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Monte Carlo simulations of excitation and electron transfer in grana membranes

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ABSTRACT

Time-resolved fluorescence measurements on grana membranes with instrumental response function of 3 ps reveal faster excitation dynamics (120 ps) than those reported previously. A possible reason for the faster decay may be a relatively low amount of "extra" LHCII trimers per reaction center of Photosystem II. Monte Carlo modeling of excitation dynamics in $C_2S_2M_2$ form of PSII–LHCII supercomplexes has been performed using a coarse grained model of this complex, constituting a large majority of proteins in grana membranes. The main factor responsible for the fast fluorescence decay reported in this work was the deep trap constituted by the primary charge separated state in the reaction center (950–1090 cm^{-1}). This value is critical for a good fit, whereas typical hopping times between antenna polypeptides (from ~4.5 to ~10.5 ps) and reversible primary charge separation times (from ~4 to ~1.5 ps, respectively) are less critical. Consequently, respective mean migration times of excitation from anywhere in the PSII–LHCII supercomplexes to reaction center range from ~30 to ~80 ps. Thus 1/4–2/3 of the ~120-ps average excitation lifetime is necessary for the diffusion of excitation to reaction center, whereas the remaining time is due to the bottle-neck effect of the trap. Removal of 27% of the Lhcb6 apoprotein pool by mutagenesis of *DEG5* gene caused the acceleration of the excitation decay from ~120 to ~100 ps. This effect may be due to the detachment of LHCII-M trimers from PSII–LHCII supercomplexes, accompanied by deepening of the reaction center trap.

1. Introduction

In grana membranes, Photosystem II occurs as a dimeric PSII–LHCII supercomplex composed of two core complexes (C_2) and two sets of symmetrical, peripheral antenna complexes surrounding the cores. Each core complex contains one reaction center and inner light-harvesting complexes CP43 and CP47 (comprising PsbB and PsbC apoproteins, respectively). The set of peripheral antenna complexes makes CP29, CP26 and CP24 which are connected directly to the core as well as two types of LHCII trimers: S trimers (strongly bound to the core, in contact with CP43 and CP26) and M trimers (moderately bound to the core, in contact with CP29 and CP24) [1]. Presumably, the largest supercomplex called $C_2S_2M_2$ – containing two LHCII S trimers and two M trimers – is the complex which is the most abundant one in *Arabidopsis thaliana* thylakoid membranes [2].

The antenna size of PSII–LHCII supercomplex plays an important role in excitation energy transfer within this complex. Namely, studies performed on PSII–LHCII supercomplexes with various antenna sizes (purified from grana membranes by separation on sucrose gradients) demonstrated that a decrease in size of the complex led to speeding up of fluorescence kinetics [3]. However, since purification of PSII–LHCII supercomplexes with different antenna sizes requires the application of detergents to solubilize thylakoid membranes, which may affect adversely the architecture and excitation energy transfer of the supercomplexes, native membranes (grana or thylakoids) remain a preferable system to study excitation energy transfer in PSII. In the case of thylakoids, however, contribution from Photosystem I makes interpretation of the experimental data of excitation energy transfer in PSII much more complex and problematic [4].

Using single photon counting approach it was shown that the average excitation lifetime in grana is about 150 ps [3,5,6], significantly less than the values reported in older papers [7,8] which came likely from

Abbreviations: FWHM, full width at half maximum; IRF, instrumental response function; MC, Monte Carlo; Hepes, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); PVDF, polyvinylidene difluoride; RC, reaction center

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grana samples "contaminated" by closed complexes (with reduced Q_A acceptor) or from uncoupled LHCII trimers. In principle in any studies performed on grana membranes with the use of fluorescence techniques there is some uncertainty associated with the presence of regions rich in "extra" LHCII trimers [9]. Precise determination of the number of LHCII trimers per PSII core may be helpful in the modeling of excitation energy transfer in native photosynthetic membranes. Recently, a coarse-grained model was successfully introduced allowing calculation of a few basic parameters influencing the excitation decay in PSII–LHCII supercomplexes and depicting interpolypeptide excitation hopping, primary and secondary charge separation, and charge recombination in PSII reaction centers [3,5,6]. These parameters allow estimation of mean migration time of excitation from the antenna system to reaction center and, in consequence, allow conclusions on the limiting steps in excitation decay.

In this paper we report significantly faster excitation decay in grana membranes than previously reported and model this decay using the Monte Carlo method of excitation and electron transfer in PSII–LHCII supercomplex. A model of significant reversibility of primary charge separation is supported and a new set of hopping and electron transfer parameters is proposed to be consistent with a short, 120-ps, average excitation lifetime. The data obtained for WT preparations are confronted with those recorded for mutant lacking ~25% of Lhcb6 apoprotein pool (CP24 complex).

2. Materials and methods

2.1. Plant material and growth conditions

A. thaliana plants (ecotype Columbia) were grown for five weeks in 42-mm Jiffy peat pellets on sphagnum peat moss and wood pulp (AgroWit, Przylep, Poland) under long-day conditions (16 h/8 h, light/dark) with an irradiance of 110 μmol photons m^{-2} s^{-1} at a constant temperature of 22 °C and 70% humidity. *A. thaliana* seeds (SALK_099162) with a T-DNA insertion in the *DEG5* gene (At4g18370) were obtained from NASC (Nottingham Arabidopsis Stock Center, Nottingham, UK). The T-DNA insertion was confirmed and hetero/homozygosity was analyzed by PCR using the following primers: forward, 5′-GCTTTTTCCTCA ATCTTCAATAC-3′ and reverse, 5′-AGGATTTAGTTCACGTCCCTC-3′ for the Deg5 sequence, and LBb1 (5′-GCGTGGACCGCTTGCTGCAACT-3′) for the insertion.

2.2. Isolation of thylakoids and grana membranes (BBY samples)

Intact chloroplasts were prepared using the Sigma Chloroplast Isolation Kit (Sigma-Aldrich, St. Louis, MO, USA) as described by us earlier [10] and the isolation of thylakoids was done according to [11]. Grana membranes were isolated from chloroplasts of wild type (WT) or *deg5* mutant plants according to the method of [12] with the modifications described by [13], using Triton X-100/Chl ratio of 21.2:1 (v/v) to solubilize thylakoid membranes. The pellet of grana membranes was resuspended in a small volume of a buffer containing 20 mM Hepes/KOH (pH = 7.5), 5 mM $MgCl_2$, 15 mM NaCl and 10% glycerol and stored at −20 °C.

2.3. SDS-PAGE, immunoblotting, protein quantitation and calculation of the number of LHCII trimers per PSII core

SDS-PAGE of thylakoids or grana membranes was performed in 14.5% acrylamide gels using the [14] buffer system. The gels were stained with 0.025% CBB G-250 by the method of [14]. The stained gels were scanned using Gelix One software (Biostep, Jahnsdorf, Germany) and the number of LHCII trimers per PSII core was calculated as [(Lhcb1 + Lhcb2 + Lhcb3) / Lhcb4] / 3. A 1.1 times stronger binding of CBB G-250 to Lhcb4 vs Lhcb1/2/3 was taken into account [15].

To identify and quantify selected PSII apoproteins electrophoretically resolved thylakoid and grana membranes were electrotransferred onto PVDF membranes (Roche, Basel, Switzerland), reacted with polyclonal antibodies raised against Lhcb1–6, PsaB, PsbC or PsbD apoproteins (Agrisera, Vannas, Sweden), detected by applying goat anti-rabbit biotinylated immunoglobulin G (Agrisera, Vannas, Sweden) and visualized by using the Enhanced Chemiluminescence System (ECL) according to the manufacturer's recommendations (Lumi-Light Western Blot Substrate, Roche Diagnostics GmbH, Mannheim, Germany). The quantification of immunostained signals was performed using Gelix One software.

2.4. Chlorophyll quantitation

The Chl concentration was assayed according to [16].

2.5. Measurements with streak camera

For streak camera measurements, 20 μl of concentrated grana membranes solution was diluted (to final OD of ~0.1 cm^{-1} at maximum of Q_y band) in 2 ml 20 mM Hepes buffer pH = 6.5 containing 15 mM NaCl, 5 mM $MgCl_2$, 0.005% β-dodecylmaltoside, and fresh 1 mM $K_3Fe(CN)_6$ in order to keep the reaction center in open state. The small amount of detergent was added in order to minimize formation of large aggregates of grana membranes.

The time-resolved fluorescence measurements were performed using the setup in Laser Centrum, Vrije Universiteit, Amsterdam described in detail elsewhere [17]. The samples were excited at 400 nm with a repetition rate of 125 kHz by vertically polarized ~100-fs pulses. The diameter of the laser spot on the sample was of about 200 μm. The typical energy of excitation was ~1 nJ per pulse, well below the level leading to annihilation.

The fluorescence signal, measured at a right angle with respect to the excitation beam by the detection system composed of spectrograph (Chromex 250IS), streak camera (Hamamatsu C5680), and CCD camera (Hamamatsu C4880), was recorded in three time windows: ~150 ps, ~350 ps, and ~1500 ps with temporal resolution (FWHM of instrument response function) of ~3 ps, ~6 ps, and ~16 ps, respectively. In order to obtain good enough signal to noise ratio, the fluorescence signal was accumulated for 4× (10–20 min), 4× (5–10 min), and 4× (1–4 min) in the respective time windows. The sample was placed in a rotating cuvette to ensure that each laser pulse illuminated a fully relaxed sample. The rotating cuvette was tilted at ~45° relative to excitation beam and the optical pathway length in the sample was ~2 mm.

The time resolved fluorescence spectra collected in the range 600–770 nm in all three time windows were analyzed simultaneously and globally using the GLOTARAN software [18] yielding decay associated spectra (DAS) [19].

2.6. Time-correlated single photon counting measurements

Time-correlated single photon counting (TC-SPC) measurements were performed at the Center for Ultrafast Laser Spectroscopy, Adam Mickiewicz University, Poznań, using the setup described elsewhere [20–22]. Grana membrane samples were suspended in a similar medium as in the case of streak camera-based measurements. The concentrated sample was diluted in a buffer of pH = 7.5 and of similar composition to that one used for the streak camera measurements. Fluorescence kinetics were recorded by thermoelectrically cooled MCP-PMT R3809U-05 (Hamamatsu), following excitation at 400 nm by 2-ps laser pulses of typical energy of 1 pJ per pulse at a repetition rate of 4 MHz. The sample was placed in a quartz fluorescence cuvette (1 cm × 1 cm) and stirred with a small magnetic stirrer during the measurements.

Fluorescence kinetics were recorded in a 2.5 ns time window with a resolution of 0.61 ps per channel (4096 channels) at 680 nm and