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Excitation energy transfer between Light-harvesting complex II and Photosystem I in reconstituted membranes

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ABSTRACT

Light-harvesting complex II (LHCII), the major peripheral antenna of Photosystem II in plants, participates in several concerted mechanisms for regulation of the excitation energy and electron fluxes in thylakoid membranes. In part, these include interaction of LHCII with Photosystem I (PSI) enhancing the latter's absorption cross-section – for example in the well-known state 1 – state 2 transitions or as a long-term acclimation to high light. In this work we examined the capability of LHCII to deliver excitations to PSI in reconstituted membranes *in vitro*. Proteoliposomes with native plant thylakoid membrane lipids and different stoichiometric ratios of LHCII:PSI were reconstituted and studied by steady-state and time-resolved fluorescence spectroscopy. Fluorescence emission from LHCII was strongly decreased in PSI–LHCII membranes due to trapping of excitations by PSI. Kinetic modelling of the time-resolved fluorescence data revealed the existence of separate pools of LHCII distinguished by the time scale of energy transfer. A strongly coupled pool, equivalent to one LHCII trimer per PSI, transferred excitations to PSI with near-unity efficiency on a time scale of less than 10 ps but extra LHCIIs also contributed significantly to the effective antenna size of PSI, which could be increased by up to 47% in membranes containing 3 LHCII trimers per PSI. The results demonstrate a remarkable competence of LHCII to increase the absorption cross-section of PSI, given the opportunity that the two types of complexes interact in the membrane.

1. Introduction

Photosynthetic organisms, particularly green plants, have evolved specialized multigene light-harvesting antenna systems that dynamically regulate the flow of excitation energy to the photosynthetic reaction centres (RCs). In vascular plants the two photosystems, Photosystem I (PSI) and Photosystem II (PSII) with their separate peripheral antenna proteins – LHCI (Lhca1–4) and LHCII (Lhcb1–6) – are preferentially located in the unstacked and stacked regions, respectively, of the chloroplast thylakoid membranes. Light harvesting is finely regulated and continuously adapted to the physiological conditions to achieve balanced energy input to the photosystems and prevent photodamage under conditions of excess light [1,2]. LHCII plays a major role in balancing and regulating the excitation energy and electron flow [3–5]. In the so-called state 1 – state 2 transitions in plants and green algae, excitation balance between PSI and PSII is maintained by shuttling of LHCII between them (for reviews see [6,7]). The classical view is that a subpopulation of the LHCII proteins, normally associated with PSII (in state 1) becomes phosphorylated by the STN7 kinase [8,9] and migrates from the PSII-enriched stacked, granal region to the PSI-containing unstacked, stromal region, interacting with PSI and forming PSI–LHCII supercomplexes [10,11]. PSI–LHCII supercomplexes have been successfully isolated and purified using detergents [12–15] or styrene-maleic acid copolymer [16]. LHCII has been shown to transfer energy to PSI in the PSI–LHCII supercomplexes with high efficiency [12,15], so that Galka et al. [12] proposed that mobile LHCII can be considered as an integral part of PSI in state 2. Recent studies have put forward the notion that a significant amount of LHCII is associated with PSI under normal conditions [15–17] in the absence of phosphorylation. According to Wientjes et al. [15] more than half of the PSI complexes could bind one LHCII trimer, depending on the growth-light conditions. More recently, Bell et al. [16] have isolated PSI–LHCII membrane fractions from stacked spinach thylakoids at estimated stoichiometry of three LHCII trimers per PSI, at least some of them being functionally coupled to the RC. On the other hand, Ünlü et al. [18] examined the antenna sizes of PSII and PSI upon state transitions in *Chlamydomonas* and found that, despite the significantly higher number of mobile LHCIIs in the green alga, only a very small fraction, less than one trimer, actually delivers excitation energy to PSI in state 2. A similar conclusion was

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drawn in ref. [19] from a comparison of the PSI antenna size in state 1 and 2 estimated from electrochromic absorbance changes.

In the present study we investigated the ability of LHCII to serve as antenna of PSI by utilising a bottom-up approach. Isolated LHCII and PSI were reconstituted into lipid membranes, allowing to test the effective antenna size of PSI under controlled conditions at varying known stoichiometries of LHCII:PSI. We investigated the reconstituted PSI–LHCII membranes by steady-state and time-resolved fluorescence spectroscopy with special focus on determining the efficiency and dynamics of excitation energy transfer from LHCII to PSI and the overall quantum yield of PSI photochemistry. We found that a large fraction of the energy absorbed by LHCII was efficiently transferred to PSI in membranes containing up to three LHCII trimers per RC, thereby significantly increasing the effective PSI absorption cross-section. Understanding and quantifying the functional coupling of LHCII and PSI in the artificial membranes may have important implications for designing future solar devices. PSI is very attractive for engineering hybrid biosolar devices due to its high efficiency and inherent stability [20–24]; the ability to optimize and extend its absorption cross-section can be of significant advantage.

2. Materials and methods

2.1. Isolation of LHCII

PSII-enriched membrane fragments (BBY) were isolated from dark-adapted leaves of green-house grown pea (*Pisum sativum*). LHCII was isolated from BBY membranes, solubilized with 0.7% dodecyl-β-maltoside (β-DM), by sucrose density gradient ultracentrifugation as in Caffarri *et al.* [25]. LHCII trimer bands collected from the gradients were washed with 10 mM Tricine buffer (pH 7.8) and concentrated with 30 kDa cutoff Amicon filters (Millipore), then frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Isolation of PSI

PSI-enriched stromal membrane vesicles were isolated from pea leaves by fractionation with digitonin, following the protocol of Peters et al. [26]. The preparations, with chlorophyll (Chl) a/b ratio of 9–10, were further purified by solubilisation with 1% β-DM and ultracentrifugation on a 0.1–1 M sucrose density gradient in a swing-out rotor at 200,000 *g* for 20 h. The green bands containing PSI–LHCI supercomplexes were collected, concentrated with Centricon devices (Millipore) and frozen in liquid nitrogen. Immunoblot analysis showed that after β-DM solubilisation and purification the PSI samples were virtually free of LHCII proteins (Supplementary Fig. S1).

2.3. PSI–LHCII membranes

Liposomes were prepared from mixtures of plant thylakoid lipids (50.0% (*w*/*v*) monogalactosyldiacylglycerol (MGDG), 31.0% digalactosyldiacylglycerol (DGDG), 10.7% phosphatidylglycerol (PG) and 8.3% sulfoquinovosyldiacylglycerol (SQDG)) as described in [27] with an additional 10-step freeze–thaw cycle prior to extrusion.

For preparing PSI–LHCII membranes, purified solubilized trimeric LHCII complexes and PSI–LHCI supercomplexes were mixed at molar Chl ratios of LHCII/PSI equal to 0.26, 0.52 and 0.78 – equivalent to approximately one, two and three LHCII trimers per PSI, respectively. The protein mixture was then added dropwise to a suspension of liposomes at molar lipid:protein ratio of 450:1, calculated based on PSI content only. After mild sonication in a bath sonicator the mixture was incubated at room temperature for 30 min. The detergent was then removed by cycled incubation with absorbent beads (Bio-Beads SM2, Bio-Rad). The sample was finally centrifuged for 30 min at 25,000 g to remove unincorporated protein aggregates. LHCII-only and PSI-only proteoliposomes were reconstituted at molar lipid:protein ratios of 100:1 or 300:1 for LHCII, and 450:1 for PSI. The orientation of the pigment–protein complexes in the reconstituted membranes was tested by measuring the linear dichroism of the samples aligned by gel compression [28].

2.4. Chlorophyll determination

The Chl content of all samples was determined spectrophotometrically from 80% acetone extract using molar absorption coefficients from Porra et al. [29].

2.5. Dynamic light scattering

The average size and size distribution of the sample components were measured by a W130 dynamic light scattering apparatus (AvidNano, UK). After appropriate dilution, 80 μl of the samples was loaded into microcuvettes and thermostated to 25 °C for the measurement. Data analysis was performed with the "i-Size" software supplied with the instrument.

2.6. Freeze-fracture electron microscopy

The freeze-fracturing and electron microscopy of reconstituted proteoliposomes was done as described in [27]. Briefly, the sample suspension, deposited onto a gold sample holder, was flash-frozen in partially solidified Freon. Fracturing was performed at −100 °C in a freeze-fracture device (BAF 400D, Balzers AG, Liechtenstein) and the surface was etched at −110 °C. Replicas were prepared by platinum-carbon shadowing, placed on copper grids and examined in a Morgagni 268D (FEI, The Netherlands) transmission electron microscope.

2.7. Absorption and circular dichroism spectroscopy

Absorption and CD spectra were recorded between 350 and 750 nm with 3 nm spectral resolution using an Evolution 500 dual-beam spectrophotometer (Thermo Scientific) and a J-815 spectropolarimeter (Jasco). Measurements were carried out at room temperature. The samples were diluted in 10 mM NaCl and 10 mM Tris/HCl (pH 7.8) buffer to absorbance of 1.0 at the red maximum. Solubilized complexes were measured in buffer containing 0.03% β-DM. Optical path length was 1 cm.

2.8. Low-temperature fluorescence spectra

For measurements at 77 K diluted samples were deposited on filter paper discs to ca. 0.5 μg cm^{-2} Chl and immersed in liquid nitrogen. Steady-state fluorescence spectra were recorded from the discs with a Fluorolog spectrofluorometer (Jobin Yvon Horiba). Emission spectra were recorded with excitation wavelength of 436 nm and 3 nm detection bandwidth. Excitation spectra were recorded at 730 nm detection wavelength.

2.9. Time-resolved fluorescence

Room-temperature fluorescence decays were recorded by time-correlated single-photon counting using a FluoTime 200 spectrometer (PicoQuant, Germany) equipped with a microchannel plate detector (Hamamatsu, Japan) and a PicoHarp 300 TCSPC system (PicoQuant). Excitation pulses at 633 nm with 6 ps temporal width, 0.1 pJ pulse energy and 20 MHz repetition rate were generated by a WhiteLase Micro supercontinuum laser (Fianium, UK). Fluorescence emission was detected through a monochromator at wavelengths between 670 and 750 nm and binned in 4 ps time channels. The sample was continuously circulated through a flow cell with 1.5 mm path length. The optical density at the excitation wavelength was 0.03. The total instrument