Energy Transfer in Isolated LHC Ⅱ Studied by Femtosecond Pump-Probe Technique *

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(Received 16 October 2002)

Excitation energy transfer in the isolated light-harvesting chlorophyll (Chl)-a/b protein complex of photosystem Ⅱ (LHC Ⅱ) was studied by the one-colour pump–probe technique with femtosecond time resolution. After exciting Chl-b by 638 nm beam, the dynamic behaviour shows that the ultrafast energy transfer from Chl-b at positions of B2, B3, and B5 to the corresponding Chl-a molecules in monomeric subunit of LHC Ⅱ is in the time scale of 230 fs. While with the excitation of Chl-a at 678 nm, the energy transfer between excitons of Chl-a molecules has the lifetime of about 370 fs, and two other slow decay components are due to the energy transfer between different Chl-a molecules in a monomeric subunit of LHC Ⅱ or in different subunits, or due to change of molecular conformation.

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

Light-harvesting complexes of photosystem Ⅱ (LHC Ⅱ), the most abundant and important pigment-protein complexes, in green plants can absorb light, which is the first step in photosynthesis process followed by electronic separation and charge transfer in a reaction centre. From the research results of the crystal structure,[1] a model of the monomeric subunit of LHC Ⅱ was obtained to reveal that each monomeric subunit of LHC Ⅱ comprises three transmembrane α-helices (hA, hB and hC) and a short amphiphilic helix (hD), 7–8 Chl-a, 5–6 Chl-b, and several xanthophyll (Xan) molecules.[2−7] In the complex, the role of xanthophylls is assumed to quench Chl triplets to prevent the formation of singlet oxygen, so that the seven chlorophylls closest to Xans are supposed to be Chl-a molecules, which have the necessity of triplet quenching.[4,6] Five other chlorophylls, i.e., Chl-b, are adjacent to Chl-a molecules, having the centre-to-centre distance of 0.8–1 nm between the tetrapyrrole rings of these two kinds of Chl molecules.[4,6,7]

Many results have shown that in isolated LHC Ⅱ, there are three ways of excitonic interactions between Chl molecules including Chl-b–a, b–b and a–a interactions.[2−7] The dynamic of excitation energy transfer between Chl-b and Chl-a are the most intriguing and have been comprehensively studied. The pump–probe technique and transient absorption measurement revealed that this process of the energy transfer is in the time scale of picosecond and subpicosecond. In 1989, Eads first reported that the transfer from Chl-b to Chl-a occurs with the lifetime of subpicosecond.[8] In 1992, component with lifetime of 5 ps was first reported by Kwa *et al.*[9] Later in 1994, Palsson reported a transfer time constant of 500 fs from Chl-b to Chl-a.[10] The transition absorption results also showed that excitation energy transfer took about subpicosecond from Chl-b to Chl-a,[11] which is much shorter than that from Chl-b to Chl-b.[7] By the calculation of the transfer rate from Chl-a to Chl-a in LHC Ⅱ, it was shown that most Chls transfer relatively rapidly, with exception of single site lifetimes of 3.5 and 2.5 ps of positions A3 and A7.[7] The excitonic mixing between the excited states of the molecules can be expressed as: $W_{DA} = C_{DA}K^2/(n^4R^6)$.[5,7] Although many appealing results have been obtained, the details of the energy transfer between Chl molecules are still not clearly elucidated and there are several different opinions about the model of the energy transfer in LHC Ⅱ complex.

In this Letter, we report the dynamical results of isolated LHC Ⅱ by using femtosecond (fs) pump–probe technique. By selective excitation of Chl-a or Chl-b with the beams at different wavelengths, a series of dynamic are obtained.

LHC Ⅱ complexes have been isolated according to the method described by Hong and Xu.[12] The preparation of LHC Ⅱ is characterized by room temperature absorption spectrum, which is shown in Fig. 1. The absorption peaks in the red side of the spectrum are at 650 and 676 nm, which are due to the absorption of Chl-b and Chl-a, respectively.

* Supported by the National Natural Science Foundation of China under Grant Nos 69977008 and 10274013, and the National Basic Research under Grant No G199810100.

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Pump-probe measurements were performed using an fs laser system. Tsunami Ti:sapphire laser generated pulses of 82 MHz repetition rate, 800 nm wavelength and 120 fs pulse duration. Spitfire (amplifier), which was pumped by a YLF laser, had the output with 1 kHz repetition rate and was used as the pump source of an optical parametric amplifier (OPA) to produce tunable infrared laser pulses at wavelength from 1.1 to 1.4 μm. By using a YCOB crystal, second harmonic beam at visible light was generated to be applied as the source in our measurements, which could be tuned from 550–700 nm. A polarizer was used to eliminate the infrared light because the infrared beam and visible SHG beam have perpendicular polarization.

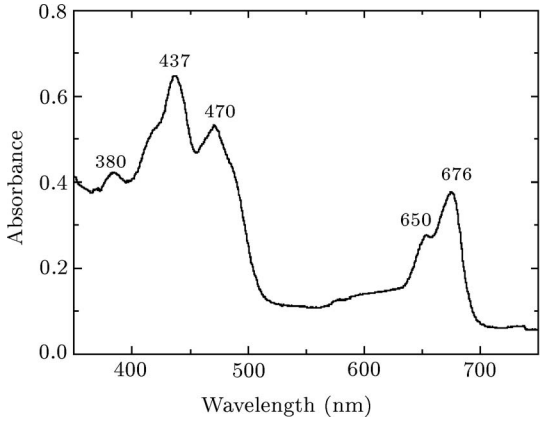


Fig. 1. Absorption spectrum of isolated LHC Ⅱ.

In our experiments, the visible beam was split into pump and probe beams. The pump beam was chopped at 500 Hz. The time delay between the probe and the pump beams was adjusted by using an optical delay line controlled by a stepping motor. A lens focused the pump and probe beams on the solution of LHC Ⅱ, which was contained in a 1-mm cell. Finally, a detector measured the intensity of the probe beam and a lockin amplifier collected the signals, which were managed by a computer. More details of our experimental set-up can be available in Refs. [13,14].

Figure 1 shows the absorption spectrum of LHC Ⅱ. The peaks at 437 nm and 676 nm are due to the absorption of Soret state and Qy of Chl-a, respectively, while the peaks at 470 nm and 650 nm are due to the absorption of Soret state and Qy of Chl-b.[15,16] The absorption between 630 and 650 nm is mainly due to Chl-b. The positions at A3, A5 and A7 of Chl-a molecules have the absorption at 670 nm while other Chl-a molecules have the peak at 676 nm.[7] Recently, the nonlinear polarization spectroscopy (NLPS) research suggested that the absorption at 649 and 657 nm are due to the Qy of Chl-b, and the absorption at 667, 675, and 679 nm belong to Chl-a molecules.[17]

In Fig. 2(a) the fluorescence spectra of LHC Ⅱ excited at different wavelengths show that when the Soret state or Qy of Chl a/b are directly excited, the fluorescence of LHC Ⅱ are nearly independent of the exciting wavelength with the peak at 682 nm. This result reflects that under different conditions, energy transfer ends at the lowest excitation state. The fluorescence at 682 nm is due to the lowest excitation state of Chl-a, which is known to be consistent with the main absorption of PS I reaction centre at 680 nm. Thus, the accordance effectively facilitates the energy transfer and photochemical reaction in green plants. Figure 2(b) shows the fluorescence excitation spectra of LHC Ⅱ probed at 682 and 730 nm, respectively. These spectra have similar shapes of the absorption spectrum of LHC Ⅱ. This similarity suggests that different excited pigments including Chl-b and Chl-a will carry out energy transfer to the ultimate energy receptor, i.e., the lowest excitation state of Chl-a.

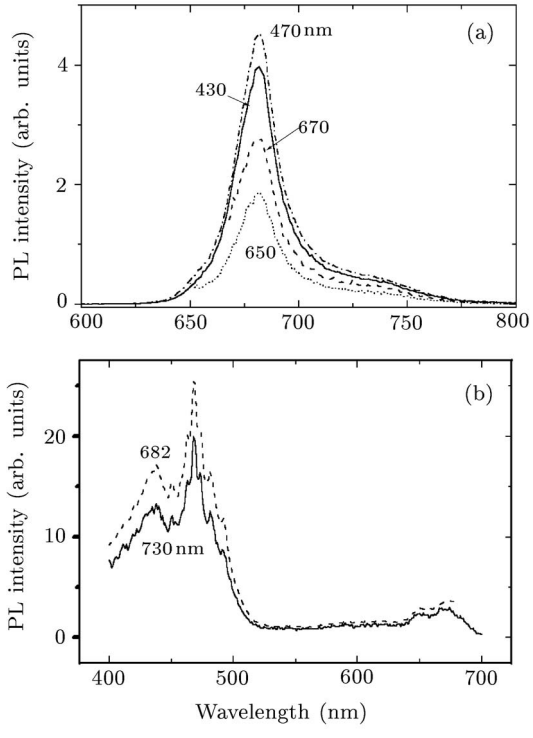


Fig. 2. (a) Fluorescence spectra of isolated LHC Ⅱ complex excited at 670, 650, 470, and 430 nm, respectively; (b) fluorescence excitation spectra of isolated LHC Ⅱ complex probed at 682 and 730 nm, respectively.

In order to study the interaction of rapid exciton processes in isolated LHC Ⅱ complexes, the fs pump–probe technique at different wavelengths was applied to study the energy transfer of Chl $a-a$, Chl $b-a$ and Chl $b-b$. Figure 3 shows the dynamical behaviour of LHC Ⅱ excited at 638 nm. From the absorption spectrum in Fig. 1, one can see that the pump pulses at 638 nm excited mainly Chl-b molecules and the energy transfer took place from Chl-b to Chl-a. By fitting this dynamic behaviour, an ultrafast process ($\sim$ 94%) at

the time scale of 230 fs and a slower process ($\sim 6\%$) at regime from 85 ps to several hundreds of picosecond were obtained. This suggests that the former is the main energy transfer from Chl-*b* to *a*. It is reported that in LHC Ⅱ, the time of energy transfer from Chl-*b* to Chl-*a* is at the scale of subpicosecond, meanwhile the energy transfer between Chl-*b* molecules of a monomeric subunit is much slower.[7] Our results are in agreement with the report of Du *et al.*[18] Because Chl-*b* and Chl-*a* molecules are compactly adjacent to each other with the centre distance of only 0.8–1.0 nm, we can express the energy transfer as the function of $W_{DA} = C_{DA}K^2/(n^4R^6)$.[5,7] Taking $k^2 = 1$, $n = 1.5$, $C = 15\,\mathrm{nm^6ps^{-1}}$,[19] and $R = 1\,\mathrm{nm}$, the calculation result is $W_{DA} = 10^{13}\mathrm{s^{-1}}$. Therefore, this ultrafast process (230 fs) is due to the energy transfer from Chl-*b* to Chl-*a* in a monomeric subunit of LHC Ⅱ, while the other slower process (85 ps) may be due to energy distribution among Chl-*b* molecules of different subunits or due to change of molecular conformation. Theoretical research suggested that the coupling intensity between Chl-*a* and Chl-*b* has the maximum of $120\,\mathrm{cm^{-1}}$, which is much smaller than the energetic separation between the Qy energy levels of the molecules ($450\,\mathrm{cm^{-1}}$),[7] so the energy transfer from Chl-*b* to Chl-*a* can be expressed by Forster theory.[5,7] Based on the calculation of the LHC Ⅱ structure, researchers know that energy transfer rates are not the same in different positions[7] with the results of $(2.6\,\mathrm{ps})^{-1}$, $(223\,\mathrm{fs})^{-1}$, $(270\,\mathrm{fs})^{-1}$, $(243\,\mathrm{fs})^{-1}$ and $(725\,\mathrm{fs})^{-1}$, respectively, from Chl-*b* at positions A6, B2, B3, B5 and B6 toward Chl-*a*. According to this, the ultrafast components in our experiments are due to the energy transfer from Chl-*b* molecules at positions B2, B3 and B5 to the corresponding Chl-*a* molecules.

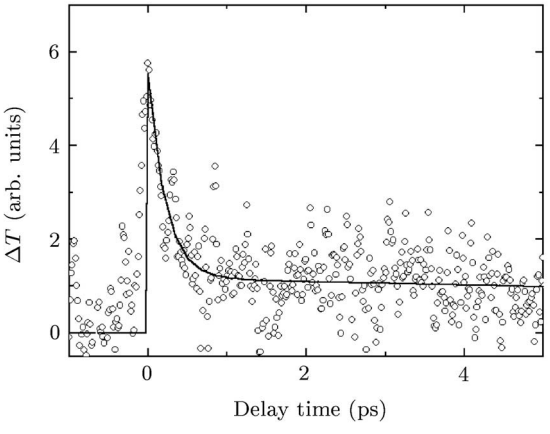


Fig. 3. Dynamic of isolated LHC Ⅱ complex excited at 638 nm by the fs pump–probe technique.

In the absorption spectrum of LHC Ⅱ, the absorption between 660–680 nm is due to absorption of Chl-*a*. To study the dynamic of excited Chl-*a* molecules, we selected pulses at 678 nm as the pump beam. Figure 4 shows the fs pump–probe result. From the analysis of the absorption spectrum, it was concluded that Chl-*a* molecules at different positions contribute to the absorption at 676 nm. As pointed out above, although excited at different wavelengths, LHC Ⅱ has the same lowest excited state, which finally captures energy from other Chl molecules. The excited Chl-*a* molecules at the blue side of absorption spectrum will continue energy transfer to those Chl-*a* molecules at the red side of absorption spectrum. Recently, Agarwal reported that the energy transfer between Chl-*a* molecules is with the lifetime scale from 300 fs to 6 ps.[20] In Fig. 4, the dynamic obtained at 678 nm has three different components: $\sim 30\%$ with a rate of $(370\,\mathrm{fs})^{-1}$, $\sim 40\%$ with a rate of $(5.4\,\mathrm{ps})^{-1}$ and $\sim 30\%$ with a rate slower than $(100\,\mathrm{ps})^{-1}$. In the LHC Ⅱ complexes, Chl molecules are so compactly adjacent to each other resulting in many exciton states. Under high-intensity laser pulses, the exciton interaction will be more observable. It is known that when the photon fluence in a single pulse is more than $8\times10^{14}\,\mathrm{cm^{-2}}$, the dynamic obtained at 680 nm is intensively dependent on the pump intensity and the energy transfer time is about several picoseconds, which suggests a process of exciton–exciton annihilation.[4] In our measurements, because the pump fluence is relatively low (lower than 10^{14} photons $\mathrm{cm^{-2}}$ per pulse), the exciton–exciton annihilation would not take place. Furthermore, the ultrafast component at lifetime of 370 fs, which belongs to dynamic obtained at 678 nm, is due to the energy transfer between excitons of Chl-*a*, while the fast component at the time scale of 5.4 ps is due to the energy distribution among different Chl-*a* molecules in monomeric subunit of LHC Ⅱ. Additionally, the slow process with lifetime longer than 100 ps is due to the energy transfer between different Chl-*a* molecules of different subunits or due to the change of molecular conformation.

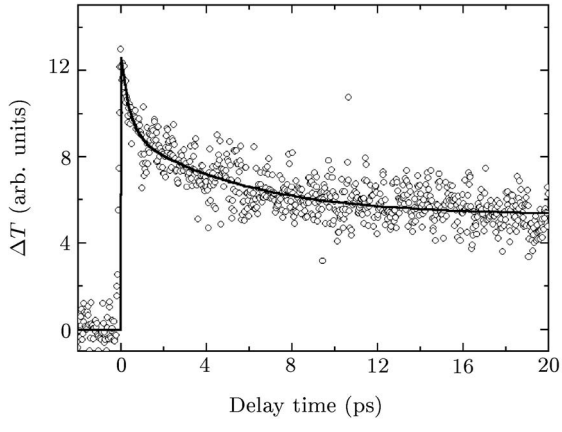


Fig. 4. Dynamic of isolated LHC Ⅱ complex excited at 678 nm by the fs pump–probe technique.

In this paper, we used the fs pump–probe technique at different wavelengths to study the dynamical behaviour of isolated LHC Ⅱ. The energy transfer in LHC Ⅱ complex can occur in three ways: Chl-*a*–*b*, Chl-*b*–*b* and Chl-*a*–*a*. Not only the difference of LHC Ⅱ structure but also the distance of Chl molecules and environment around Chls have effect on the energy transfer. In our experiments the results of dynamic show that in monomeric subunit of LHC Ⅱ, the energy transfer from Chl-*b* at positions B2, B3 and B5 to the corresponding Chl-*a* is at the time scale of 230 fs. The energy transfer between Chl-*a* excitons in monomeric subunit has lifetime of several hundreds of fs, while the energy transfer between Chl-*a* of different subunits is with the time scale about several picoseconds.

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