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Review

Hierarchical organization and structural flexibility of thylakoid membranes ☆

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ABSTRACT

Chloroplast thylakoid membranes accommodate densely packed protein complexes in ordered, often semi-crystalline arrays and are assembled into highly organized multilamellar systems, an organization warranting a substantial degree of stability. At the same time, they exhibit remarkable structural flexibility, which appears to play important – yet not fully understood – roles in different short-term adaptation mechanisms in response to rapidly changing environmental conditions. In this review I will focus on dynamic features of the hierarchically organized photosynthetic machineries at different levels of structural complexity: (i) isolated light harvesting complexes, (ii) molecular macroassemblies and supercomplexes, (iii) thylakoid membranes and (iv) their multilamellar membrane systems. Special attention will be paid to the most abundant systems, the major light harvesting antenna complex, LHCII, and to grana. Two physical mechanisms, which are less frequently treated in the literature, will receive special attention: (i) thermo-optic mechanism —elementary structural changes elicited by ultrafast local heat transients due to the dissipation of photon energy, which operates both in isolated antenna assemblies and the native thylakoid membranes, regulates important enzymatic functions and appears to play role in light adaptation and photoprotection mechanisms; and (ii) the mechanism by which non-bilayer lipids and lipid phases play key role in the functioning of xanthophyll cycle de-epoxidases and are proposed to regulate the protein-to-lipid ratio in thylakoid membranes and contribute to membrane dynamics. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

The light reactions of oxygenic photosynthesis, the absorption of light, the regulated supply of excitation energy into the photochemical reaction centers and the functioning of the vectorial electron and proton transport system as well as the synthesis of the primary products of the energy conversion depend largely on the architecture of the photosynthetic machinery. In all organisms the photosynthetic apparatuses are highly organized systems with well recognizable hierarchical architecture. They also exhibit astounding variations between different organisms and habitats and, the topic of this review, possess substantial structural and functional flexibilities of the structural complexity in response to rapidly changing environmental conditions. This will be discussed in detail in this review, the major aim of which is to provide deeper insights into the mechanisms and physiological significances of different reorganizations at different levels of the structural complexity. When looking for examples, light-harvesting complex II (LHCII) and the granal thylakoid membranes (TMs) are the evident choices. This is justified by their high abundance in nature and the rich literature available. With regard to the molecular mechanisms the review will focus on two topics, which have received relatively little attention in the literature: thermo-optic effect, an excitation energy-dissipation-assisted mechanism of structural changes, and the role of non-bilayer lipids and lipid phases in the structure and dynamics of TMs.

2. Structural hierarchy and flexibility at different levels of structural complexity

As most biological entities, oxygenic photosynthetic organisms are organized in a hierarchical manner, satisfying the conditions that "each level in the hierarchy represents an increase in organizational complexity, with each object being primarily composed of the previous level's basic unit" and "the concept of emergence —the properties and functions found at a hierarchical level are not present and irrelevant at the lower levels" [1].

Abbreviations: CD, circular dichroism; Chl, chlorophyll; DDE, diadinoxanthin de-epoxidase; DGDG, digalactosyl-diacylglycerol; EPR, electron paramagnetic resonance; FCP, fucoxanthin-chlorophyll protein complex; H_{II}, inverted hexagonal phase of lipids; LHCI, light-harvesting complex of Photosystem I; LHCII, light-harvesting complex II; LHCII-HL, LHCII isolated from high light treated leaves; MGDG, monogalactosyl-diacylglycerol; NPQ, non-photochemical quenching; PG, phosphatidyl-glycerol; PLL, poly-L-lysine; psi, polymer or salt-induced; PSI, Photosystem I; PSII, Photosystem II; qE, energy-dependent quenching; RD, repeat distance; SANS, small-angle neutron scattering; SQDG, sulfoquinovosyl diacylglycerol; TM, thylakoid membrane; VDE, violaxanthin de-epoxidase; WT, wild type

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2.1. Pigment–protein complexes

With regard to the light reactions of photosynthesis, the basic building blocks, the pigment and protein molecules are assembled into pigment–protein complexes. In these the dense packing and non-random orientation of the pigment molecules at well defined binding sites give rise to numerous interactions, e.g. short-range pigment–pigment (dipole–dipole) excitonic interactions as well as different pigment–protein interactions [2]. The molecular architecture of the complexes generally determines the fate of excitation energy and the photophysical pathways within the complexes. The correlation between structure and function at the level of light harvesting complexes can be demonstrated best on LHCII, the most abundant membrane protein in the Biosphere, which is also one of the most studied proteins. Its high resolution crystal structure is known [3,4]. LHCII also participates in important regulatory functions [5], which require structural flexibility of the protein. Some features of this functional and structural plasticity of LHCII can be recognized in vitro, at the level of isolated complexes.

2.1.1. Light-induced changes and protein dynamics in LHCII

Jennings and coworkers [6] were the first to show that isolated, detergent-solubilized LHCII is capable of undergoing light-induced reversible fluorescence quenching with rates proportional to the intensity of the excitation. Thermodynamic analyses of the transients measured both on solubilized complexes and lamellar aggregates have shown that they are associated with conformational changes in the protein complexes —with an estimated 8.8 kJ/M enthalpy of activation for the forward direction [7]. The changes were more pronounced in lamellar aggregates than in trimers, showing that a single quenching center, presumably a trimer that had undergone a (reversible) conformational change, was capable to quench a large domain in the aggregate [8].

The light-induced reorganizations of LHCII appeared not to affect the excitonic interactions, as shown by the invariance of the excitonic CD bands —suggesting that the conformational changes are minor [9]. In lamellar aggregates of LHCII, which undergo substantial reorganizations in the long-range chiral order of the complexes (see Section 2.2), light-induced reversible release of Mg^{2+} and flash-induced fast photoelectric signal attributed to charge-displacement currents were also observed — suggesting that the changes occur in the hydrophilic loops responsible for cation binding [10,11]. Direct experimental evidence for the involvement of the stromal side loop was provided by in vitro phosphorylation experiments. It has been shown that the phosphorylation site of isolated LHCII, which can be cleaved by trypsin, is sensitive to the preillumination of the complexes [12]. These data are also in perfect harmony with recent findings that the amino acids at the N-terminus possess high structural flexibility. Pulsed EPR measurements on spin-labeled LHCII revealed that LHCII possesses rigid cores and flexible hydrophilic domains at the N-terminus [13]. (The N-terminus is missing from the LHCII crystal structure either due to disorder or heterogeneity [4].) Using single particle fluorescence microscopy, it has been shown that detergent-solubilized LHCII trimers immobilized on poly-L-lysine (PLL) undergo rapid conformational changes, switching between fluorescing and dark states [14–16]. These conformational switches, although observed with excitation light intensities much higher than in the macroscopic experiments, might explain the ability of LHCII to participate in light-induced reversible reorganizations. As suggested earlier by a different study, using time-resolved fluorescence and hydrostatic pressure on solubilized LHCII, there is a local conformational switch between the quenched and unquenched state; the estimated free energy difference of 7.0 kJ/mol is allowing the switch to operate in a controlled way [17].

2.1.2. pH stability of LHCII

Since the acidification of the lumenal pH is known to induce qE, the energy-dependent NPQ component in vivo [5], the effect of lowering the pH on LHCII is of special interest. Krüger and coworkers [14–16] have conducted systematic studies on PLL-immobilized detergent solubilized LHCII trimers and observed changes in the operation of the conformational switch using the single-molecule spectroscopy technique. The observed low-pH induced changes, with immobilized trimers allowing no aggregation, were, however, accounted for by the effect of pH on the interaction between LHCII and PLL rather than on the protein structure. In good agreement with this conclusion, it has been shown that reconstituted wild type LHCII exhibited virtually no sensitivity to pH variations; in contrast, the S123G lumenal section mutant showed a low-pH-induced reversible quenching [18]. Further, it has been shown that the lumenal loop segment between helix B and helix C plays a key role in maintaining the structural stability of the complex under acidic conditions, and the negatively charged residues in this loop regulate the pigment conformation and the structural stability under different pH environments [19]. Exchanging glutamic acid at position 94 with glycine (E94G), far from the pigment binding sites, destabilizes the 3_{10} helix in the lumenal loop structure and leads to an acquired pH sensitivity of the LHCII, and also affects the excitonic CD bands assignable to the neoxanthin-binding domain. This conclusions, i.e. that a subtle change in the lumenal loop of LHCII, induced by single amino acid mutagenesis, can generate a sensitivity to low pH and establish NPQ, have recently been confirmed [20].

2.2. Oligomers, aggregates and supercomplexes

Different pigment–protein complexes readily self-assemble into oligomeric forms and supercomplexes. At this level of hierarchy, in addition to the complexes themselves, different other compounds play important roles. For instance, digalactosyl-diacylglycerol (DGDG) facilitates the formation of the extended arrays of LHCII [21]. Different lipid molecules are found both in PSII and PSI supercomplexes in stoichiometries similar to that of the host TMs [22]. This might, in part, be the consequence of their embedding in the lipid membranes but it is more likely to indicate that bound lipid molecules play special roles in the assembly and functional regulation of the reaction center complexes, as shown e.g. for PG in the case of formation of fully functional PSII supercomplexes in vivo [23] or in the trimerization of LHCII monomeric complexes [4]. Other compounds which appear to play important roles in the self-assembly and stability of supercomplexes include small intrinsic membrane proteins, such as the Ycf4 for PSI [24] and PsbW for PSII [25] —their roles are not fully understood. The supply of reaction centers with excitation energy is regulated by varying the peripheral light harvesting antenna [26]. Most prominently, in green algae and higher plants, variations in LHCII arrays play important regulatory roles, and thus their spectroscopic features and light-induced reorganizations deserve special attention.

2.2.1. Spectral signatures of LHCII aggregates

With the assembly of isolated LHCII into large, ordered aggregates, novel spectral properties emerge. One of the most prominent change occurs in the fluorescence quantum yield of LHCII, which drops by an order of magnitude upon the formation of aggregates, when compared to the solubilized complexes [27]. This is also manifested in substantial shortening of the lifetime of the Chl-*a* singlet excited state, from about 4 ns to a few hundred picoseconds [28–30].

Significant aggregation-induced changes have been reported to occur also in the LD and CD spectra [31]. However, at least part of the differences between the detergent solubilized trimer and the LHCII aggregate might originate from a perturbation of the molecular architecture of the complexes by the detergent, as indicated first by triplet-minus-singlet transient spectroscopy [32], and also by a more recent CD and LD spectroscopic study, when comparison was made to the native TMs and small aggregates [33]. Detergents appear to affect mainly the orientation of carotenoids, and a few excitonic CD bands arising from carotenoid:chlorophyll interactions. In general, these

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