doi:10.3788/gzxb20144308.0832001

# 假根羽藻外周天线 LHC II 寡聚体的瞬态吸收光谱

贺俊芳[1],朱长军[2],赵小侠[1],李院院[1]
(1 西安文理学院 物理与机械电子工程学院,西安 710065)
(2 西安工程大学 理学院物理系,西安 710048)

**摘 要**:采用时间分辨瞬态吸收光谱技术研究了假根羽藻外周天线寡聚体的光保护机制.分别以667 nm飞秒激光脉冲和白光脉冲作为泵浦光和探测光,探测光与泵浦光之间的延时范围和准确度分别为 340 ps 和 134 fs.实验结果表明在泵浦光激发之后外周天线对探测光的吸收是动态变化的.对瞬态吸收光谱进行多指数拟合,并结合外周天线的荧光发射谱和激发谱进行分析,结果表明:500～600 nm 的瞬态吸收谱主要来源于类胡萝卜素分子,外周天线寡聚体至少包含四种具有光保护作用的类胡萝卜素分子,对应的 $S_0 \rightarrow S_n$ 跃迁光谱为 511 nm 和 554 nm,522 nm 和 541 nm,530 nm 和 563 nm(对应管藻黄素),536 nm 和 575 nm;类胡萝卜素分子以两种方式参与到光保护过程中:一种是直接方式,在几皮秒范围内猝灭叶绿素三重态;另一种是间接方式,在几百皮秒范围内猝灭从叶绿素分子获得能量的单线态氧.

**关键词**:瞬态吸收光谱;光合作用;泵浦探测;外周天线;光保护;类胡萝卜素;叶绿素;单线态氧
**中图分类号**:Q631;Q632 **文献标识码**:A **文章编号**:1004-4213(2014)08-0832001-6

# Transient Absorption Spectra of Aggregated LHC II in Broypsis Corticulans

HE Jun-fang[1], ZHU Chang-jun[2], ZHAO Xiao-xia[1], LI Yuan-yuan[1]
(1 *School of Physics and Mechatronics Engineering, Xi'an University of Arts and Science, Xi'an, 710065, China*)
(2 *Department of Physics, School of Science, Xi'an Polytechnic University, Xi'an, 710048, China*)

**Abstract**: A time resolved transient absorption spectroscopy was performed to investigate the photoprotection mechanism in light harvesting complex II of broypsis corticulans. A 667 nm femotosenond laser and white light pulse were used as pump and probe lights. The range and accuracy of the delay line between probe and pump were 340 ps and 134 fs, respectively. The experiment results show that the absorption of the probe is dynamically changing after pump light exciting. The results of the multi-exponential fitting, fluorescence emission and excitation spectra show that the transient absorption spectra from 500 nm to 600 nm mainly originate from carotenoids and the aggregated light harvesting complex II contains at least four types of carotenoids functioning photoprotection which have $S_0 \rightarrow S_n$ transition spectral properties 511 nm and 554 nm, 522 nm and 541 nm, 530 nm and 563 nm (corresponding to siphonaxanthin), 536 nm and 575 nm. Carotenoids play the photoprotection role not only in a direct manner by quenching the triplet state of chlorophyll *a* in several picoseconds, but also in an indirect manner by quenching the singlet state of oxygen in several hundreds of picoseconds.

**Key words**: Transient absorption spectrum; Photosynthesis; Pump-probe; Light harvesting complex II; Photoprotection; Carotenoids; Chlorophyll; Singlet state oxygen
**OCIS Codes**: 320.7120; 320.7100; 170.1420; 170.7160

**Foundation item**: The National Natural Science Foundation of China (No. 60978038)
**First author**: HE Jun-fang(1971—), female, research fellow, Ph. D. degree, mainly focuses on ultrafast phenomena. Email:amilyhjf@163.com
**Received**: Jan. 1, 2014; **Accepted**: Apr. 1, 2014

# 0 Introduction

Photosynthesis is of fundamental significance in the conversion of solar energy to energies utilized by living things which are adapted to different environments and may be modulated in response to changing light environments. Light Harvesting Complex (LHC II) in green plant and alga possesses the most abundant light harvesting pigment-protein complex. In essence, it is believed to be organized in trimeric units and it binds about 50% of the chlorophyll present in the chloroplast. Besides chlorophylls, LHC II contains carotenoids which are in close contact with chlorophyll molecules[1-5]. Carotenoids can absorb light in the blue-green and yellow regions and, then, transfer the energy to chlorophyll, which increases the efficiency of collection of solar energy in the wavelength region where chlorophyll does not absorb light[6]. On the other hand, the reverse energy transfer process may occur, that is, carotenoids can quench both triplet excited states in chlorophyll and singlet state in oxygen, protecting the photosynthetic apparatus from photodamage.

So far, intensive researches have been conducted on the energy transfer from carotenoids to chlorophyll[7-8]. Solar energy can be transferred from carotenoids to chlorophyll $S_1$ or $S_2$ state on picosecond time scale, or even femtoseconds time scale[9-10]. Much attention has also been paid to the photoprotection mechanism caused by carotenoids in photosynthesis when the photosystem is under high light illumination[11-12]. There were currently four models suggesting how to quench excess excited state Chl a[13-16]: 1) via energy transfer to the carotenoid $S_1$ state, 2) via electron transfer, resulting in a Chl-a anion and a carotenoid cation that recombine on a picosecond time scale, 3) it is achieved via carotenoid-Chl exciton coupling that provides a pathway for deactivation of excess excited Chl via the rapidly decaying $S_1$ state of the carotenoid, and 4) it results from formation of Chl/Chl exciton pairs which undergo charge transfer as the pathway for Chl excited state deactivation. Except the forth model, the first three models involve carotenoids directly. Some researches[11,17-19] showed that xanthophyll cycle play an important role in photoprotection. Zeaxanthin can accept energy from Chl $Q_y$ states in all possible orientations, whereas violaxanthin can do so only at very close distances by charge transfer.

Little effort, however, has been dedicated to the dynamics of the photoprotection process. This paper focuses on the revelation of the dynamics of photoprotection using a time resolved transient absorption spectroscopy.

# 1 Experiment

## 1.1 Materials

Bryopsis corticulans is a siphonous green alga growing in intertidal areas, where periodic changes of light accepted by the green alga during a cycle of tides may demand that LHC Ⅱ of the alga operate with some mechanisms to adapt the drastically changing light. Thus it is a suitable material for investigating the photoprotection information. The LHC II aggregates were obtained from bryopsis corticulans. The complex contains a specific carotenoid, siphonaxanthin, as well as Chl *a*, Chl *b*, neoxanthin and violaxanthin. Siphonaxanthin which is present in the light-harvesting siphonaxanthin-chlorophyll *a*/*b*-protein complex is responsible for enhanced absorption in blue-green region (530 nm). The light harvesting chlorophyll *a*/*b*-protein complex with a Chl *a*/Chl *b* ratio of 1.1 is similar to that of higher plants.

## 1.2 Experiment setup

Experiment setup for transient absorption difference measurements in visible spectral range using a femtosecond pump-probe scheme was described previously[20]. Briefly, a Ti: Sapphire laser acting as master oscillator was pumped by the intracavity frequency doubled cw Nd : $YVO_4$ laser and emits ultrashort pulses of about 80 fs at 800 nm. The laser pulses were subsequently stretched, amplified and then recompressed at 1 kHz repetition rate by a regenerative amplifier (Spectra Physics, Spitfire). The amplified pulses have high average power (about 640 mW) and short pulse duration (about 100 fs). The amplified pulses were used to drive an Optical Parametric Amplifier (OPA), tunable in UV-Vis-IR and to generate a white light continuum in a 1 mm glass plate used as the probe. The output of OPA was used as pump light. The probe light traveled through sample in a separate time with respect to the pump light, which was controlled by a high precision optical delay line. The range and accuracy of the delay line were 340 ps and 134 fs. The two lights overlapped spatially inside the sample. The probe light was polarized under magic angle (54.7°) with respect to the pump light. In order to explore the photoprotection mechanisms related to carotenoids, the wavelength of pump light was tuned around 667 nm. The pump light intensity was about 15 nJ/pulse. The probe intensity was typically 10 times lower. Absorption difference spectra were recorded by a high sensitivity micro-channel plate photo-multiplier tube and time correlation single photon count technology.

Experiment was carried out at room temperature

and atmospheric pressure. The sample was prepared in the near natural state, without treatments of deoxidize or saturated oxygen.

## 2 Results and discussions

Fig. 1 shows the transient absorption spectrum of aggregated LHC II in bryopsis corticulans. A femtosecond continuum light was used to illuminate the LHC II aggregates. To avoid photo-saturation of LHC II, the femtoseconds light was very weak, with pulse energy of 1 nJ. The absorption spectrum obtained in our experiments shows more detailed information than traditional absorption spectra. In the spectrum, almost twenty absorption peaks were identified at 457, 463, 469, 478, 482, 489, 493, 511, 522, 530, 536, 541, 548, 554, 563, 569, 575 and 592 nm, respectively. The carotenoid absorption wavelength ranges from 400 nm to 600 nm[21]. Therefore, the peaks shown in Fig. 1 correspond to the absorption lines of carotenoids and chlorophylls of the aggregated LHC II.

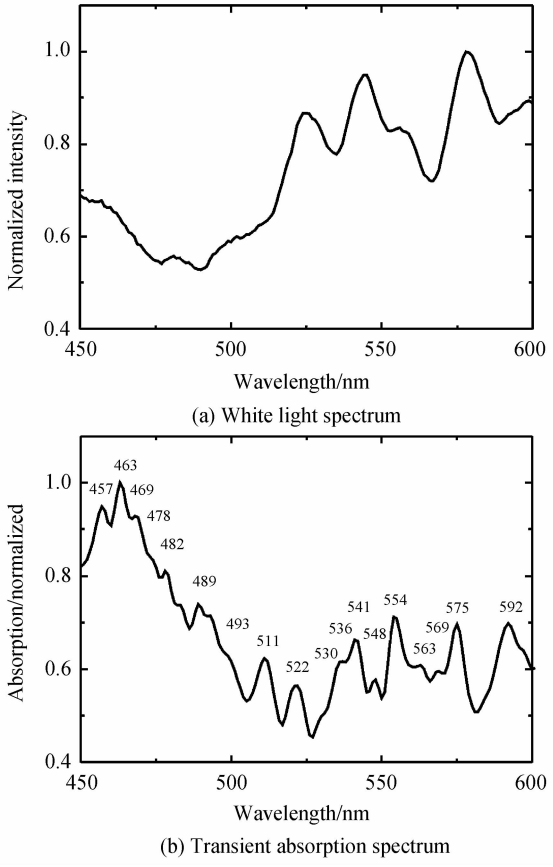


Fig. 1 White light spectrum and transient absorption spectrum of aggregated LHC II

When chlorophyll molecules are illuminated, they are excited to a high singlet state, and then intercrossed to their first excited triplet state. The triplet-excited chlorophylls can react with molecular oxygen to produce singlet oxygen, which is a powerful oxidising agent and rapidly kills those cells exposed to it. Carotenoids are able to overcome this effect in two ways, as shown in Fig. 2. The first way is to quench

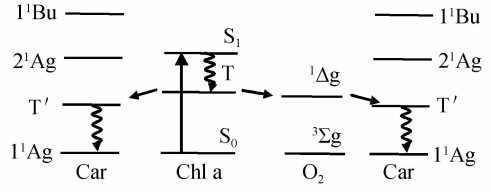


Fig. 2 Direct and indirect quenching channels

the triplet state of chlorophyll *a* directly, which can be expressed as

$$^3\text{Chl } a^* + \text{Car} \rightarrow \text{Chl } a + {}^3\text{Car}^* \quad (1)$$

The second way is to quench singlet oxygen, preventing the production of singlet oxygen, which can be written as

$$O_2(^1\Delta_g) + \text{Car} \rightarrow O_2(^3\Delta_g^-) + {}^3\text{Car}^* \quad (2)$$

In order to investigate the protecting role of carotenoids, the samples were excited at 667 nm, corresponding to the absorption of chlorophyll $Q_y$ band. Time varying characteristics of 4 representative absorption peaks were analysed using the absorption difference spectra shown in Fig. 3.

The time varying characteristics of absorption peaks can be analysed by

$$y = y_0 + a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3) \quad (3)$$

where $y_0$, $a_i$, $\tau_i$ are background, pre-exponential factors and lifetimes, respectively. The multi-exponential model is often used on energy transferring between pigments of photosynthesis[22-23]. In this fitting model, the pre-exponential factors, $a_i$, can be either positive or negative. A positive $a_i$ represents a decay process, while negative $a_i$ values are characteristic for growth processes. The multiple exponential fittings reveal that all the kinetics curves have a fast rise component with several hundred of fs, corresponding to the excitation of chlorophyll *a*. Meanwhile, two delay components, one fast process of several ps and one slow process of hundreds of ps, correspond to the two energy transfer processes displayed in Eqs. (1) and (2). It can be seen from Fig. 3 that the time durations of direct energy transfer process and indirect energy transfer process are on several ps and hundreds of ps time scales. Pumped at 667 nm, chlorophyll *a* molecules were first excited and, then, transfer energy in an optimized way to a reaction center where the energy was transformed in the form of chemistry. But in the LHC II aggregates, there exists no acceptor transforming energy in the form of chemistry. Thus, the energy must be dissipated in other manners. The first manner is emitting fluorescence directly from chlorophyll *a*. The second is transferring energy in direct and indirect manners to other pigments, e. g.,

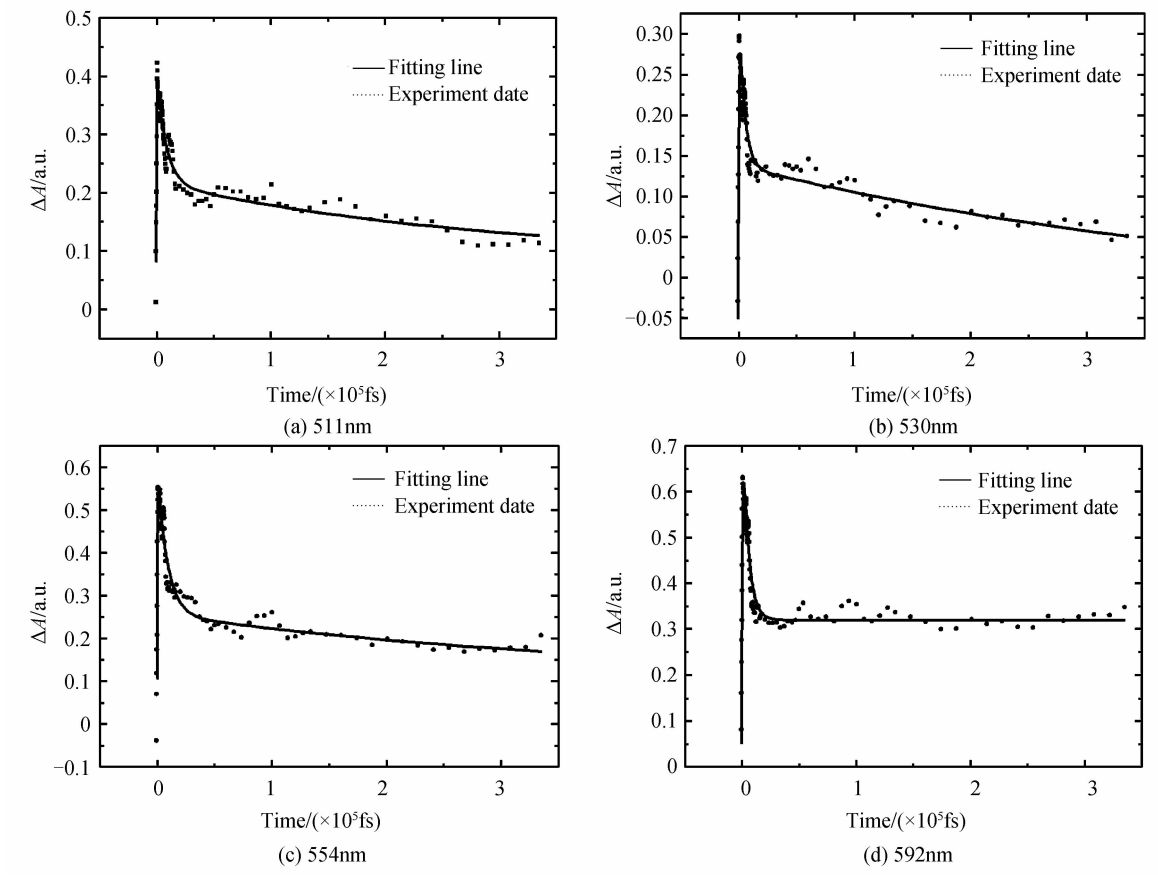


Fig. 3 Time dependence of absorption difference spectra of LHC II pumped at 667 nm

carotenoid and chlorophyll *b*. The energy transferred directly from chlorophyll *a* to carotenoid, or indirectly from chlorophyll *a* though oxygen to carotenoid, was eventually dissipated thermally, leading to photoprotection.

In the case of excitation with 436 nm, the emission spectrum was detected and was fitted by six Gaussian bands, i. e., 658 nm, 680 nm, 686 nm, 696 nm, 726 nm, 736 nm(dot lines), as displayed in Fig. 4. Thereinto, the band 658 nm is originated from chlorophyll *b* absorbing at 650 nm, whereas, the 436 nm light is likely to excite chlorophyll *a*, implying that energy can be transferred from chlorophyll *a* to chlorophyll *b*. To confirm this process, the excitation spectrum at emission 658 nm was also detected in the red region, as shown in Fig. 4. It is clear that the light from 665 nm to 690 nm, corresponds to the chlorophyll *a* $Q_y$ absorption, which induces chlorophyll *b* emitting fluorescence.

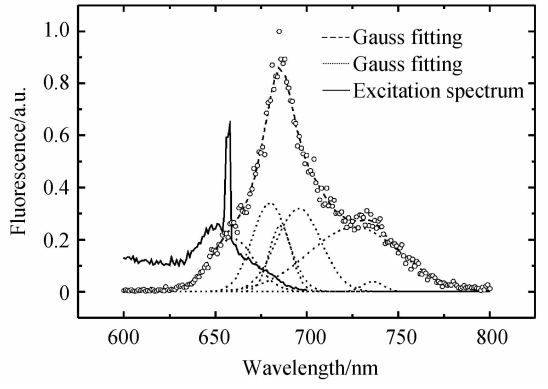


Fig. 4 Fluorescence emission and Gauss fitting, and excitation spectra of aggregated LHC II

Previous research results show that there is a large range of overlapping region between chlorophylls and carotenoids' absorption spectra in the region below 500 nm[24]. Therefore, it is possible that energy transfer from chlorophyll *a* to chlorophyll *b* and, thus, the difference absorption dynamics curves below 500 nm are originated not only from carotenoid but also from chlorophyll *b* molecules, causing the faster delay lifetimes below 500 nm. Consequently, only the absorption kinetic curves in the region 500～600 nm are originated from carotenoids.

An interesting phenomenon appears, namely, 511 nm and 554 nm have similar lifetimes and percentages in the decay process, as shown in Table 1. These peaks corresponds to the same molecule. Similar situation can be found 522 nm and 541 nm, 530 nm and 563 nm, 536 nm and 575 nm. Since the $S_0 \rightarrow S_1$ transition is optically forbidden, these wavelengths might be due to the $S_0 \rightarrow S_n$ transition. According to previous report[3], the 530 nm and 563 nm are due to siphonaxanthin. In the calculation model, A+B/(N+C), 511 nm and 554 nm, 522 nm and 541 nm, 536 nm

and 575 nm originate from three carotenoids with 10 or 11 carbon-carbon double bonds. Herein, the three carotenoids are named as Car511, Car522 and Car536. The two delay lifetimes of the curves correspond to the two photoprotection ways described in Eqs. (1) and (2). Fig. 5 shows the transient absorption difference spectra developing with time increasing after pump light exciting. At about 1.3 ps, the absorption difference spectra from 500 nm to 600 nm reach the maximum. Then with the time increasing, the transient absorption difference spectra are decreasing .

**Table 1 Lifetime parameters of the kinetic curves**

| Wavelength /nm | $\tau_1$(fs) with negative $a_1$ | $\tau_2$(ps) with $a_2$ (percentage in delay processes) | $\tau_3$(ps) with $a_3$ (percentage in delay processes) |
|---|---|---|---|
| 478 | 670 | 1.06(12%) | 44.2(88%) |
| 482 | 676 | 1.24(6%) | 60.8(94%) |
| 489 | 361 | 1.44(5%) | 38.7(95%) |
| 493 | 328 | 1.18(3%) | 51.6(97%) |
| 511 | 354 | 8.22(4%) | 302(96%) |
| 522 | 706 | 5.83(12%) | 88.4(88%) |
| 530 | 640 | 4.77(1%) | 487(99%) |
| 536 | 488 | 5.62(3%) | 320(97%) |
| 541 | 843 | 5.64(15%) | 91.9(85%) |
| 548 | 1 871 | 3.01(24%) | 117(76%) |
| 554 | 353 | 8.73(5%) | 386(95%) |
| 563 | 649 | 4.52(1%) | 634(99%) |
| 569 | 1 683 | 2.85(3%) | 541(97%) |
| 575 | 546 | 7.46(3%) | 717(97%) |
| 592 | 549 | 6.15 | / |

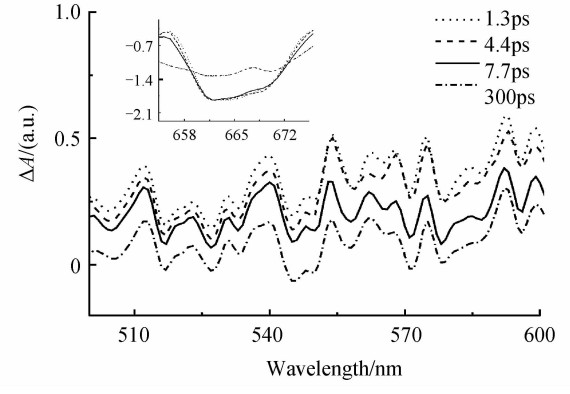


Fig. 5 The transient absorption difference spectra of aggregated LHC II at four time constant. (The inset is for about 667 nm)

Correspondingly, the transient absorption difference at about 667 nm (exciting wavelength) has little change in several picoseconds, then increase sharply at several hundred picoseconds (in the inset). The results show that the quenching process of excited state Chl a mostly occur in or over several hundred picoseconds. So combined with the lifetimes parameters of the kinetic curves, it is clear that the four carotenoids quench the triplet state of chlorophyll *a* in several picoseconds with low percentages, while they mostly quench the singlet oxygen state on several hundreds picoseconds timescale. Car522 seems to faster protect photosynthetic apparatus than the other three carotenoids, and has a larger percentage in quenching triplet state of chlorophyll *a*.

# 3 Conclusion

Photoprotection mechanism in LHC II of broypsis corticulans was studied using time resolved transient absorption spectroscopy. Transient absorption spectrum of LHC II was obtained using femtoseconds continuum light and, furthermore, transient absorption difference spectra of aggregate LHC II were obtained using pump-probe scheme with the pump light centered at 667 nm. The results indicate that carotenoids play an important role in the photoprotection process in two fashions, in a direct manner by quenching the triplet state of chlorophyll *a* in several picoseconds and, in the meantime, in an indirect manner by quenching the singlet state of oxygen in several hundreds of picoseconds.

**References**

[1] KÜHLBRANDT W, WANG D N, AND FUJIYOSHI Y. Atomic model of plant light-harvesting complex by electron crystallography[J]. *Nature*, 1994, **367**(17): 614-621.

[2] LIU Zhen-feng, YAN Han-chi, WANG Ke-bin, *et al*. Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution[J]. *Nature*, 2004, **428**(6980): 287-292.

[3] CHEN Hui, SHEN Shi-hua, HE Jun-fang, *et al*. A light-harvesting siphonaxanthin-chlorophyll a/b-protein complex of marine green alga[J]. *Chinese Science Bulletin*, 2004, **49**(18): 1936-1941.

[4] PENG Ju-fang, WANG Shui-cai, HE Jun-fang, *et al*. Study on energy transfer kinetics of homotrimer and heterotrimer of the light-harvesting complex Ⅱ from bryopsis corticulans[J]. *Acta Photonica Sinica*, 2005, **34**(3): 395-399.

[5] HE Jun-fang, WANG Shui-cai, REN Zhao-yu, *et al*. Energy transfer between pigment molecules of outer antenna[J]. *Acta Photonica Sinica*, 2002, **31**(6): 668-671.

[6] MONTENEGRO M A, NAZARENO M A., DURANTINI E N, *et al*. Singlet molecular oxygen quenching ability of carotenoids in a reversemicelle membrane mimetic system[J]. *Photochemistry and Photobiology*, 2002, **75**(4): 353-361.

[7] MIMURO M, TETZUYA K. Carotenoids in photosynthesis: absorption, transfer and dissipation of light energy[J]. *Pure and Applied Chemistry*, 1991, **63**(1): 123-130.

[8] HE Jun-fang, WANG Shui-cai, CAI Xia, *et al*. Kuang tingyun. characteristics of β-car pigments in isolated photosystem Ⅱ reaction center studied by selective excitation [J]. *Acta Photonica Sinica*, 2004, **33**(10): 1187-1191.

[9] MÜLLER P, LI X P, NIYOGI K K. Non-photochemical quenching. a response to excess light energy[J]. *Plant Physiology*, 2001, **125**(4): 1558-1566.

[10] BARZDA V, PETERMAN E J, VAN GRONDELLE R, *et al*. The influence of aggregation on triplet formation in light-harvesting chlorophyll a/b pigment-protein complex II of

green plants[J]. *Biochemistry*, 1998, **37**(2): 546-551.

[11] STANDFUSS J, VAN SCHELTINGA A C, LAMBORGHINI M *et al*. Mechanisms of photoprotection and nonphotochemical quenching in pea lightharvesting complex at 2.5Å resolution[J]. *The European Molecular Biology Organization Journal*, 2005, **24**(5): 919-928.

[12] ANDREW J. Y, DENISE P, ALEXANDER V. R, *et al*. The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis[J]. *Pure and Applied Chemistry*, 1997, **69**(10): 2125-2130.

[13] TOMÁŠ P, PAVEL C, CHERYL A K. Carotenoid - protein interaction alters the S1 energy of hydroxyechinenone in the orange carotenoid protein [J]. *Biochimica et Biophysica Acta*, 2013, **1827**(3): 248-254.

[14] RUBAN A V, BERERA R, ILIOAIA C, *et al*. Identification of a mechanism of photoprotective energy dissipation in higher plants[J]. *Nature*, 2007, **450**(7169): 575-578.

[15] HOLT N, ZIGMANTAS D, VALKUNAS L, *et al*. Carotenoid cation formation and the regulation of photosynthetic light harvesting [J]. *Science*, 2005, **307** (5708): 433-436.

[16] BODE S, QUENTMEIER C C, LIAO P N, *et al*. On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, **106**(30): 12311-12316.

[17] DONG Gao-feng, YANG Cheng-wei, XU Zhi-fang, *et al*. Illumination of xanthophyll cycle and non radiative energy dissipation[J]. *Progress in biochemistry and Biophysics*, 2002, **29**(1): 91-93.

[18] DIANA K, CHERYL A K. The orange carotenoid protein in photoprotection of photosystem II in cyanobacteria [J]. *Biochimica et Biophysica Acta*, 2012, **1817**(1): 158-166.

[19] ROSANNA C, KERSTIN A N, BERND K, *et al*. Effects of altered a- and b-branch carotenoid biosynthesis on photoprotection and whole-plant acclimation of Arabidopsis to photo-oxidative stress[J]. *Plant, Cell and Environment*, 2013, **36**(2): 438-453.

[20] HE Jun-fang, WANG Min-qiang, LUO Zhi-hui, *et al*. Ultrafast dynamics of electron and hole in ZnSe quantum dots in gel glasses[C]. SPIE, 2005, **5635**: 190-193.

[21] POLLI D, CERULLO G, LANZANI G, *et al*. Carotenoid-bacterialchlorophyll energy transfer in $LH_2$ complexes studied with 10-fs time resolution[J]. *Biophysical Journal*, 2006, **90**(7): 2486-2497.

[22] NOVODEREZHKIN V I, PALACIOS M A. Energy-transfer dynamics in the LHCII complex of higher plants: modified redfield approach[J]. *Journal of Physical Chemistry B*, 2004, **108**(29):10363-10375.

[23] ZIGMANTAS D, HILLER R G., SUNDSTRÖM V, *et al*. Carotenoid to chlorophyll energy transfer in the peridinin-chlorophyll-a-protein complexinvolves an intramolecular charge transfer state [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2002, **99**(26): 16760-16765.

[24] VAN AMERONGEN H, VAN GRONDELLE R. Understanding the energy transfer function of LHCII, the major light-harvesting complex of green plants[J]. *Journal of Physical Chemistry B*, 2001, **105**(3): 604-617.