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Two photon absorption energy transfer in the light-harvesting complex of photosystem II (LHC-II) modified with organic boron dye

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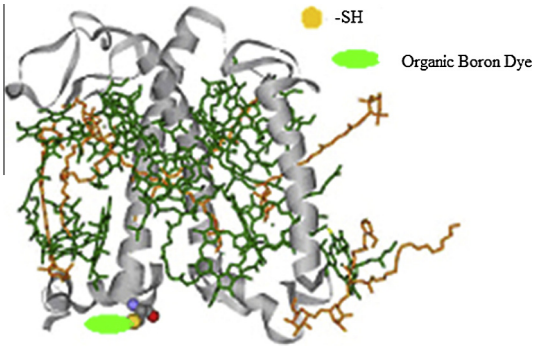
HIGHLIGHTS

- A two-photon absorption compound, DMDP-CH_2Br, was synthesized and linked to LHC-II.
- The LHC-II-dye complex can absorb two photons of the laser light effectively.
- The absorbed energy in the LHC-II-dye complex can transfer to chlorophyll a.

GRAPHICAL ABSTRACT

A two photon absorption compound, 4-(bromomethyl)-N-(4-(dimesitylboryl)phenyl)-N-phenyl-aniline (DMDP-CH_2Br), was synthesized and covalently linked to the LHC-II in formation of a LHC-II-dye complex. The LHC-II-dye complex can absorb two photons of the laser light effectively. The absorbed excitation energy is then transferred to chlorophyll a with an obvious fluorescence enhancement. The work may be interesting and give potentials for developing hybrid photosystems.



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ABSTRACT

The plant light-harvesting complexes of photosystem II (LHC-II) play important roles in collecting solar energy and transferring the energy to the reaction centers of photosystems I and II. A two photon absorption compound, 4-(bromomethyl)-N-(4-(dimesitylboryl)phenyl)-N-phenylaniline (DMDP-CH_2Br), was synthesized and covalently linked to the LHC-II in formation of a LHC-II-dye complex, which still maintained the biological activity of LHC-II system. Under irradiation with femtosecond laser pulses at 754 nm, the LHC-II-dye complex can absorb two photons of the laser light effectively compared with the wild type LHC-II. The absorbed excitation energy is then transferred to chlorophyll a with an obvious fluorescence enhancement. The results may be interesting and give potentials for developing hybrid photosystems.

Introduction

Collecting the solar energy and transferring the energy to the reaction centers of photosystems I and II are the most important

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role in plant light-harvesting complex of photosystem II (LHC-II) [1–3]. Isolated from different plants, such as pea, spinach and bamboo, each LHC-II polypeptide bound with chlorophyll a, chlorophyll b and carotenoid still remains the light-harvesting function [4,5]. Actually, the chlorophyll a and chlorophyll b are the main chemical pigments for light-harvesting. The absorption coefficients of LHC-II in blue portion of the sunlight spectrum are high enough, followed by the red portion. However, the absorption coefficient is poor in the green and near-infrared portions, which means that the LHC-II can not sufficiently capture the green and near-infrared light from the sun light [6]. Because of the solar energy is partly absorbed by the atmosphere, IR light of solar radiation reaching the Earth may have about 60% energy fractions. In order to absorb the solar energy efficiently, organic dye-modified complexes of LHC-II or its peptide fragments, such as LHCb1, LHCb2 and LHCb3 proteins (LHC-II-dye complex) had been prepared [7]. The excitation energy flow in the LHC-II system had been studied in different techniques for the targets of interpreting high energy efficacy of LHC-II as the major plant antenna and fabricating the potential artificial photosynthesis [8–10]. For efficient conversion of solar energy to chemical energy, the artificial photosynthetic systems had been studied for several decades [11]. The studies of artificial photosynthesis may involve introduction of different organic dyes to the photosystems I and II as a model to mimic the process and mechanism of photosynthesis, which will be a useful contribution to better understanding the photosynthesis and solar energy conversion.

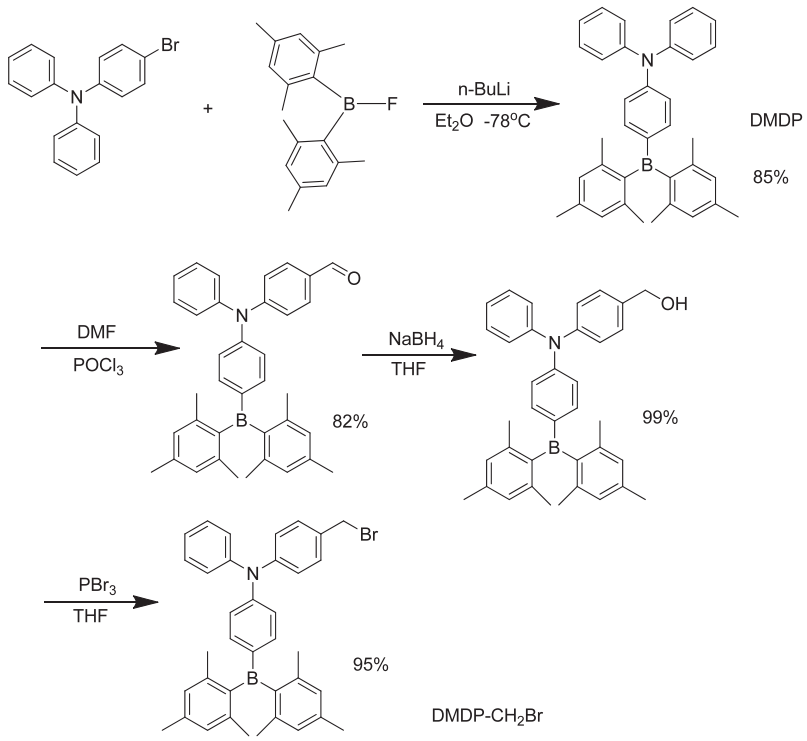
In the polypeptide sequences of the LHC-II [5], a sulphydryl group on cysteine residue could be modified by reaction with some organic dyes. Triarylboron compounds have attracted much attention due to their high fluorescence quantum yields and two photon absorption properties [12,13]. Recently, we synthesized a series of triarylboron compounds, i.e. 4-(dimesitylboryl)-N,N-diphenylaniline (DMDP) and its bromomethyl-linked derivative, 4-(bromomethyl)-N-(4-(dimesitylboryl)phenyl)-N-phenylaniline (DMDP-CH_2Br), as two photon fluorescence dyes in near-IR or IR region. The organic dye, DMDP-CH_2Br, can react with the sulphydryl group on the peptide fragment of LHC-II. After protein recombinant in vitro, a new light-harvesting photosystem II complex modified with organic boron dye (LHC-II-dye complex) was obtained, which retains its biological activity, and does not change the basic function of the LHC-II polypeptide. Under irradiation with femtosecond laser pulse at 754 nm, the modified LHC-II can absorb two photons of the laser light more effectively than the wild LHC-II. The energy is then transferred to chlorophyll a, resulting in an obvious fluorescence enhancement compared with the wild type LHC-II.

This work concerns the collection of the solar energy and conversion of the energy to the reaction centers of the organic boron dye-modified photosystems II, which may be a potential candidate for artificial photosynthesis. The corresponding research gives an approach to improve the energy transfer yield in artificial photosynthesis by two photo techniques, which contributes a better understanding of photosynthesis and also gives potentials in developing new hybrid photosystems.

Materials and methods

Synthesis of the organic boron dye, DMDP-CH_2Br

4-(Dimesitylboryl)-N,N-diphenylaniline (DMDP) was synthesized from 4-bromo-N,N-diphenylaniline as starting material in the temperature at -78 °C in 85% yield [14,15]. The DMDP was transferred to corresponding benzaldehyde and phenylmethanol derivatives in usual methods with the yields of 82% and 99%, respectively [16,17]. And then, the corresponding phenylmethanol derivative was bromized with PBr_3 to get the target organic boron compound [18], 4-(bromomethyl)-N-(4-(dimesitylboryl)phenyl)-N-phenylaniline (DMDP-CH_2Br), with the yield of 95%. The synthetic route is shown in Scheme 1.



Scheme 1. The synthesis scheme of the organic boron compound, DMDP-CH_2Br.

Preparation of light-harvesting complex of photosystem II modified with organic boron dye, LHC-II-dye complex

The LHCb1 apoprotein was isolated from pea using our published method [19], the sulphydryl group of cysteine residue in the sequence of LHCb1 acts as the reaction site to connect the organic boron dye at benzyl bromide active group. The reaction is illuminated in Scheme 2. LHCb1 60 mg was dissolved in 5 mL 0.5% sodium dodecyl sulfate (SDS) solution, to this aqueous solution DMDP-CH_2Br 4.2 mg in 0.3 mL THF was added in dropwise at room temperature. After addition of 0.3 mg $NaHCO_3$, the mixture was stirred for 3 days. The LHCb1-dye was precipitated by adding 6 mL ethanol, purified by centrifuging and washing with ethanol and water [20–22]. And then the LHCb1-dye was reconstituted to get the LHC-II-dye complex using published method [22,23]. Fig. 1 gives the results of sucrose density gradient ultracentrifugation of the wild type LHC-II and LHC-II-dye complexes. The clear difference in the results indicates that the organic boron dye has been connected to the LHC-II protein. The further experiments show that the artificial mutation of LHC-II with a covalently bonded organic dye has the similar biological activities with the wild type LHC-II protein. The chemical modified apoprotein LHCb1 does not interfere with LHC-II assembly, function and stability. The structure of the LHC-II-dye complex is shown in Fig. 2 for interpretation of the complex.

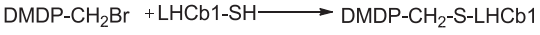
Absorption and fluorescence measurements

The absorption spectra were recorded using Hitachi U-3010 spectrophotometer, the fluorescence emission spectra were recorded with a Hitachi F-2500 fluorescence spectrophotometer. The two photon absorption induced emission spectra were carried using Coherent Chameleon XR Ti-Sapphire femtosecond laser (78 MHz) as excited light source and detected using Edinburgh FLS 900 spectrophotometer [24,25].

Results and discussion

Absorption spectra

LHCb1 apoprotein in 0.5% sodium dodecyl sulfate (SDS) aqueous solution does not show obvious absorption in visible range. The



Scheme 2. Reaction of the organic boron dye and the LHCb1 apoprotein.

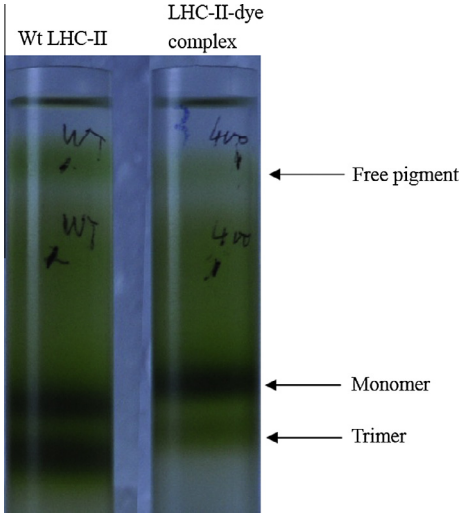


Fig. 1. Sucrose density gradient ultracentrifugation of wild type and LHC-II-dye complex.

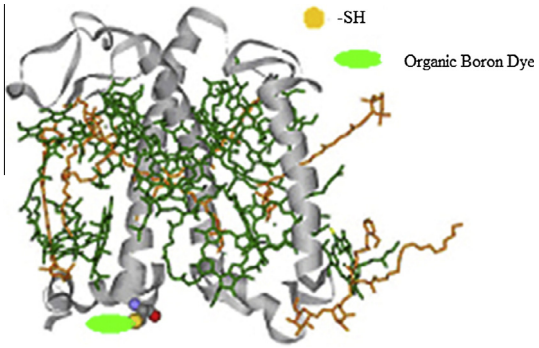


Fig. 2. The structure of the LHC-II-dye complex.

new formed LHCb1-dye has maximum absorption at 382 nm (Fig. 3), which is homologous with the absorption of the organic dye, DMDP-CH_2Br. The result indicates that the organic boron dye is connected to the LHCb1 apoprotein.

The LHCb1 and LHCb1-dye were reconstituted respectively in the same conditions, resulting in formation of wild type LHC-II and LHC-II-dye complex. Fig. 4 shows the absorption spectra of the wild type LHC-II and the LHC-II-dye complex in 0.5% SDS solutions. The absorption spectra of the two proteins are similar, which means that the LHC-II-dye complex may have similar optical absorption property with wild type LHC-II. As consistent with other published results, the absorptions are mainly contributed from the chlorophyll a and chlorophyll b [6,22]. The contribution from organic boron dye to the absorption is not obvious due to its low molar ratio in the LHC-II polypeptide system. This result indicated that the LHC-II-dye complex could retain the biological activity of normal wild LHC-II polypeptide in efficient capture of light energy without affect from the labeled dye.

Fluorescence spectra

The fluorescence spectra of LHCb1 and LHCb1-dye in SDS solutions were measured and shown in Fig. 5. The apoprotein LHCb1 has not obvious fluorescence emission under excitation at 377 nm, while the LHCb1-dye has a fluorescence maximum at 446 nm, which is the typical fluorescence of the organic boron dye.

The fluorescence spectra of the recombined wild type LHC-II and LHC-II-dye complex showed in Fig. 6, which were obtained at the excitation wavelengths of 377, 438 and 471 nm, near the absorption maximum of organic boron dye, chlorophyll a and

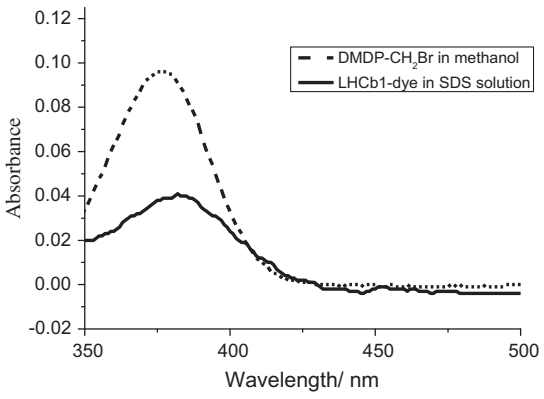


Fig. 3. The absorption spectra of LHCb1-dye in 0.1% dodecyl β-D-maltoside solution and DMDP-CH_2Br in methanol.

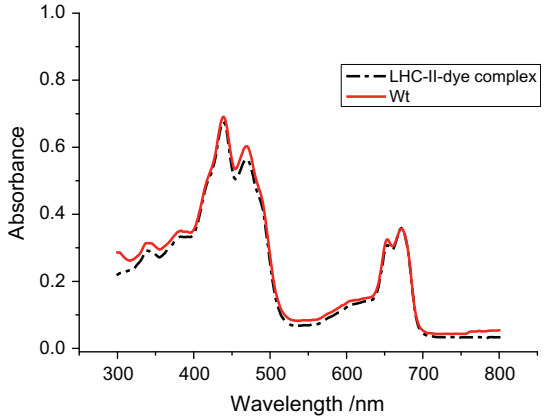


Fig. 4. Absorption spectra of wild type LHC-II and LHC-II-dye complex in SDS solutions (at same concentration).

chlorophyll b, respectively. In all the fluorescence spectra with different excitation wavelengths, the fluorescence maximums of the wild type LHC-II and LHC-II-dye complex are the same at 685 ± 1 nm as the emission from chlorophyll a [22]. The result indicates that the introduction of organic boron dye in the LHC-II protein will not change the energy transfer path in the light harvesting process (from chlorophyll b to chlorophyll a). Meanwhile, the excitation of the organic boron dye with 377 nm gave only the emission of chlorophyll a. It indicated clearly that the energy transfer is from the boron dye to the chlorophyll a or chlorophyll b.

Two photon excited fluorescence spectra

In order to investigate the energy transfer in the organic boron dye-labeled LHC-II-complex, femtosecond laser at 754 nm was used to excite the organic boron fragment for evaluating the change of the two photon excited fluorescence spectra (Fig. 7). Under excitation of the femtosecond laser, the relative fluorescence intensity of LHC-II-dye complex is obviously enhanced with 1.44 times higher than the wild type LHC-II, which presumes that the LHC-II-dye complex can absorb two photons of the laser more effectively compared with the wild type LHC-II, and then the energy is transferred to chlorophyll a. The results also suggest that chemical modified LHC-II system such as introduction of an organic dye to the LHC-II system could improve the light harvesting properties and the solar energy absorption efficiency.

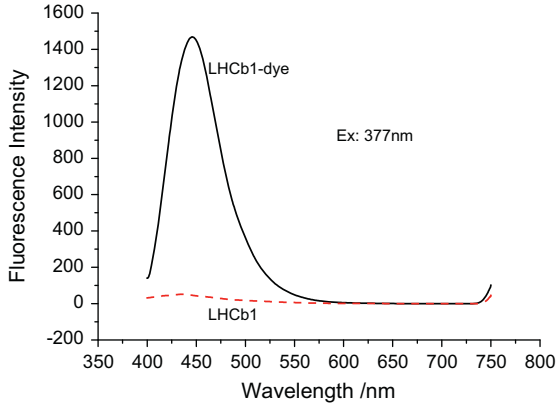


Fig. 5. Fluorescence spectra of LHCb1 and LHCb1-dye in SDS solutions (at same concentration).

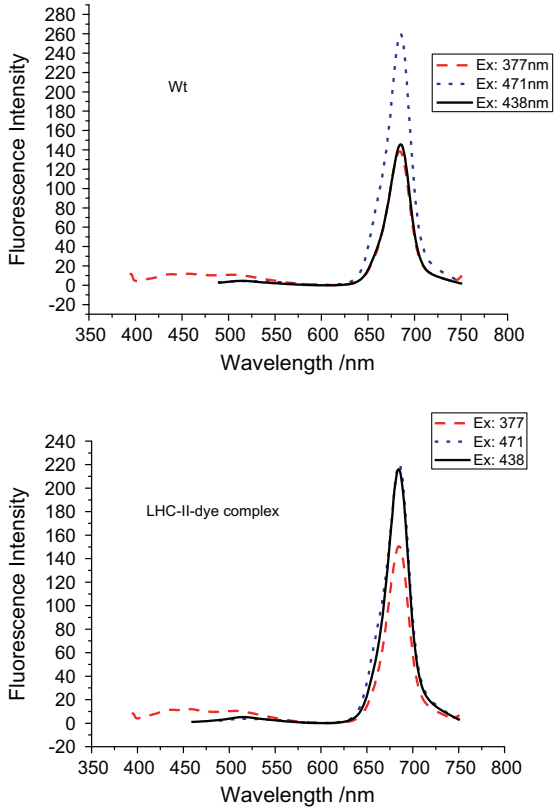


Fig. 6. Fluorescence spectra of wild type LHC-II (top) and LHC-II-dye complex (bottom) (at same concentration).

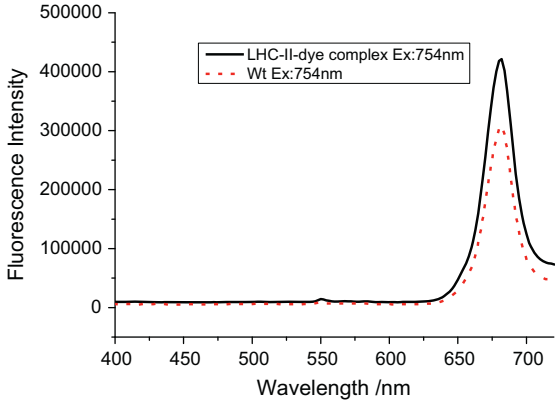


Fig. 7. Two photon excited fluorescence spectra of wild type LHC-II and LHC-II-dye complex (at same concentration).

Conclusions

We have synthesized a two photo absorption compound, 4-(bromomethyl)-N-(4-(dimesitylboryl)phenyl)-N-phenylaniline (DMDP-CH_2Br). It is clearly shown that the organic boron compound can be covalently linked with the LHC-II to get LHC-II-dye complex. The LHC-II-dye complex maintains the biological activity of the LHC-II system, and the organic boron dye does not change the basic function of the LHC-II polypeptide. Under irradiation with 754 nm femtosecond laser, the LHC-II-dye complex can absorb two photons of the laser more effectively compared with the wild type LHC-II. The absorbed excitation energy is then transferred to chlorophyll a with an obvious

fluorescence enhancement. Further researches on improvement of the energy transfer efficiency are in progress. The work may be interesting and give potentials for developing hybrid photosystems.

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