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Protonation-induced changes in the macroorganization of LHCII monolayers

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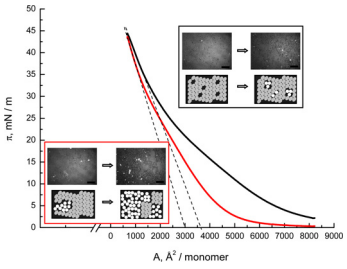
HIGHLIGHTS

- Protonated and partly deprotonated LHCII monolayers have different macroorganization.
- Protonated LHCII monolayer has higher order of organization than partly deprotonated.
- Protonated LHCII monolayer is more stable than partly deprotonated.
- Conformation and/or organization changes take place during monolayers compression.
- Monolayer of protonated is much more heterogeneous than of partly deprotonated LHCII.

GRAPHICAL ABSTRACT



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ABSTRACT

The major light-harvesting complex of photosystem II (LHCII) is an important regulatory protein in photosynthetic membranes. *In vivo* LHCII forms stable trimers and is found either associated to photosystem II or in LHCII-only containing domains. It was suggested that in native thylakoid membrane LHCII changes its conformation and macroorganization upon switching from light-harvesting to photoprotective state. Herein we have analyzed LHCII Langmuir monolayers at different subphase salt composition and in two different states – partly deprotonated (LHCII), at low basic pH 7.8, and highly protonated (p-LHCII), at pH 5.2, mimicking the functional light-harvesting and light-protective states of the protein, respectively. We have found strong difference in the supramolecular organization of the protein in these two functional states, the protonated monolayer exhibiting higher order of organization and significantly higher stability compared to the partly deprotonated one. Both LHCII and p-LHCII monolayers are composed of trimers self-assembling in aggregates with different packing density – loosely packed compiling homogeneous well-ordered monolayer areas and tightly packed organized in heterogeneous disordered phase. These two types of macroorganization are found in different proportions in protonated and partly deprotonated LHCII monolayers, the p-LHCII monolayer being much more heterogeneous than LHCII one.

Abbreviations: LHCII, major light-harvesting complex of photosystem II in partly deprotonated state; $LHCII_{0/150/500}$, LHCII spread on subphase supplemented with 0, 150 or 500 mM NaCl; p-LHCII, major light-harvesting complex of photosystem II in protonated state; $p\text{-}LHCII_{0/150/500}$, p-LHCII spread on subphase supplemented with 0, 150 or 500 mM NaCl; β-DM, n-dodecyl β-D-maltoside; A, mean (LHCII) monomer area; A_0, limiting (LHCII) monomer area; π_{int}, initial surface pressure after spreading; A_π, mean (LHCII) monomer area at a given surface pressure; BAM, Brewster angle microscopy; C_s^{-1}, compressibility modulus.

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1. Introduction

The major light-harvesting complex of photosystem II (LHCII) is a chlorophyll a/b pigment-protein complex that plays a key role in capturing and utilizing sun light, in the macroorganization of the thylakoid membrane and in the dissipation of the excess excitation energy [1–3]. *In vivo* LHCII is found either associated with photosystem II (PSII) in PSII-LHCII supercomplexes or in LHCII-only containing domains [4].

LHCII is responsible for the formation of stacked membrane areas, grana stacks [5–7], and stabilizes the bilayer structure of photosynthetic membranes. It forms stable trimers [8], which self-organize into aggregates both *in vivo* and *in vitro* [9]. When isolated it forms highly organized multilamellar membrane systems [5,8,10] that exhibit remarkable structural and functional flexibility, strongly affected by the lipid microenvironment [11–15].

The light-induced protonation of LHCII has been identified as a primary event in the sequence of molecular events that finally assure plants photoprotection, generally referred to as non-photochemical quenching (NPQ) [16,17]. *In vivo* it is triggered by the high light intensity-induced increase in the transmembrane potential and acidification of the thylakoid lumen. *In vivo* the switch from light-harvesting to photoprotection is achieved *via* structural changes involving the LHCII complex: (i) light-driven protonation of lumen exposed Glu residues (which *per se* induces LHCII conformational changes [18–21]) followed by activation of the violaxanthin deepoxidase which converts the non-covalently bound to LHCII violaxanthin to zeaxanthin *via* deepoxidation [18,22,23]; (ii) depending on the light intensity and spectral features LHCII can also undergo a light-activated conformational change allowing for phosphorylation of the stroma exposed residues which leads to structural change in the N-terminus of the complex [24–27], LHCII dissociation from PSII and its consequent association with PSI [28–31]. LHCII phosphorylation was proposed to enhance the monomerization of the trimeric LHCII complexes [32,33]. It was suggested that LHCII harbors two structural domains – a transmembrane backbone domain responsible for the energy dissipation and a surface-exposed one regulating the association of the complex to the photosystems' macromolecular complexes [27].

The relationship between the conformation and macroorganization of the photosynthetic complexes has often been addressed and dynamic architectural switches are now considered a part of the regulatory mechanisms of plants [34,35]. Monolayer studies on LHCII supramolecular structure at air/water [36] and argon/water [37–40] interface provided information on the 2D macroorganization and structural reorganizations of LHCII complexes under different experimental conditions. For example by means of Langmuir and Langmuir–Blodgett monolayers it was demonstrated that LHCII exhibits a conformational change at 22–25 °C related to increase in its molecular area that was accompanied by increased energy consumption [39]; this LHCII conformation was assigned to the photoprotective state of the protein. Later studies revealed that the light-induced switch of LHCII to its photoprotective conformation affects the overall membrane organization for the case of model lipid-LHCII membranes – trans-layer, rivet-like LHCII structures were identified [41].

Here, we have explored the structure and macroorganization of LHCII monolayers at air/buffer interface mimicking the LHCII light-harvesting and photoprotective states by adjusting the pH of the LHCII suspension and the subphase media to pH 7.8 and 5.2, respectively. Our aim was to study the structural changes in LHCII-only domains using isolated lamellar aggregates of LHCII as a model system, which resembles, particularly in structural flexibility, the native membrane [42]. To avoid or reduce submerging of LHCII that tend to form aggregates, we studied the effect of the subphase salt content on the organization and stability of the monolayers. Furthermore, for the first time we report Brewster angle microscopy (BAM) images of LHCII monolayers that allow label-free and real-time visualization of the lateral domain organization and the morphology of LHCII monomolecular films and its change upon compression.

2. Materials and methods

2.1. Isolation of LHCII

LHCII macroaggregates were isolated from pea thylakoid membranes according to the protocol of Krupa et al. [43], modified by Simidjiev et al. [13]; 0.7% Triton X100 was used for solubilization of the membrane fractions. The chlorophyll concentration was determined spectrophotometrically [44]. The chlorophyll a/b ratio of the LHCII preparations was 1.16 ± 0.04. For adjusting the pH of the LHCII suspension, the samples were washed three times in Tricine buffer with pH 7.8 or 5.2. Mild solubilization of those preparations was achieved by adding 0.02% n-dodecyl β-D-maltoside (β-DM) (Sigma–Aldrich, >98% GC) and incubation of 1–2 min before measuring 77 K fluorescence spectra [45] or spreading on the air/buffer interface.

2.2. Steady-state fluorescence measurements

77 K steady state fluorescence was recorded on Jobin–Yvon JY3 spectrofluorimeter in a quartz capillary (3 mm diameter) upon 436 nm excitation using 4 nm excitation and emission slits. The chlorophyll concentration of the samples was 10 μg chl/mL. The spectra were corrected for the photomultiplier sensitivity and buffer contribution.

2.3. Preparation of LHCII monolayers

LHCII was dissolved in 50 mM Tricine buffer, pH 7.8 or 5.2, to a final protein concentration of 0.04 mM. The LHCII concentration was estimated on the basis of the chlorophyll concentration, assuming that a single LHCII monomer binds 14 chlorophyll molecules (eight chlorophyll *a* and six chlorophyll *b*). 25 μL of the solution was spread on the surface of the corresponding buffer subphase containing 50 mM Tricine and different salt concentration (0, 150 or 500 mM NaCl) at initial monomer area of 8300 Å^2; prior each compression the protein complexes were left for 30 min to relax on the air/buffer interface. The monolayers were compressed and expanded at a speed of 900 Å^2/monomer·min. The surface pressure/monomer area isotherms, π/A, the monomer area/time stability curves, A/t, and the compression–expansion hysteresis loops, π/A, were recorded on a Langmuir film balance with a Teflon trough and Wilhelmy dynamometric system measuring surface pressure with a strip of filter paper. The following parameters were determined from the π/A isotherms: (i) average monomer area, A, occupied by LHCII molecules in a specific phase or conformational state; it is determined by extrapolation of the region of the isotherm corresponding to this phase/state to zero surface pressure [46,47]; (ii) limiting area per monomer, A_0, which is the minimum area to which the monolayer can be compressed without collapsing; it is determined by extrapolation of the steep high-pressure region of the isotherm to zero surface pressure; (iii) compressibility modulus ($C_s^{-1} = -A_\pi(\partial\pi/\partial A)_T$), obtained as numerical derivative of π/A isotherms.

All experiments were performed at 20 °C. Water from Purelab Option-Q purification system with specific resistance of 18 MΩ/cm was used for buffer subphase preparation. At least five independent measurements were preformed for each experimental condition.

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