The observation of excited-state dynamical evolution in light-harvesting complex LH2 from *Rhodobacter sphaeroides* 601

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Abstract With selective excitation around BChl–B800 and BChl–B850 absorption bands, we observed the evolution of excited-state dynamics in LH2 from *Rhodobacter sphaeroides* 601. The dynamical traces demonstrate a dominant excited-state absorption (ESA) followed concomitantly by an ultrafast transmission increase and decay with pulse-width limited time scale at 818 nm and 828 nm excitation. The ESA occurring prior to excitonic thermalization or ground-state bleach was observed at 840 nm as well. These experimental results indicate the competition between the transition from excitonic states to higher-lying excited states and interexciton relaxation, which are of physical significance for understanding excitation transfer and related mechanisms in LH2. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Light-harvesting complex; Excited-state dynamics; Photosynthetic system

1. Introduction

Photosynthetic organisms contain pigment–protein antenna complexes that absorb light and transfer energy efficiently and rapidly to the photochemical reaction center. Photosynthetic purple bacteria are frequently used for studying excitation transfer and related mechanisms in photosynthesis due to their relative simplicity. They contain two types of light-harvesting antennae, the core antenna LH1 surrounding the reaction center and the peripheral antenna LH2. The structure of the peripheral light-harvesting antenna complex LH2 of *Rhodopseudomonas acidophila* revealed a densely packed bacteriochlorophyll–protein system. Between two concentric cylinders of protein subunits in LH2, there are two parallel rings of bacteriochlorophyll molecules, which have two absorption bands around 800 nm and 850 nm. For the LH2 complex from *Rhodobacter sphaeroides*, the pigment arrangement is very similar to that of *R. acidophila*, with two rings of pigments in the C_9 symmetrical arrangement [1]. The ultrafast excitation transfer pathways and mechanism in light-harvesting complex LH2 have been the subjects of experimental and theoretical research workers (see [2] and references therein). Excitation hopping transfer and excitonic relaxation are two limiting cases of the general description for excitation dynamics. Intermolecular excitation transfer in the photosynthetic system among the weakly coupled BChl–B800 molecules, and from BChl–B800 to BChl–B850 molecules, is generally described by the incoherent hopping mechanism [3]. On the other hand, the exciton concept has been applied to the strongly coupled BChl–B850 ring in light-harvesting complexes LH2, in which the excitation is delocalized over a number of pigment molecules and the dynamics take place through relaxation between different excitonic states in the BChl–B850 ring. However, the extent of delocalization, the energetic level picture of excitonic states and the energy relaxation channels among the excitonic levels of BChl–B850s are still a matter of debate [4–8].

Time-resolved techniques have been extensively applied to follow the dynamics of optical excitations within LH2 complex in order to investigate the delocalization process of excitation energy. In our present work, we present the results of time-resolved dynamics of excited BChl–B850 with selective excitation on the blue side of its absorption band by using the conventional pump-probe technique. These observations of BChl–B850 excited-state dynamics might have physical significance for better insight into the excitonic picture and provide useful information for the investigation of energy transfer channels in LH2 of *R. sphaeroides* 601.

2. Materials and methods

LH2 isolated from *R. sphaeroides* 601 was dissolved in 50 mM Tris–HCl buffer (pH 8) and the optical density was set to 0.5 at 850 nm in a 1 mm cuvette. The one-color femtosecond pump-probe experiments were performed on a home-built system [9]. A Ti:sapphire laser (Tsunami, Spectral Physics), pumped by a diode laser, served as the source of ultrashort pulses in our experiment. The output pulses were of 80–100 fs duration with a repetition rate of 82 MHz. The wavelength of the output pulses can be tuned from 750 to 870 nm with 10 nm bandwidth (fwhm) by changing the position of the mode-locked slit across the beam profile. The pump power used in our measurement was less than 0.2 nJ/pulse with a beam diameter of about 50 μm in the beam overlap region. This corresponded to a maximum photon flux of 5×10^8 photons per pulse, and approximately 10% of the BChl–B850 molecules were excited. In the polarized pump-probe measurement, the anisotropy is obtained by monitoring the probe transmission as a function of temporal delay and polarization relative to the pump pulses. All measurements in this work were carried out at room temperature.

3. Results and discussion

Fig. 1 shows the absorption spectrum in the near-IR region of LH2 from *R. sphaeroides* 601, the two bands at 800 nm and

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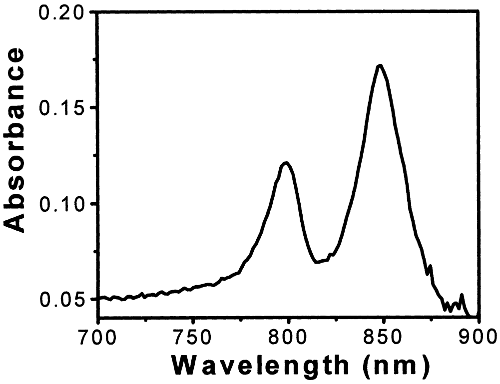

Fig. 1. The near-IR absorption spectrum of LH2 isolated from *R. sphaeroides* 601.

850 nm of LH2 pigment–protein complexes originate from interactions between bacteriochlorophyll molecules, and of the pigments with surrounding protein. Assuming a similar structure of LH2 from *R. sphaeroides* with that of *R. acidophila* [1], the band around 800 nm originates mainly from nine BChl–B800 molecules and secondly from the excitonic states of BChl–B850 molecules. Theoretical calculated spectroscopic properties of the tightly packed, highly symmetric molecule arrangement of the BChl–B850 ring in LH2 indicate that the absorption transitions in BChl–B850 can span over the region from 780 to 880 nm [10]. According to the excitonic model, the major absorption near 850 nm results from two degenerate delocalized exciton levels ($k = \pm 1$), while transition from the ground state to the lowest exciton level ($k = 0$) is formally forbidden. Due to the energetic and structural disorder effect, the originally degenerate exciton levels may be distorted and the transition energy is then distributed to other originally not allowed transitions [11]. However, the detailed nature of this 850 nm absorption band is still under debate. Recent experimental results showed that there is a further small band (located at about 800 nm) in the blue side of the 850 nm band of BChl–B800 depleted complex, which implies that in native LH2 this BChl–B850 side band is masked by the main BChl–B800 transition [5].

For the energy transfer from BChl–B800 to BChl–B850, several experiments have shown that this process takes about 0.7 ps at room temperature. In our measurement with 800, 810 and 815 nm excitation, the time-resolved dynamics are presented in Fig. 2. The transient dynamics at 800 nm excitation demonstrated a decay component with a 0.7 ps time constant, plus a small excited-state absorption (ESA) due to the energy transfer from BChl–B800 to BChl–B850, which was also observed in previous studies. We also measured the dynamical behaviors of excited BChl–B800 molecules under different pump power ranging from 1×10^8 to 2×10^9 photons per pulse (not shown), and found that the normalized traces at 800 nm excitation were independent on the pump power. With the excitation pulses shifting to the red side of the BChl–B800 absorption band, an increasing magnitude of the ESA relative to the bleaching was observed. This evolution mainly arises from the variation of absorption cross section for BChl–B800 and BChl–B850 at the different spectral regions. The broad overlapping region with the BChl–B850 band results in stronger excitation coupling and more efficient energy transfer, or other mechanisms referring to excitation energy relaxation. It is also obvious from Fig. 2 that the bleaching prior to the ESA was getting faster as tuning the excitation wavelength to the red side of the BChl–B800 band, reaching a pulse-width limited decay at 815 nm excitation. Together with the evolution of ESA near 800 nm and the excitonic model, the above-mentioned results implicate a strong overlap between the lower excited state of BChl–B800 and the higher excitonic state of BChl–B850, which results in the ultrafast and efficient excitation transfer from BChl–B800 to BChl–B850 molecules.

In the next section, we will limit the focus to the evolution of the ESA with a different pump-probe configuration and the excitation wavelength. It is expected to get more information about the excited states of BChl–B850. Fig. 3 shows the transient dynamics trace for LH2 of *R. sphaeroides* 601 with the 818 nm pump-probe pairs. A distinct difference can be seen from that in Fig. 2. A rapid absorption and an ultrafast transmission increase can be clearly observed at near zero time point. This trace could be decomposed into two processes, a

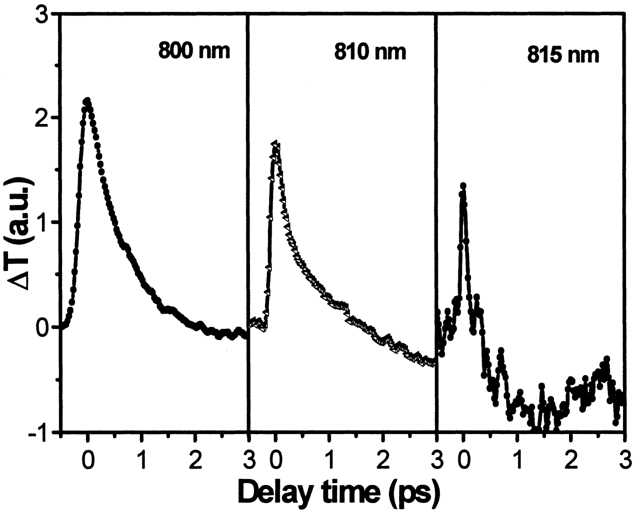

Fig. 2. Transient dynamic traces of LH2 up the excitation at 800 nm (left), 810 nm (middle) and 815 nm (right) with parallel pump-probe polarization.

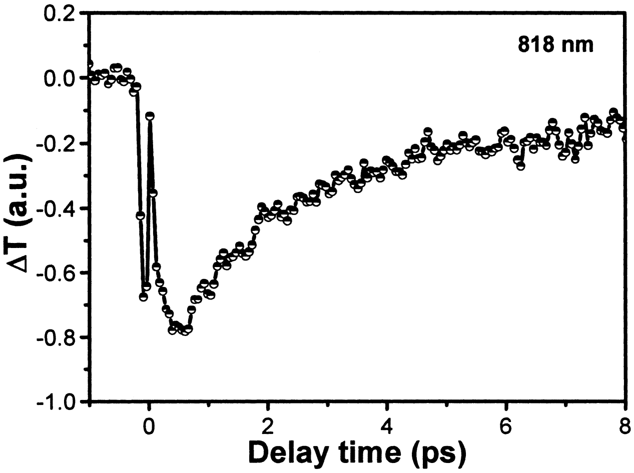

Fig. 3. Time-resolved dynamics of excited BChl–B850 at 818 nm excitation with parallel polarization.

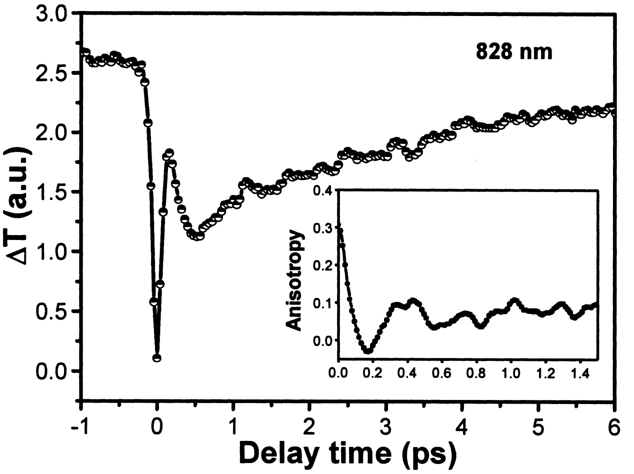

Fig. 4. Transient dynamics of excited BChl–B850 at 828 nm excitation with magic angle (54.7°) polarization. The inset is the evolution of anisotropy.

decay of absorption with about 3 ps time constant plus a 'bleaching' signal with pulse-width limited time scale. The negative signal could be simply assigned to ESA of BChl–B850 molecules, which decays with a several picoseconds time constant. To our knowledge, the new ultrafast 'bleaching' shown in Fig. 3 has not been reported up to now. Fig. 4 shows the time-resolved transmission change of excited BChl–B850 with 828 nm excitation at magic angle polarization, which demonstrates a similarity to that at 818 nm excitation. It seems that the appearance of the new bleaching locates at a little longer delay time with respect to the largest ESA. We also performed the measurements at parallel and perpendicular polarization, and calculated the evolution of anisotropy value shown as inset in Fig. 4. The initial anisotropy value is 0.3 and decays to a small negative value in about 100 fs and then rises to a stable value of 0.1 in about 200 fs. Further tuning the laser pulses to longer wavelength, the transient dynamics of excited BChl–B850 are shown in Fig. 5 for 840 nm and 848 nm. It can be observed that the ESA gradually disappears and a strong bleaching dominates the transient trace. Additionally, no apparent pump-intensity dependence was observed from the dynamical traces under different pump power at 848 nm excitation in our experiment (data not shown), thus the excitation annihilation process can be neglected.

From our above observation, it is clearly demonstrated that the dynamical evolution of excited BChl–B850 molecules differs with excitation wavelength. Generally, the change of transmission results primarily from a combination of ground-state bleaching, ESA and stimulated emission. Their contribution to the signal depends on their relative cross sections at the particular probe wavelength and the populations of different states involved. On the basis of the excitonic level picture mentioned above, the negative signal could be assigned to the excitonic state absorption of BChl–B850. The excitation energy was first absorbed by the allowed transition resulting in the formation of excited BChl–B850 molecules, and then absorbed another photon (reverse saturation absorption) for the next transition to higher-lying excited state. If this is the case, the result indicates that the first excited state involved has a larger cross section of absorption than that of ground state. A similar result has been reported in a previous study with non-linear absorption spectroscopy [12]. On the other hand, since the ESA was observed in a wider spectral region from 790 to ∼840 nm, both the first and second excited states of BChl–B850 should be inhomogeneously broadened, which makes the higher electronic transitions allowed. Meanwhile, the excited excitonic state of BChl–B850 involved should have a longer lifetime of about several picoseconds as shown in Figs. 3 and 4 at 818 nm and 828 nm, respectively. In this case, the ultrafast bleaching component should arise from another state, whose related ground state also has absorption ranging at least from 815 nm to 840 nm. This kinetic component with about 100 fs lifetime is unlikely to arise from stimulated emission, which is expected at longer wavelengths with respect to the excitation, and as a slower process with a picoseconds or longer time scale. There may be the possibility that this new bleaching originates from the decrease of induced ESA, due to excitation relaxation to the next lowest excitonic state ($k = \pm 1$), which is expected in the femtoseconds time domain. Obviously, the above assumption needs to be further supported.

An alternative interpretation for our results refers to the energy transfer sequence in the functional photosynthetic unit. It has been realized previously that with the formation of excited BChl–B850, either by direct excitation or by energy transfer from BChl–B800, the excitation energy will transfer to the next energy acceptor. The energy transfer pathway in the photosynthetic unit should be that the excitation energy is first localized in the next lowest exciton state ($k = \pm 1$) and then redistributed to the lowest state in LH2, then transferred to LH1 and finally captured by the reaction center. So the excitation energy probably experiences an equilibration among excitonic levels in the BChl–B850 ring before the next transfer to LH1. The absorption might then correspond to the absorption of the next lowest excited excitonic states, which is populated by interexciton relaxation. With the fast population of the next lowest excitonic state, the localized exciton in this state would thermalize rapidly, and as a result the ESA of BChl–B850 were to be weakened. The anisotropy evolution with about 100 fs time scale as shown in the inset of Fig. 4 might also correspond to this process, which is consistent to the result at 850 nm excitation in [8]. After the estab-

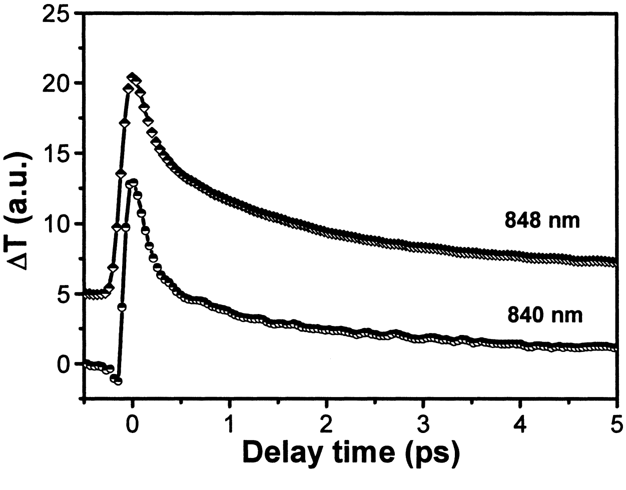

Fig. 5. Time-resolved change of transmission at 840 nm and 848 nm excitation with parallel pump-probe pairs.

lishment of a new equilibrium of excitation energy, the absorption decayed with several picoseconds.

With the excitation wavelength shifting to the region of the BChl–B850 absorption band, the ground-state bleaching/excitonic thermalization dominate in the first hundreds of femtosecond. From the transient dynamics at 840 nm excitation shown in Fig. 5, the initial absorption signal could still be observed, indicating the ESA is prior to ground-state bleaching or the local heating of exciton. At 848 nm excitation, this prominent process of ground-state bleaching/exciton thermalization conceals the ESA and demonstrates a triexponential decay.

In this letter, we reported the results of wavelength-dependent transient dynamics of the excited BChl–B800 and BChl–B850 molecules in LH2 of *R. sphaeroides* 601 at different excitation wavelengths. It is clearly demonstrated that the evolution of excited-state dynamics of BChl–B800 and BChl–B850 is strongly dependent on the excitation wavelength, indicating the coexistence and competition of ESA and ground-state bleaching/exciton thermalization or stimulated emission in the first hundreds of femtosecond. At the excitation wavelengths ranging from 818 nm to 840 nm in our experiment, the initial excited-state population probably experiences a very rapid equilibration by interexciton relaxation, most likely to the next lowest excitonic states. The experimental data also show that the ESA precedes the thermalization of local excitons or ground-state bleaching in the blue region of the BChl–B850 band. These new observations are of physical significance for the insight into the excitonic energy level scheme and for the understanding of the mechanism of excitation transfer in photosynthetic antenna complexes.

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