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Direct energy transfer from the major antenna to the photosystem II core complexes in the absence of minor antennae in liposomes

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ABSTRACT

Minor antennae of photosystem (PS) II, located between the PSII core complex and the major antenna (LHCII), are important components for the structural and functional integrity of PSII supercomplexes. In order to study the functional significance of minor antennae in the energetic coupling between LHCII and the PSII core, characteristics of PSII–LHCII proteoliposomes, with or without minor antennae, were investigated. Two types of PSII preparations containing different antenna compositions were isolated from pea: 1) the PSII preparation composed of the PSII core complex, all of the minor antennae, and a small amount of major antennae (MCC); and 2) the purified PSII dimeric core complexes without periphery antenna (CC). They were incorporated, together with LHCII, into liposomes composed of thylakoid membrane lipids. The spectroscopic and functional characteristics were measured. 77 K fluorescence emission spectra revealed an increased spectral weight of fluorescence from PSII reaction center in the CC–LHCII proteoliposomes, implying energetic coupling between LHCII and CC in the proteoliposomes lacking minor antennae. This result was further confirmed by chlorophyll *a* fluorescence induction kinetics. The incorporation of LHCII together with CC markedly increased the antenna cross-section of the PSII core complex. The 2,6-dichlorophenolindophenol photoreduction measurement implied that the lack of minor antennae in PSII supercomplexes did not block the energy transfer from LHCII to the PSII core complex. In conclusion, it is possible, in liposomes, that LHCII transfer energy directly to the PSII core complex, in the absence of minor antennae.

1. Introduction

Photosystem (PS) II is a multisubunit pigment–protein complex that utilizes the solar energy to catalyze water splitting and plastoquinone reduction in the thylakoid membrane of cyanobacteria, algae and higher plants. It supplies oxygen and energy for life on earth and is therefore called "the engine of life" [1]. The subunits of PSII can be classified into two groups: the PSII core complex and the peripheral antenna system. The PSII core complex mainly consists of three structural domains: the PSII reaction center (RC) composed of D1 and D2 proteins that stabilize the carriers for charge separation and primary electron transport, the inner antennae CP47 and CP43, and a set of extrinsic proteins on the lumen side involved in oxygen evolution. There are also several other low molecular subunits in the core complex [2]. Detailed structural information on the PSII core complex of cyanobacteria was obtained from high resolution X-ray diffraction [3–8]. The PSII core complex of higher plants has been studied at lower resolution by electron crystallography [9–11].

The peripheral antenna system of PSII is responsible for light harvesting, for energy transfer to PSII RC, for dissipating excess energy as heat, and for regulating the distribution of excitation energy between PSI and PSII [12]. In higher plants, the major antenna of PSII (the light-

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-Dichlorophenol indophenol; β-DDM, dodecyl-β-D-maltoside; DGDG, digalactosyl diacylglycerol; DPC, 1,5-Diphenylcarbazide; LHCII, major antenna of photosystem II; MGDG, monogalactosyl diacylglycerol; TLC, thin-layer chromatography; NPQ, non-photochemical quenching; OEC, oxygen evolution complex; OG, n-octyl-β-D-glucopyranoside; PG, phosphatidylglycerol; PL, proteoliposomes; PS, photosystem; RC, reaction center; SQDG, sulfoquinovosyl diacylglycerol; MCC, PSII preparation with PSII core complex, minor antennae, and small amounts of major antennae; CC, purified PSII core complex without peripheral antenna; MCC PL, MCC proteoliposomes; CC PL, CC proteoliposomes; LHCII PL, LHCII proteoliposomes; L2MCC PL, MCC–LHCII proteoliposomes co-inserted with 2 mol LHCII trimers per PSII RC dimer; L2CC PL, CC–LHCII proteoliposomes co-inserted with 2 mol LHCII trimers per PSII RC dimer; L6MCC PL, MCC–LHCII proteoliposomes co-inserted with 6 mol LHCII trimers per PSII RC dimer; L6CC PL, CC–LHCII proteoliposomes co-inserted with 6 mol LHCII per PSII RC dimer; MCC PL + L2 PL, mixture of MCC PL and LHCII PL with the same LHCII/PSII RC ratio as L2MCC PL; CC PL + L2 PL, mixture of CC PL and LHCII PL with the same LHCII/PSII RC ratio as L2CC PL; MCC PL + L6 PL, mixture of MCC PL and LHCII PL with the same LHCII/PSII RC ratio as L6MCC PL; CC PL + L6 PL, mixture of CC PL and LHCII PL with the same LHCII/PSII RC ratio as L6CC PL

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harvesting complexes of PSII, LHCII), composed of the nuclear-encoded proteins Lhcb1-3, also play important roles in lateral segregation of the main pigment-protein complexes in thylakoid membrane and maintaining the grana structure [13]. Three minor antennae, CP29, CP26 and CP24, the products of Lhcb4-6 genes, are present as monomers in vivo. The antenna system of PSII is highly dynamic, undergoing flexible changes in coping with the changing environments [14]. Electron microscopic analyses show that the PSII–LHCII supercomplexes are composed of several LHCII trimers associated with the PSII core complex, with minor antennae located between them [15–18]. Normally, two strongly bound LHCII trimers (S-LHCII, S_2) and two moderately bound LHCII trimers (M-LHCII, M_2) are associated with dimeric PSII core complex to form $C_2S_2M_2$ supercomplexes. This is proposed to represent the general organization of PSII–LHCII supercomplexes and to act as the structural and functional basic unit for PSII [19,20]. In addition, there are also abundant "extra" LHCII trimers [21].

Studies on the functions of minor antennae have mainly been focused on three aspects: 1) the assembly of PSII supercomplexes; 2) energy transfer from LHCII to the PSII core complex and 3) photoprotection or photoinhibition. It is reported that CP29 plays a crucial role in the assembly and stability of the supercomplexes since no PSII–LHCII supercomplex could be found upon mild detergent solubilization of thylakoid membrane from plants lacking CP29 [22]. Nevertheless, de Bianchi et al. [23] presented the evidence that it was still possible to form C_2S_2M and $C_2S_2M_2$ particles without CP29 in the *koLhcb4* mutant. Functionally, an *Arabidopsis thaliana* mutant lacking CP29 showed decreased maximal photosynthetic efficiency of PSII and reduced capacity for non-photochemical quenching (NPQ) [23,24]. Recently, a possible path for NPQ in CP29 was assigned based on its high-resolution crystal structural analysis [25]. Picosecond fluorescence kinetics measurement revealed that lack of CP29 decreased the energy migration kinetics from LHCII to PSII RC [26]. Conflicting observations have been presented regarding the structural significance of CP24. In *A. thaliana*, it is reported to be necessary for the association of the M-LHCII to the PSII core and the electron transport in the thylakoid membrane [27,28], While in *Chlamydomonas reinhardtii*, each dimeric PSII core can still bind up to six LHCII trimers in the absence of CP24 [29]. Furthermore, it has been observed that the energy migration kinetics from LHCII to the PSII core is slowed down in thylakoids lacking CP24 [26]. In contrast to CP24 and CP29, CP26 seems to be neither important in the assembly of PSII supercomplexes, nor have any effect on the energy migration in PSII [22,26,27,30]. Furthermore, CP26 can adopt a trimeric structure and function as major antenna in case Lhcb1-2 are lacking [31]. The picosecond-fluorescence spectroscopy measurement showed that the migration time from LHCII to the PSII core increased enormously in the thylakoid membrane of the *A. thaliana* line depleted of all the minor antennae [32].

The photosynthetic membrane is a complex biological membrane system containing densely packed proteins in a lipid bilayer, about half of which consists of non-bilayer lipids. The lipids not only function as a matrix stabilizing different photosynthetic pigment–protein supercomplexes, but also influence the structure and function of the proteins via specific or non-specific interactions with the membrane proteins [33,34]. As a complement to in vivo studies, the incorporation of membrane proteins into artificial membranes has become an important tool for evaluating the functions of membrane proteins and their interactions with one another and with lipids [35]. It is based on pioneering work with liposomes composed of different lipids which showed that different photosynthetic membrane proteins could be incorporated and interact with one another in liposome membranes [36–39].

The technique has been used in this work for studying functions of all the minor antennae as a complete set. Two types of PSII preparations with different antennae compositions were isolated from pea and co-reconstituted with LHCII into liposomes composed of thylakoid membrane lipids. The spectroscopic and functional characteristics of different PSII–LHCII proteoliposomes were compared to mixtures of liposomes containing only PSII preparations with liposomes containing LHCII, but of similar net composition. The results show that in this in vitro system, energy transfer is possible from LHCII to PSII RC in the absence of any minor antennae.

2. Materials and methods

2.1. Isolation of PSII complexes and LHCII

Pea (*Pisum sativum* L.) was grown in a 14-h photoperiod under an irradiance of 100 μmol photons/m^2/s. The temperature and relative humidity were set at 23/19 °C (day/night) and 70%, respectively. BBY membranes were isolated from 2-week-old pea leaves according to Berthold et al. [40] with proper modifications due to the characteristic compositions of pea thylakoid in contrast to spinach. The whole isolation process was performed on ice in a dark room under a 520 nm LED illumination. The BBY membrane with chlorophyll (Chl) concentration of 2 mg/mL was resuspended in the storing buffer (15 mM NaCl, 5 mM $CaCl_2$, 0.5 M betaine, 0.4 M sucrose, 20 mM MES–NaOH, pH 6.5), then frozen in liquid nitrogen and stored at −80 °C. The Chl concentration was determined according to Porra et al. [41].

PSII preparations (MCC) composed of PSII core, all the minor antennae, and a small amount of major antennae were purified according to the modified method of Hankamer et al. [42]. Frozen BBY membrane containing 120 mg Chl was slowly thawed on ice and dispersed in 180 mL buffer A (0.5 M sucrose, 40 mM MES NaOH, pH 6.0) and centrifuged for 15 min at 48,000 ×*g*. The pellet was resuspended in 24 mL buffer B (1.8 M sucrose, 18 mM NaCl, 72 mM $MgCl_2$, 72 mM MES–NaOH, pH 6.0) and centrifugation tubes were quickly rinsed with 17 mL 346 mM n-octyl-β-D-glucopyranoside (OG) (Merck, Germany). The suspension was homogenized and incubated, under stirring, on ice for 75 min. Then 70 mL buffer A was added into the suspension and the mixture was centrifuged for 10 min at 48,000 ×*g*. The supernatant was combined with 195 mL buffer C (40 mM MES–NaOH, pH 6.0) and centrifuged for 1 h at 150,000 ×*g*. The supernatant was diluted by a half volume of buffer C and then centrifuged for 30 min at 50,000 ×*g*. The pellet was resuspended in a small volume of liposome buffer (10 mM NaCl, 25 mM MES–NaOH, pH 6.5) and homogenized in a glass homogenizer. MCC with a final Chl concentration of 1 mg/mL was frozen in liquid nitrogen and stored at −80 °C.

Purified PSII core complexes (CC) were isolated starting from MCC according to Hankamer et al. [42] with some modifications. The MCC was incubated with 25 mM dodecyl-β-D-maltoside (β-DDM) (Anatrace, Inc., UK) with stirring for 10 min on ice. The suspension was layered on the top of a continuous sucrose density gradient (0.5 M sucrose, 0.03% (w/v) β-DDM, 10 mM NaCl, 5 mM $CaCl_2$, 25 mM MES–NaOH, pH 6.5) formed by freeze–thaw method and centrifuged at 90,000 ×*g* for 5 h in a VTi 50 rotor (Beckman Coulter, Inc., USA). The band corresponding to dimeric PSII core complex was collected and then concentrated at 5000 ×*g* for 30 min with an Amicon Ultra®-15 membrane filter with a 100 kDa cutoff (Millipore, USA). CC was immediately adjusted to proper Chl concentration and used for assays.

Large amounts of crude LHCII were prepared from pea by the method described in Rühle and Paulsen [43]. The crude LHCII was further purified to avoid the contamination from minor antennae according to Liu et al.. The crude LHCII was solubilized completely with 1% (w/v) OG at a Chl concentration of 4 mg/mL and subjected to gel-filtration chromatography using a Superdex® 200 Hiload® 16/600 column (General Electric Company, USA) in an ÄKTA® Purifier system (General Electric Company, USA) [44]. The fractions of LHCII trimers were collected and precipitated with 100 mM KCl. After it was rinsed twice using distilled water, LHCII was frozen in liquid nitrogen and stored at −80 °C.