), were used in our experiment. These two LH2 complexes show quite different behaviour in absorption and femtosecond dynamics. By using the femtosecond pump–probe technique, the energy transfer processes occurring in two complexes were studied. Because of removing the B800 pigment from the LH2 in B800-released LH2 complex, the energy transfer between the B800 to B850 pigment was completely eliminated, while the pure internal energy transfer within the exciton states of B850 pigment could be carefully investigated. The results show that, at B800 absorption band, B800-released LH2 obviously shows a dominated transient absorption different from the photobleaching observed in RS601; while at the B850 band, these two complexes show similar photobleaching behaviour.

PACS: 34. 30. +h, 42. 65. Re, 78. 47. +p

The primary process in the photosynthesis starts from the absorption of the light by the peripheral antenna complexes and creates excited states that then proceed to the core complexes containing reaction centre. The Rhodobacter sphaeroides LH2 complex acts as pigment antennas to absorb sunlight and transfer the excitation energy to the reaction centres efficiently. It has a nonametric ring structure with ninefold symmetry, each monomeric unit contains an α-apoprotein, a β-apoprotein and carotenoid (Rhodopin glucoside) molecules.[1,2] The complex has its longest wavelength electronic transitions in the near-infrared region with two absorption bands located around 800 and 850 nm, respectively. The 18 BChl molecules are sandwiched between the α- and β-apoprotein helices and form a continuous overlapping ring. These molecules absorb mainly at about 850 nm and are called the BChl-B850 molecules. Another nine BChl molecules are located more peripherally in the complex and are situated between the β-apoproteins. These molecules absorb light at about 800 nm and are correspondingly denoted as the BChl-B800 molecules. In addition, the carotenoids span the membrane, making van der Waals contact with both the BChl-B800 and B850 molecules.

The research on the ultrafast energy transfer dynamic processes in intra/inter B800 pigments, B800 to B850 and in the intra/inter B850 has been carried out by different techniques, which include femtosecond pump–probe,[3−5] femtosecond transient absorption (TA),[6,7] fluorescence up conversion,[8] and transient grating technique.[9] The study of the photodynamic process in the bacterial antenna complex at different wavelengths for excitation of B800 and/or B850 band has resulted in a good understanding on the energy transfer happening in this complex. It is reported that the light energy absorbed by B800 pigments can be rapidly transferred to the B850 ring on a time scale of 0.7 ps at room temperature. There are many theoretical works intended to account for these dynamics,[10,11] but perfect agreement has not yet been obtained. From many experimental studies and theoretical calculations, it was suggested that the B800-B850 interband transfer and the B850 intraband transfer could be strongly affected by high lying exciton levels of the B850 band. The electronic coupling among these states may accelerate the transfer process between B800 and B850 pigments. However, no experimental result on the ultrafast energy transfer process from the high lying exciton levels has been reported.

Recently, it was reported that B800 molecules within the LH2 protein for Rhodobacter sphaeroides and Rps acidophila can be selectively released, which provides useful samples for studying the energy transfer process in the antenna system. In fact, the upper exciton components in the exciton state of the B850 pigment, which were supposed to be extended

* Supported by the National Natural Science Foundation of China under Grant Nos 69977008 and 10274013, and the State Key Basic Research and Development Programme of China under Grant No 1998010100.

** To whom correspondence should be addressed.

©2003 Chinese Physical Society and IOP Publishing Ltd

to around 750 nm,[12] are in the vicinity of the absorption band of B800 pigments. The absorption of B850 to these high lying exciton levels is hidden under the B800 Q_y absorption and it even has about 20% contribution to the absorption around 800 nm. By using the B800-released samples, we are in a position to study the excitation and related energy transfer processes of upper exciton levels of B850 near the B800 absorption peak.

LH2 of Rhodobacter sphaeroides is an ideal system to study the physical mechanism of energy transfer. It has been demonstrated recently that its pigment arrangement is similar to that of Rps acidophila.[13] In order to study the mechanism of energy transfer within LH2, two kinds of Rhodobacter sphaeroides LH2 were used in the measurement. One was the B800-released LH2 obtained by releasing B800-BChl from LH2 of RS601. The other was wild type of Rhodobacter sphaeroides 601 (RS601). A dynamical evolution of high lying exciton levels in B850 rings around 790 nm was first measured in this study. The research results offer a new insight into the investigation of the energy transfer mechanism in the LH2.

For preparation of the B800-released LH2, LH2 of RS601 was changed from suspension in TL buffer to LM buffer (10 mmol/L Tris-HCl, 0.9% n-dodecyl-β-maltosid, pH8.0) on a DEAE-cellulose column. Then LM buffer containing ascorbic acid (5 mmol/L) was acidified with acetic acid to pH4.8. B800 was released after 90 min incubation at 25°C. Conditions for the release of the B800 molecules were optimized as in Ref. [12].

Femtosecond (fs) pump–probe experiments were carried out by using a Spectra Physics Ti:sapphire laser at room temperature. The output from the oscillation stage of the laser was used as the laser source for pump and probe beams, with pulse duration of about 120 fs, repetition rate of 82 MHz and average power of about 500 mW. The wavelength of the laser beam could be tuned from 760 nm to 850 nm by moving the slit in the laser system, while the group velocity dispersion was also adjusted to keep the better mode-locked condition of the laser oscillation. The pump–probe set-up was similar to that described in Ref. [5].

Figure 1 shows the absorption spectra of Rhodobacter sphaeroides for RS601 (curve a) and B800-released LH2 (curve b) at room temperature. For native RS601, there are the carotenoid absorption peaks at 451, 479 and 512 nm, one Q_x band absorption peak at 590 nm, and the typical B800 and B850 absorption bands of RS601 located at 800 and 848 nm, respectively. For the B800-released complex, the absorption peak of the B850 band is red-shifted about 1.5 nm relative to that for native RS601. Absorption spectrum of B800-released LH2 also shows that the absorption band of B800 pigments completely disappears due to the fact that B800 were removed. In comparison with the absorption peaks of carotenoids in RS601, those of B800-released LH2 were blue shifted 6 nm, which might be caused by the loss of the interaction between carotenoids and B800.[14] The absorption spectra of two samples obviously confirm the fact that there is a large difference in the complex structure between them and the feature of B850 in B800-released is maintained as in RS601.

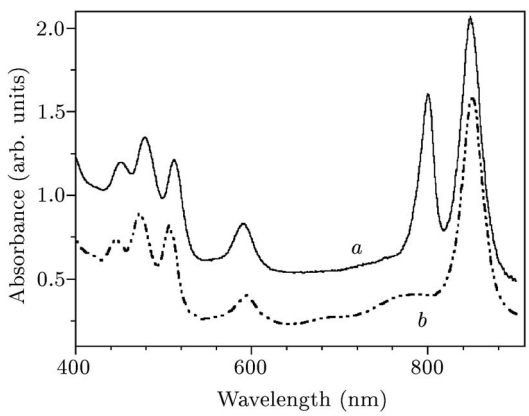


Fig. 1. Absorption spectra of LH2 of Rhodobacter sphaeroides: (a) RS601 and (b) B800-released at room temperature. The spectrum b is magnified and the spectra are vertically shifted for clarity.

By using the tunable fs laser beams from 795 nm to 850 nm, very interesting results were observed. At B800 absorption band, the fs dynamics for two LH2 complexes show quite different behaviour in this region, which would be helpful to understand the real photosensitive primary processes of these two different complexes under light absorption.

When the excitation wavelength was tuned at 795 nm, which is near the peak absorption peak of B800 pigments, for RS601 (Fig. 2(a)) the transient behaviour is dominated by photobleaching (PB) caused by the saturated absorption (SA) in the B800 band, which was followed by a transient absorption tail about 2 ps later caused by the B850 pigments. The ultrafast decay time of PB signal, which results from the energy transfer from B800 to B850 and the internal conversion in B800 band, is about 550 fs, while the measurement taken around 795 nm for B800-released, as shown in Fig. 2(b), obviously shows that there is a dominated transient absorption coming from B850 pigments instead of the dominated PB signal in RS601. As the concentration of B800-released LH2 is about six times lower than that of RS601 and the absorption of B850 pigments is weaker than that of B800 pigments at 795 nm, its fs pump–probe signal is much weaker than that of RS601. As seen from Fig. 1, when

all B800 pigments were removed from the antenna complexes LH2, no strong absorption around 800 nm exists, while the remaining absorption is from the tail of B850 absorption band, which is weak in this wavelength region. The nonlinear absorption measurement for the B800-released complex reveals that the nonlinear absorption at 795 nm is related to a transition from B850 rings to an upper level in the manifold. Our measurement also confirms that the B850 pigments can be excited to the high lying one-exciton levels with a 800 nm beam.

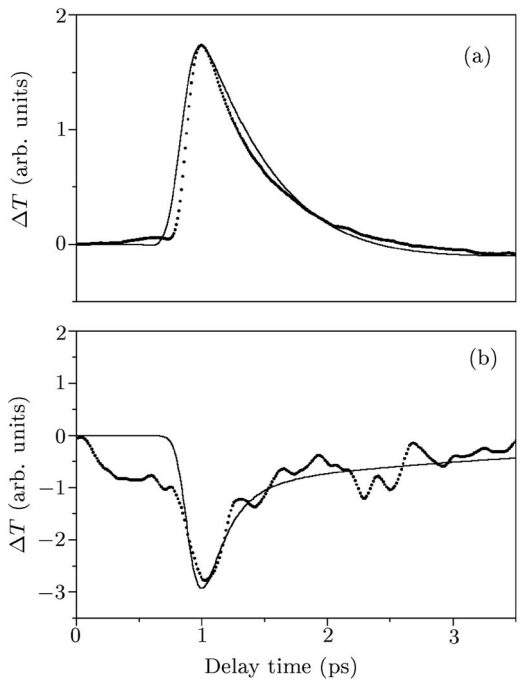


Fig. 2. Femotsecond pump–probe dynamics of Rhodobacter sphaeroides: (*a*) RS601 and (*b*) B800-released samples at 795 nm. The dots represent the experimental data and the solid lines are the fitting results with the eight-level model.

In order to analyse the data, rate equations based on eight energy levels model were utilized to describe the energy transfer in the LH2 complex (Fig. 3). In this energy level scheme, four levels on the left (a_1–a_4) are related to B800, while the other four levels (b_1–b_4) on the right represent the excitation and energy relaxation in B850. The initial values of the parameters used in the calculation were taken from our measurement and other papers, and were finally obtained from the fitting procedure. The laser pulse duration at sample position was determined to be 120 fs from the autocorrelation measurement and the power density at the sample was of 100 MW/cm^2. When the excitation wavelength is near the B800 band, for RS601, the B800 pigment was effectively excited. As the absorption cross section σ_{a1} of the excited state level is less than σ_a of the ground state, the photodynamic is a distinct photobleaching. However, for B800-released, only B850 pigments are excited. This leads to an excited state absorption in contrast to the strong bleaching observed from the RS601 LH2 complex due to the fact that the absorption cross sections σ_{b11} and σ_{b12} of one-exciton level are larger than σ_b of the ground state. The solid line in Fig. 2 is the simulated result using this model. Some parameters at 795 nm obtained from the fitting are: absorption cross section σ_a of 3.8×10^{-15} cm^2, σ_b of 7×10^{-16} cm^2, σ_{b11} of 7×10^{-16} cm^2, σ_{b12} of 1.2×10^{-15} cm^2, decay rates k_{b11} of 2.1×10^{13} s^{-1}, k_{b2} of 2.5×10^{13} s^{-1} and k_{b1} of 3.1×10^{11} s^{-1}. Some data obtained from the fitting are in agreement with those obtained from the absorption and published values, but the values of σ_{b11} and k_{b2} are obtained from our work.

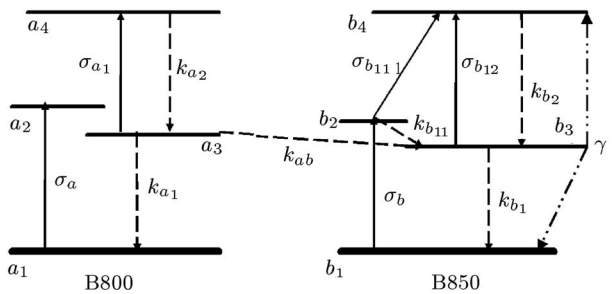


Fig. 3. Eight levels for RS601. The left four levels belong to the B800 pigments (a_1, a_2, a_3, a_4) and the right part is related to B850 (b_1, b_2, b_3, b_4).

The fs pump–probe measurement with the wavelength tuned between 800 nm and 850 nm was also carried out. From 800 nm to about 815 nm, the B800-released complex shows a similar evolution of transient absorption as that at 795 nm, but the TA signal decreases with the increase of the wavelength of the fs pulse. When the wavelength was tuned to the further red side of the B800 band, these two complexes have similar photodynamic behaviour mainly coming from the B850 pigments. Figure 4 shows the dynamics processes of two complexes excited at 850 nm, where the transient response is from B850 pigments. B800-released LH2 shows a distinct photobleaching similar to RS601, which has two components in the decay of the photobleaching. By using the deconvolution treatment, the decay time of the ultrafast component was determined to be 375 fs for RS601 and 290 fs for the B800-released complex, respectively. Based on the energy level model and the simulation procedure, this ultrafast photobleaching signal was attributed to the population in the two-exciton state (level b_4) of the B850,[15] whose absorption cross section is much less than that from the exciton level, σ_{b12}. Due to the large absorption cross section of the ground state in

B850 and the intense pump laser beam, the exciton in B850 can be effectively excited to the two-exciton state, leading to this ultrafast PB signal. The fast rise of PB signal reflects the rapidly increased population in the two-exciton state and the decay of this ultrafast PB signal reflects the fast depopulation from two-exciton state to the one-exciton states. The slow decay component comes from the stimulated emission from the exciton state by the probe beam due to the large fluorescence emission cross section of B850 in this wavelength region. The decay time of this slow component gives an indication of the decay of the population in the exciton state of B850.

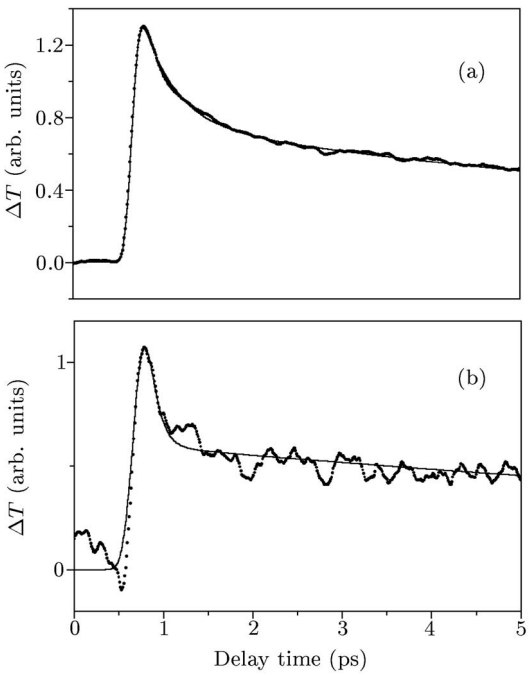


Fig. 4. Femotsecond pump–probe dynamics of LH2 of Rhodobacter sphaeroides at 850 nm: (*a*) RS601 and (*b*) B800-released. The dots represent the experimental data and the solid lines are the fitting results.

In conclusion, the optical absorption properties and the femtosecond dynamics of the B800-released LH2 sample were measured at room temperature in comparison with that for native RS601. The mutant B800-released LH2 shows a distinct difference in the absorption spectrum and the fs dynamics at the wavelength near the B800 band from the RS601. This result shows that the high lying exciton states in B850 can be excited by 800 nm pulse and has a large upward absorption probability, leading to a distinct photoabsorption dynamics. However, near the peak of the B850 absorption band, two LH2 samples show the similar photodynamic behaviour with two photobleaching decay components, which are caused by the two-exciton state excitation and the efficient stimulated emission from the exciton state in B850, respectively. From the simulation based on the rate equations and the calculation of the differential transmittance, these measured dynamics were found in good agreement with the simulated results.

References

[1] Freer A et al 1996 *Structure* **4** 449
[2] Walz T et al 1998 *J. Mol. Biol.* **282** 833
[3] Polivka T et al 2000 *J. Phys. Chem.* B **104** 1088
[4] Kumble R et al 2001 *J. Photochem. Photobiol.* A **142** 121
[5] Guo L J et al 2001 *Chin. Phys. Lett.* **18** 522
[6] Nagarajan V et al 1999 *J. Phys. Chem.* B **103** 2297
[7] Ihalainen J A et al 2001 *J. Phys. Chem.* B *105* 9849
[8] Jimenez R 1996 *J. Phys. Chem.* **100** 6825
[9] Salverda J M et al 2000 *J. Phys. Chem.* B *104* 11395
[10] Pullerits T, Hess S, Herek J L and Sundstrom V 1997 *J. Phys. Chem.* B **101** 10560
[11] Scholes G D, Harcourt R D and Fleming G R 1997 *J. Phys. Chem.* B **101** 7302
[12] Wu H M et al 1997 *Mol. Cryst. Liq. Cryst.* **291** 163
[13] Thomas W et al 1998 *J. Mol. Biol.* **282** 833
[14] Gall A et al 1999 *FEBS Lett.* **449** 269
[15] Stiel H et al 1997 *Chem. Phys. Lett.* **276** 62