Study on Energy Transfer in LHC Ⅱ of Bryopsis Corticulans by Femtosecond Spectra*

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Abstract Based on the technique of Time Correlated Single Photon Counting (TCSPC), energy transfer kinetics among chlorophylls in a monomeroic unit of major light-harvesting complex (LHC Ⅱ) from bryopsis corriculan were studied. In the chlorophyll *Q* region, six characteristic molecules marked as Chlb_{630}, Chlb_{642}, Chla_{652}^{653}, Chla_{664}^{667}, Chl$a_{675}^{676,680}$, Chla_{682}^{683} were discriminated. Fluorescence kinetics measured in the chlorophyll *Q* region was excited by pulsed light of 630nm (70 fs). After analyzing, results of energy transfer among chls were obtained as following: only~20% of the energy absorbed by Chlb_{630} is initially transferred directly to other Chls with time constants shorter than 150 fs. Overwhelming part of the energy transfer among chlorophylls occur with time constants longer than 76 ps. Energy transfer pathway with time constants of several hundred femotoseconds and tens of picoseconds were also obtained. The fluorescence lifetimes of Chlb_{652}^{653}, Chla_{664}^{667}, Chl$a_{675}^{676,680}$, Chla_{682}^{683} were determined to be 1.41 ns, 1.39 ns, 676 ps, 709 ps respectively. The percentages of energy dissipation in the pathway of fluorescence emission are no more than 40% in the monomeric unit of LHC Ⅱ.

Keywords Energy transfer; LHC Ⅱ; Femtosecond spectra; Fluorescence kinetics

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0 Introduction

Bryopsis corticulans is a siphonous green alga growing in intertidal areas. It can be survival during the periodic tide. LHC Ⅱ of photosystem II, the outermost and most abundant antenna complex of chloroplasts, exists as a trimer and binds half of the thylakoid chlorophyll molecules, which has an essential role in harvesting solar energy and transfer in the process of photosynthesis.

Measured by the absorption kinetics in the LHC Ⅱ of high plants, there now exists a general agreement in the field about the Chl-Chl energy transfer in the LHC Ⅱ complex. At temperatures near room temperature the fastest Chl*b* to Chl*a* transfer seems to occur with a lifetime of ~150~200 fs[1~4]. Further components have lifetimes of ~500~600 fs and 5~7 ps[1~4]. Energy transfer among the Chlas occur on a time scale typically 1 ps and longer[2~5]. Given higher spectra sensitivity and temporal resolution, measured by the fluorescence kinetics, more detailed information on energy transfer can be obtained. In the present work, the pigment organization of monomeric LHC Ⅱ by combination of transient absorption spectrum and transient fluorescence emission spectrum was investigated. Then the energy transfer between Chls of monomeric LHC Ⅱ was studied by the fluorescence kinetics. All these results are important for a further understanding of the relation between structure and function of LHC Ⅱ.

1 Materials and methods

1.1 Isolation of monomeric LHC Ⅱ

A light-harvesting chlorophyll *a*/*b*-protein complex was isolated directly from thylakoid membranes of marine green alga, Bryopsis corticulans, by two consecutive runs of liquid chromatography. The monomeric form of LHC Ⅱ was obtained by the procedure as described in the Reference[6].

1.2 Experimental apparatus

Femtosecond transient absorption and fluorescence emission measurements were performed by using a titanium-sapphire laser system. The master oscillator is a Ti : sapphire laser excited by a CW diode pumped intracavity doubled Nd : YVO. The laser provides a train of femtosecond pulses (56 fs) at 82 MHz repetition rate with 0.4W of average power at the central wavelength of 800 nm. Seed pulses were amplified by a Ti : sapphire regenerative amplifier (Spitfire/Hurricane) pumped at 1 kHz by a *Q*-switched,

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intracavity doubled Nd : YLF running at the second harmonic wavelength, 527 nm (model 527 DP-H, Evolution). The white light used in OPA systems (OPA-800CF) (Spectra-Physics) as a seed was created by focusing a few μJ of energy (800 nm) into sapphire. Thus the broad spectral coverage of white light continuum provides an ideal seed source for an OPA. As the visible light range (<800 nm) in the range of seed light is no worthy in the process of parametric amplication, this range was split off with dichroic mirror and utilized for detection for transient absorption spectroscopy. The OPA stage converted the wavelength to ~630 nm with a pulse width of ~70 fs. Pulse energy of ~0.1 μJ in a 1mm diameter spot was used. The Edinburgh Instruments FLS920 was chosen for measuring spectra and the kinetic of fluorescence emission. Based on time correlated single photon counting technique (TCSPC) equipped with the ultrafast photo-detectors of micro-channel plate photomultiplier MCP-PMT (HAMAMATSU R3809U-50 with the cooling system C4878), the measuring temporal resolution of this system fluorescence emitting can be obtained to ~20 ps.

1.3 Data analysis

As exciting pulse is not a real Dirac function in the experiments, the measured time resolved fluorescence emission spectrum $h(t)$ is the convolution integral of real fluorescence emission spectrum $g(t)$ with instrument response $f(t)$. Such situation was represented by the following integral: $h(t)=\int g(t-\tau)f(\tau)d\tau=\int g(\tau)f(t-\tau)d\tau$. So the numerical procedure required the use of the convolution integral to extract the lifetime parameters. The model of numerical fit was expressed in mathematical terms as follows: $Y=y_0+A_i\sum_{i=1}^{n}\exp(-x/t_i)$, with pre-exponential factors A_i, the characteristic lifetime t_i and an additiona background y_0. The pre-exponential factors can be either positive or negative. A positive A_i value represented a decay process (energy dissipation), while negative A_i value was characteristic for growth process (accepting energy). The numerical routine to extract the parameters A_i and t_i was following in a matlab procedure based on marquardt-levenberg algorithm compiled by ourselves. The reduced chi-square of fitting result was calculated to evaluate the quality of the fit results.

2 Results

2.1 Pigment composition and its Spectroscopic characteristic in the chlorophyll *Q* region

Understanding of the energy transfer of monomeric LHC Ⅱ required recognition of the pigment composition and its spectroscopic characteristic. The pigment composition of LHC Ⅱ was analyzed by using reversed-phase High Performance Liquid Chromatography (HPLC) method[7]. The ratios of Chl*a*/Chl*b* trimer was 1.2, which were similar to that of the native LHC Ⅱ reported in higher plants before[8]. The knowledge of spectroscopic characteristic has been obtained from transient absorption spectrum (Fig. 1) and transient fluorescence emission spectrum (Fig. 2). The transient fluorescence emission spectrum was excited by 630 nm in case the excitation of carotenoids. The absorption and fluorescence spectra of the samples were measured before and after the femtosecond lifetime measurements, showing no changes due to photochemical or other damage. From the analysis of on the spectroscopic charactistic (Fig1, Fig2.) combined with the absorption spectral of individual pigments[9,10] there were six characteristic molecules marked as $Chlb_{630}$, $Chlb_{642}$, $Chla_{652}^{653}$, $Chla_{664}^{667}$, $Chla_{675}^{676,680}$, $Chla_{682}^{683}$ (the subscript denotes the center wavelength of absorption, the superscript denotes the center

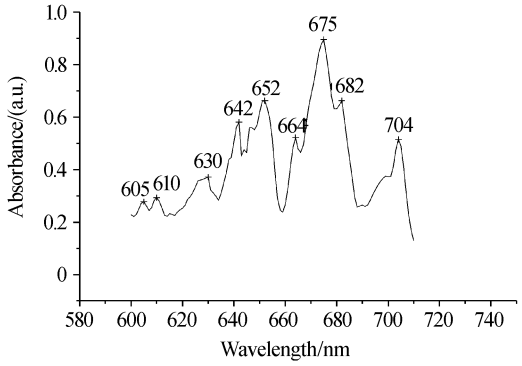

Fig. 1　Transient absorption spectrum of LHC Ⅱ monomer at room temperature

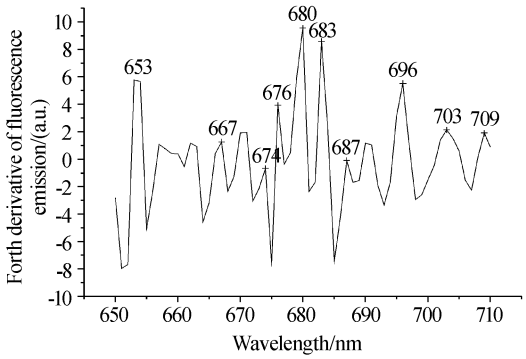

Fig. 2　The fourth derivative of fluorescence Emission spectrum of of LHC Ⅱ trimer at room temperature excited by 630 nm with a pulse width of ~70 fs

wavelength of fluorescence emission.) were discriminated in the chlorophyll *Q* region.

2.2 Energy transfer between Chls

There appeared many peaks in the fluorescence emission spectrum (Fig. 2), but only during the range of 655 nm～695 nm, fluorescence emission can be detected in the time resolved experiments. Considering the correspondence with the transient absorption spectrum (Fig. 1), four representative time-resolved fluorescence spectra characterized with the pigments were analyzed following the routine as describing in the data analysis. After reconvolution simulation, the most appropriate fitting curve (Fig. 3.) and results log (not shown) were created. The reduced chi-square of fitting results were all in the level of 10^{-4}, and the quality of fitting results were seem to be well accepted. As the fitting curve is also a combination of growth process (accepting energy) and decay process (dissipation energy). Therefore the fitting lifetimes in the results log were also divided into two major processes: accepting energy process and energy dissipation process. Among each process, considering pigment composition of LHC Ⅱ and its spectroscopic characteristic in the chlorophyll *Q* region, which allowed us to specifically assign these lifetimes and differentiate in detail the energy transfer paths between Chls. The corresponding percentage of each subprocess was calculated as $\frac{A_i}{\sum A_i}$ (pre-exponential factors A_i with the same sign).

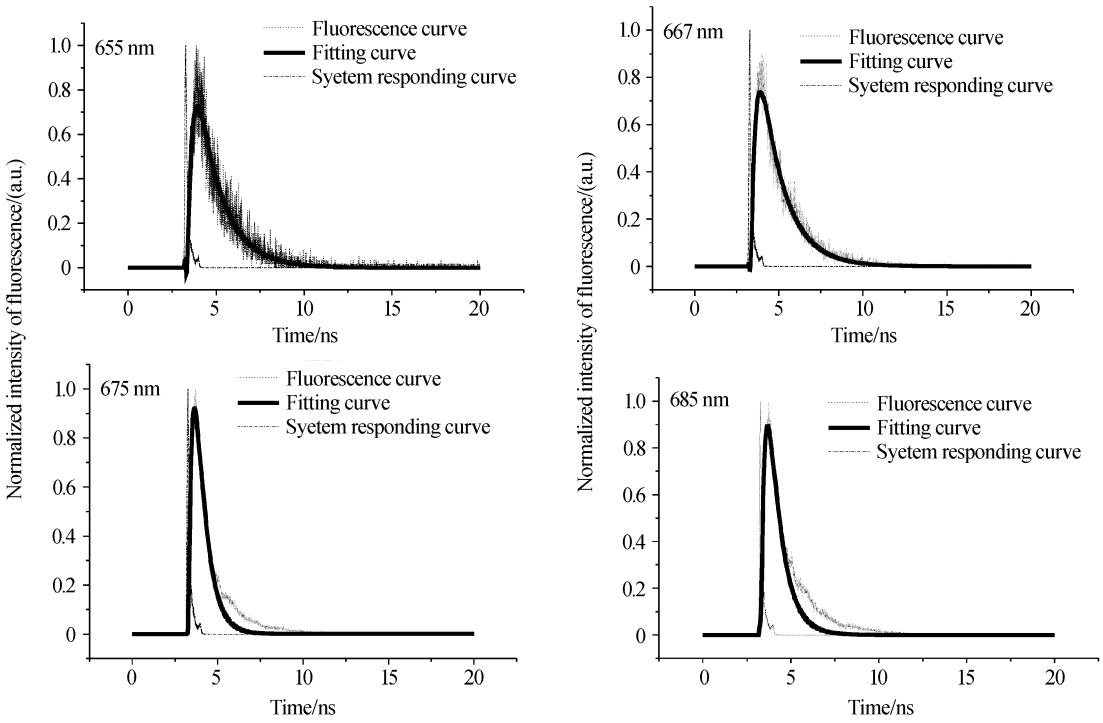

Fig. 3 Time resolved fluorescence spectra of LHC Ⅱ excited by 630nm measured at different waveband

All the results of lifetimes and percentage of energy transfer between Chls were summarized clearly in Table. 1. Results can be reduced to five points: 1) Only～20% of the energy absorbed by Chlb_{628} was initially transferred directly to other Chls with time constants shorter than 150 fs: Chlb_{630} $\xrightarrow{90\text{ fs}}$ Chlb_{652}^{653}, Chlb_{630} $\xrightarrow{153\text{ fs}}$ Chla_{664}^{667}, Chlb_{630} $\xrightarrow{124\text{ fs}}$ Chl$a_{675}^{676,680}$, Chlb_{630} $\xrightarrow{127\text{ fs}}$ Chla_{682}^{683}; 2) Overwhelming part of the energy transfer among chlorophylls occur with time constants longer than 76 ps: Chlb_{630} ⋯→ Chlb_{642} ⋯→ Chla_{652}^{653} (137 ps), Chlb_{630} ⋯→ Chlb_{642} → Chla_{664}^{667} (148 ps), Chlb_{630} ⋯→ Chlb_{642} ⋯→ Chl$a_{675}^{676,680}$ (106 ps), Chlb_{630} ⋯→ Chlb_{642} ⋯→ Chla_{682}^{683} (76 ps), which denotes that before transfer energy to another Chls, the excited Chl must exchange energy to vibrations of a lower state with different multiplicity (intersystem crossing); 3) Energy transfer directly from Chla_{652}^{653} to Chl$a_{675}^{676,680}$ and Chla_{64}^{666} to Chl$a_{674}^{677,680}$ were trifling (< 0.02 %), yet the transfer time were determined to be 197 fs and 93 fs. So energy transfers from Chlb_{628} to Chl$a_{674}^{677,680}$ via Chl$a_{652}^{654,657}$ with time constant of 83 fs were also subtle. However, energy transfers from Chlb_{628} to Chla_{664}^{666} via Chl$b_{652}^{654,657}$ with lifetime of 174 fs, Chlb_{628} to Chla_{682}^{683} via Chl$b_{654,657\,652}$ and Chla_{664}^{666} with lifetime of 217 fs were considerable (>10 %). The difference can be owed to the differences in the arrangement and direction of dipole moment of Chls in LHC Ⅱ; 4) Some

slower energy transfer such as $Chlb_{653\,652} \xrightarrow{13.8\ ps} Chla_{682}^{683}$, $Chla_{664}^{667} \xrightarrow{8.4\ ps} Chla_{682}^{683}$, $Chla_{674}^{676,680} \xrightarrow{9.1\ ps} Chla_{682}^{683}$ were also obtained, which can be attributed to internal conversion before transferring energy to another Chls; 5) The fluorescence lifetimes of $Chla_{652}^{653}$, $Chla_{664}^{667}$, $Chla_{675}^{676,680}$, $Chla_{682}^{683}$ were determined to be 1.41 ns, 1.39 ns, 676 ps, 709 ps respectively. The percentages of energy dissipation in the pathway of fluorescence emission are no more than 40% in the monomeric unit of LHC Ⅱ.

Table. 1　Lifetimes and percentage of energy transfer between Chls

C_{652}^{653}(Chl*b*)	C_{664}^{667}(Chl*a*)	$C_{675}^{676,680}$(Chl*a*)	C_{682}^{683}(Chl*a*)
$C_{630}\rightarrow C_{652}^{653}$	$C_{630}\rightarrow C_{664}^{667}$	$C_{630}\rightarrow C_{675}^{676,680}$	$C_{630}\rightarrow C_{682}^{683}$
90 fs(22%)	153 fs(16%)	124 fs(19%)	127 fs(18%)
$C_{630}\cdots\rightarrow C_{642}\cdots\rightarrow C_{652}^{653}$	$C_{630}\cdots\rightarrow C_{642}\cdots\rightarrow C_{664}^{667}$	$C_{630}\cdots\rightarrow C_{642}\cdots\rightarrow C_{675}^{676,680}$	$C_{630}\rightarrow C_{652}^{653}\rightarrow C_{664}^{667}\rightarrow C_{682}^{683}$
149 ps(78%)	148 ps(69%)	106 ps(69%)	217 fs(10%)
$C_{652}^{653}\rightarrow C_{664}^{667}$	$C_{630}\rightarrow C_{652}^{653}\rightarrow C_{664}^{667}$	$C_{630}\rightarrow C_{652}^{653}\rightarrow C_{675}^{676,680}$	$C_{630}\cdots\rightarrow C_{642}\cdots\rightarrow C_{682}^{683}$
89 fs(27%)	125 fs(27%)	68 fs(27%)	76 ps(71%)
$C_{652}^{653}\rightarrow C_{675}^{676,680}$	$C_{664}^{667}\rightarrow C_{675}^{676,680}$	$C_{630}\rightarrow C_{664}^{667}\rightarrow C_{675}^{676,680}$	$C_{682}^{683}\rightarrow C$
197 fs(0.02%)	93 fs(0.02%)	174 fs(10.3%)	128 fs(0.002%)
$C_{652}^{653}\cdots\rightarrow C_{682}^{683}$	$C_{664}^{667}\cdots\rightarrow C_{682}^{683}$	$C_{675}^{676,680}\cdots\rightarrow C_{682}^{683}$	$C_{682}^{683}\cdots\rightarrow C$
13.8 ps(48%)	8.4 ps(60.9%)	9.1 ps(48%)	9.55 ps(58.9%)
$C_{652}^{653}\rightarrow S_0$	$C_{664}^{667*}\rightarrow S_0$	$C_{675}^{676,680*}\rightarrow S_0$	$C_{682}^{683*}\rightarrow S_0$
1.41 ns(25%)	1.39 ns(37%)	676 ps(39%)	709 ps(41%)

3　Discussion

The consistent understanding of the LHC-II absorption in an overall substructure model, including possible excitonic effects, was thus complicated by the unknown origin of the observed spectral heterogeneity of at least 10 subbands[11]. In the process of detecting the absorption and emission spectra of LHC-II exicted by steady excitation sources, considering the nonconservative contributions to the biological sample induced by consistent irradiation, the spectra would be distorted by the final bleaching and excited state absorption signal in Chls range[1]. Therefore, in this article, a new approach to assess the spectroscopic characterization of monomeric LHC Ⅱ by probing the absorption peaks and emission peaks directly by femtosecond pulses was presented. All the measured spectras were excited by the laser with a pulse width of ~70 fs at 1 kHz, which ensured the restoration of sample when excited by utrafast light. However, there also was somewhat smaller part of involved subbands hindered in transient spectra. For complementarity, some derivative spectras were made to explore the hindered subbands (Fig. 2). There are at least six characteristic molecules in the *Q* range of chls marked as $Chlb_{628}$, $Chlb_{646}$, $Chlb_{652}^{654,657}$, $Chla_{664}^{666}$, $Chla_{674}^{677,680}$, $Chla_{682}^{683}$, which were recognized by transient spectroscopic technique. They were mostly consistent with the results studied by other groups with different techniques that there exits absorption peak at 648 nm、660 nm、669 nm、678 nm、684 nm、695 nm[1,3,12~14].

In Table. 1, it was noticed that when energy transfer between Chls via C_{642}, the transfer time would last up to 100 ps, and this pathway was the main channel of energy transfer between Chls for its higher percentage. It implicated that $Chlb_{642}$ must be assembled in a special position in the structure of monomeric LHC Ⅱ; or $Chlb_{642}$ maybe always went through the intersystem crossing and exists in a triplet state before exchange energy to others. Some slower energy transfer time of 10 ps, assigned to the accepting energy time of $Chla_{682}^{683}$ from other Chls directly, and it occupied mostly in the energy transfer percentage. It also implicated $Chla_{682}^{683}$ was the main acceptor in LHC Ⅱ. With the longer of wavelength, the more it dissipated energy in the mode of fluorescence. That is to say, the efficiency of energy transfer was decreasing with the turn of $Chlb_{657\,652}^{654}$, $Chla_{664}^{666}$, $Chla_{674}^{677,680}$, $Chla_{682}^{683}$.

4　Conclusion

This study was the first demonstration to discriminate the pigments composition in monomeric LHC Ⅱ by its transient spectrum characteristic. In the lifetime data analysis, as the fluorescence emission curve including the growth process (accepting energy) and decay process (dissipate energy); a 6-exponential fit was verified to be appropriated to fit these data. This allowed us

to specifically assign and differentiate in detail the energy transfer paths among Chls. And all the lifetime was assigned to different pathway of energy transfer reasonably. The long-term objective for this project is to provide answers to key questions on the structure-function relationships of the apparatus enabling efficient utilisation of light for the synthesis of metabolic energy in oxygenic photosynthesis.

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假根羽藻外周天线内能量传递的飞秒光谱研究

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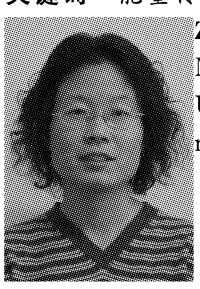
摘 要 在时间相关单光子技术的基础上,对假根羽藻外周天线内叶绿素分子间的能量传递进行研究. 采用瞬态吸收与荧光发射谱识别样品内的具有特征光谱组分的分子,得到在叶绿素分子的 Q 带区主要存在以下六个特征分子:$Chlb_{630}$, $Chlb_{642}$, $Chla_{652}^{653}$, $Chla_{664}^{667}$, $Chla_{675}^{676,680}$, $Chla_{682}^{683}$. 在 630 nm 的飞秒脉冲光的激发下,通过对不同特征发射峰出的荧光动力学进行解析得到: 1)$Chlb_{628}$ 分子所吸收的能量仅有大约 20%被直接传递给其他叶绿素分子,传能时间小于 150 fs;2)叶绿素间大部分的能量传递发生在长于 76 ps 的时间范围内;3)传能时间常量在几百 fs 以及 10 ps 左右的间接传能可能与具有不同光谱组分特征的叶绿素分子在外周天线内的排列方式以及偶极距的取向有关;4)$Chlb_{654,657\,652}$, $Chla_{664}^{666}$, $Chla_{674}^{677,680}$ 和 $Chla_{682}^{683}$ 以荧光形势耗散能量的时间常量分别为 1.41 ns, 1.39 ns, 676 ps, 709 ps,这部分在整个能量耗散中占的比例不超过 40%.

关键词 能量传递;外周天线;飞秒光谱;荧光动力学



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