

Supermolecular organization of photosystem II and its associated light-harvesting antenna in the wild-type and npq4 mutant of *Arabidopsis thaliana*

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Introduction

The thylakoid membranes of green plants build a highly complex and dynamic membrane system. Part of these membranes form the so-called grana stacks, which are enriched in PSII and LHCII. PSII-LHCII supercomplexes have been isolated by gentle detergent solubilization of spinach grana membranes or thylakoids, and their structures have been analysed by electron microscopy and image analysis of single particles at a resolution of about 20 Å (see, e.g., Boekema et al., 1995, 1999ab; Nield et al., 2000a). The results revealed three types of binding sites of trimeric LHCII around a dimeric PSII core complex (designated S, M and L, from strongly, moderately and loosely-bound LHCII, respectively) and also three ways by which the PSII-LHCII supercomplexes are laterally associated to each other (to give the so-called megacomplexes). Analysis of semi-crystalline arrays in grana membranes revealed the supramolecular organization of the PSII-LHCII supercomplexes in the membranes (Boekema et al., 2000a).

In this contribution we report an analysis by electron microscopy and image analysis of PSII-LHCII megacomplexes and grana membranes from *Arabidopsis thaliana*, both from the wild-type plant (Yakushevska et al., 2001) and from the npq4 plant. This mutant lacks the PsbS protein and cannot dissipate excess light energy by non-photochemical quenching (Li et al., 2000). A structural characterization of PSII of this mutant could shed more light on this physiologically very important regulation mechanism (Horton et al., 1996).

Materials and methods

PSII-LHCII super- and megacomplexes and native grana membranes were isolated from BBY-preparations or thylakoid membranes from *Arabidopsis thaliana* (wild-type or npq4 mutant) as described before (Boekema et al., 1999a, 2000a; Yakushevska et al., 2001). Samples of single particles or membranes were negatively stained with 2% uranyl acetate and imaged using a Philips CM10 electron microscope at 52000 x magnification. Image analysis (multi-reference alignment, statistical analysis and classification) was carried out as before (Boekema et al., 1999a, 2000a).

Results and discussion

Electron microscopy and image analysis of PSII-LHCII supercomplexes from wild-type *Arabidopsis* has recently been presented by Yakushevska et al. (2001). The most obvious difference with the results from spinach was the much larger number of supercomplexes with attached M-LHCII trimer. For instance, the relative amount of C₂S₂M₂ complexes was about 20 times larger in *Arabidopsis* than in spinach. M-LHCII trimers could also bind in the absence of S-LHCII trimers, which could not be observed in spinach. L-LHCII trimers could, however, not be detected. An analysis of PSII-LHCII supercomplexes from the npq4 mutant of *Arabidopsis* did not reveal significant differences with that of the wild-type (not shown). Both the relative occurrence of the various types of supercomplexes, as well as the structural organization of these complexes were identical within the error limits.

In Fig. 1 averaged projections are shown of megacomplexes from the npq4 mutant and wild-type *Arabidopsis*. These projections are very similar and represent the only megacomplex found in *Arabidopsis*. This megacomplex, however, differs from the three types of megacomplexes observed before in spinach (Boekema et al., 1999ab), of which two are shown in Fig. 1. The right part of the complex is shifted downwards by 2.5-3 nm compared to the type II complex from spinach, or upwards by 4.5-5 nm compared to type I.

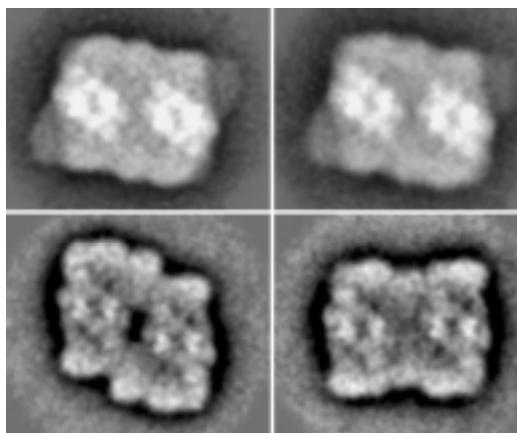


Fig. 1. Results of multi-reference alignment and classification of top-view projections of PSII-LHCII megacomplexes. Upper left: average of 250 C₄S₄M₂₋₄ projections obtained from the npq4 mutant of *Arabidopsis*. Upper right: average of 250 C₄S₄M₂₋₄ projections obtained from wild-type *Arabidopsis*. The smaller density at the corners of both projections arises from the partial absence of the M-LHCII + CP24 and CP26 structures. Averages of the spinach C₄S₄M₂ type I megacomplex (lower left) and type II megacomplex (lower right) are shown for comparison (Boekema et al., 1999a). The stronger contrast in the spinach projections originates from a stronger stain embedding.

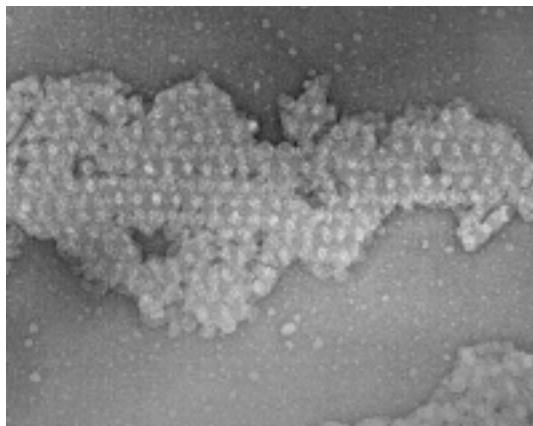


Fig. 2. Electron micrograph of a semi-crystalline membrane fragment, obtained from the npq4 mutant of *Arabidopsis thaliana*.

The differences between the megacomplex projections from the wild-type and npq4 mutant are much smaller than observed before when either the extrinsic 23 kDa protein was removed from spinach membranes by salt washing, or the extrinsic 33 kDa protein was additionally removed by Tris-washing (Boekema et al., 2000b). The absence or presence of a relatively large protein with four transmembrane helices, such as the PsbS protein, should in principle have a larger impact on the top view projections of the supercomplexes than the absence or presence of extrinsic proteins, which in the absence of conformational rearrangements only add to the relative densities. These results suggest that the PsbS protein is not located between the supercomplexes. This confirms the previous conclusion of Nield et al. (2000b) that PsbS is not located in the C₂S₂ supercomplex, and extends this conclusion by showing that it is also not located near the additional M-LHCII and CP24 subunits, present in our complexes.

Fig. 2 shows an electron micrograph of a membrane fragment in which the complexes are ordered in a semi-regular fashion. About 10% of the membranes revealed this type of order. Image analysis (Fig. 3) showed a unit cell of 25.6 x 21.4 nm (angle 77°), which is considerably larger than the unit cell of the most common regular array in spinach grana membranes (27.3 x 18.3 nm, angle 74.5° - Boekema et al., 2000a). The repeating unit consists of C₂S₂M₂ (Yakushevska et al., 2001), whereas that of spinach was shown to consist C₂S₂M (Boekema et al., 2000a). In spinach, the number of semi-crystalline membrane fragments was 50-60%, much higher than in *Arabidopsis*. However, in spinach the majority of the crystalline arrays occurred only in one of the two membranes of the stacks (the other mainly occupied by LHCII), while all crystalline arrays in *Arabidopsis* occurred in both layers, at specific angles of either 34 or 58 degrees (not shown). It is possible, however, the *Arabidopsis* membranes with only one crystalline layer were unstable under our experimental conditions and that the amount of membranes with crystalline arrays in both layers is similar in both plants.

The most surprising result of our analysis is that the regular arrays appeared to be almost identical in the wild-type *Arabidopsis* and in the npq4 mutant (Fig. 3). The dimensions of the unit cell are virtually the same for both types of membranes, and differences between densities (not shown) are probably dominated by differences in resolution, which is slightly higher in the arrays from the wild-type plants, despite the same number of fragments. These results suggest that the PsbS protein is not located within the regular arrays of PSII and LHCII.

Based on these results we suggest that the PsbS protein is located in the LHCII-enriched regions of the grana membranes. Such regions were observed in almost every pair of stacked

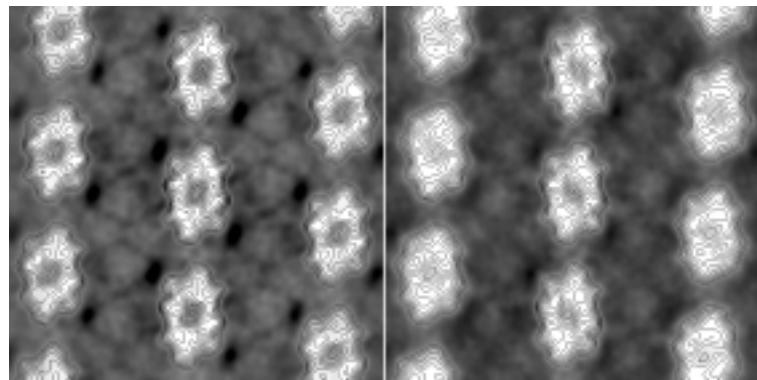


Fig. 3. Result of image analysis of crystalline fragments. Left: sum of 450 aligned fragments from wild-type *Arabidopsis*. Right: sum of 512 aligned fragments from the npq4 mutant of *Arabidopsis*.

grana membranes and could even be localized in the membrane opposing a regular unit of PSII-LHCII supercomplexes (Boekema et al., 2000a). Small aggregates of trimeric LHCII have been observed by EM in partially solubilized grana membranes from spinach (Dekker et al., 1999) and *Arabidopsis* (not shown), and biochemical procedures for the purification of small LHCII-enriched fragments have been reported (Ruban et al., 1999). A localization of PsbS in regions other than of the ordered PSII-LHCII supercomplexes would be consistent with its non-stoichiometric and highly variable contents (Ottander et al., 1995; Li et al., 2000).

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