Structural constraints for protein repair in plant photosynthetic membranes

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The thylakoid membrane system inside plants chloroplasts defines the structural framework for photosynthetic conversion of sunlight into metabolic energy forms (ATP, NADPH + H+). An architectural hallmark of these thylakoid membranes is the tight stacking of part of the membrane into cylindrical flat grana thylakoids, with a diameter of about 500 nm, that are interconnected by unstacked stroma lamellae forming a complex 3D network of alternating grana piles and stroma lamellae. The structural differentiation in the stacked and unstacked thylakoid regions is the basis for a pronounced spatial separation of multisubunit pigment-protein complexes that catalyze energy transformation. The main part of photosystem II (PSII) associated with light-harvesting complex II (LHCII) is concentrated in the grana thylakoids whereas PSI-LHCl and the ATPase complex are excluded from the stacked grana and accumulate in the unstacked thylakoid regions. The fifth protein complex, the cytochrome b6f complex, is assumed to be homogenously distributed. It is important to recognize that this structural arrangement is not static but highly dynamic and responsive to environmental factors like light intensity and quality or temperature. Knowledge about the interplay between dynamic structural features of the intricate thylakoid architecture, and the functionality, regulation, repair and biogenesis of the photosynthetic machinery is essential for understanding the plasticity of energy conversion in plants living in a fluctuating multi-factorial environment.

PSII Repair Cycle

A prime example for the dynamic response of the photosynthetic apparatus is the structural changes triggered by high light stress. Recently, we showed that high light induces changes in the thylakoid membrane architecture and connected these changes to the repair of photodamaged PSII.1 PSII is vulnerable to photo-oxidative damage caused by toxic side products (reactive oxygen species) of primary photochemical processes within PSII. The main target of this damage is the D1 subunit buried in the ~1.4 MDa dimeric LHCII-PSII holocomplex.2 Efficient repair of the damaged D1 subunit is of utmost importance for the survival of plants.3 Therefore, plants have evolved a sophisticated PSII repair cycle that is one of the most efficient repair machineries in nature.4,5 The PSII repair cycle includes a number of different steps catalyzed by a distinct set of kinases, phosphates and proteases.6-9 A central step in the repair process is the proteolytic degradation of damaged D1 (and other PSII subunits) by specific proteases. The D1 subunit contains five transmembrane α-helices connected by polar protein parts facing both aqueous phases surrounding the thylakoid membrane, i.e., the stroma side and the thylakoid lumen side.9 Proteolysis of D1 occurs at both sides6 catalyzed by either FtsH proteases (stroma side) or Deg proteases (lumenal side). Interestingly, both proteases form hexamers that are essential for their functionality and the degradation of the D1 subunit.7,8,10 The FtsH hexamer consists of a membrane-integral part and a bulky stromal part (height ~6.5 nm).11 For Deg1, it was shown that hexamerization is controlled by a low pH in the thylakoid lumen,10 i.e., under conditions where light-induced photosynthetic electron transport build a proton motive force across the thylakoid membrane. The diameter of the hexamer is 8 to 9 nm and its height is about 7 nm.10 An open question is how damaged PSII in stacked grana get access to the proteases.

Structural Constraints for PSII Repair

Understanding degradation of damaged PSII by FtsH and Deg proteases has to take structural constraints of the thylakoid membrane system into account. In this respect, three structural features have to be considered (see Fig. 1, upper part). First, the width of the stromal gap between adjacent grana membranes was determined to be 3 to 4 nm.12,13 In consequence, this will hinder access of the bulky stromal domain of the FtsH protease to stacked grana (see Fig. 1, upper part, #1). Second, a similar steric constraint as for FtsH is realized for the Deg hexamer in the thylakoid lumenal space. The width of the narrow lumen in dark-adapted plants is ~4.5 nm12,13 and may restrict the access of...
Deg proteases to stacked grana. The restriction is aggravated by macromolecular crowding in the lumen of stacked grana, i.e., by lumenal protrusions of PSII (mainly the water-splitting complex). We found that the mobility of the small lumen-hosted plastocyanin (~3 x ~3 x ~4 nm) in grana stacks is confined to small diffusion microdomains in dark-adapted plants. Based on these models, it is expected that the much larger Deg protease is excluded from the lumen in grana thylakoids (Fig. 1, top panel, #2). Third, it was shown by diffusion measurements that the mobility of grana hosted proteins within the membrane plane (in particular of the PSII holocomplex) is very low. Again, this is caused by the very high protein packing density (70% of the grana membrane area belongs to proteins and 30% to lipids) in stacked grana that restricts damaged PSII in stacked grana from reaching the repair machinery in unstacked thylakoid regions (Fig. 1, top panel, #2). In summary, the narrow lumenal and stromal gaps in stacked grana as found for dark-adapted plants in combination with the low PSII mobility questions how damaged PSII gets access to proteases that are most likely excluded from grana to initiate the repair process by D1 proteolysis.

**Light-Induced Architectural Changes**

A potential solution to these problems is that thylakoid membranes undergo significant structural rearrangements that could alleviate accessibility between damage PSII and the proteases as summarized in the bottom panel of Figure 1 (high light induced structural changes are indicated by orange arrows). We found that high light treatment leads to lateral shrinkage of the grana diameter from about ~370 nm (dark) to ~300 nm (60 min high light). The advantages of grana diameter shrinkage concerning the PSII repair cycle are: (1) damaged PSII localized in former stacked regions now has direct access to FtsH in the new unstacked regions; (2) the diffusion distance of damaged PSII in the remaining grana core to unstacked thylakoid membranes becomes shorter; and (3) the grana perimeter to area ratio increases, i.e., there are more contact zones between stacked and unstacked membranes. A further change found in thylakoid membranes isolated from high light treated material is that the overall protein mobility in core grana increases significantly. Thus, it may be easier for damaged PSII to escape from the grana core. We found that both the lateral shrinkage as well as the higher protein mobility in grana core is triggered by protein phosphorylation by the stn8 kinase highlighting the importance of posttranslational protein modification. Two other structural changes may facilitate the physical contact between PSII and proteases but it has to be verified whether they are realized under high light stress. The first one is a swelling of the thylakoid lumen that we detected under moderate light intensities (500 μmol quanta m⁻²s⁻¹). Under this condition, the luminal width expands from ~4.7 nm to ~9.2 nm. This may allow the Deg-hexamer to enter the lumen in stacked grana. The second structural change is that in addition to lateral destacking, transversal destacking also occurs under high light, i.e., the width of the stromal gap increases. Transversal destacking could give FtsH access to grana stacks and could also facilitate the migration of damaged PSII in grana core due to a weakening of interactions between adjacent membranes in grana stacks. In conclusion, high light stress triggers architectural changes of the thylakoid network most likely controlled by protein phosphorylation. These changes include partial destacking of the grana stacks (both laterally and transversal) and widening of the thylakoid lumen in combination with higher protein mobility in grana core that allows efficient PSII repair in protein crowded thylakoid membranes.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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