Specific association of photosystem II and light-harvesting complex II in partially solubilized photosystem II membranes

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Abstract   In this study, we report the structural characterization of photosystem II complexes obtained from partially solubilized photosystem II membranes. Direct observation by electron microscopy, within a few minutes after a mild disruption of the membranes with the detergent *n*-dodecyl-α,D-maltoside, revealed the presence of several large supramolecular complexes. Images of these complexes were subjected to multivariate statistical analysis and classification procedures, resolving a new complex consisting of the previously characterized dimeric supercomplex of photosystem II and light-harvesting complex II [Boekema et al., Proc. Natl. Acad. Sci. USA 92 (1995) 175–179] and two additional, symmetrically organized protein masses each containing a second type of trimeric light-harvesting II complex. We conclude that large and labile integral membrane proteins, such as photosystem II, can be quickly structurally characterized without extensive purification.
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Key words: Photosystem II; Photosynthesis; Electron microscopy

1. Introduction

Photosystem II (PS II) is a large supramolecular pigment-protein complex embedded in the thylakoid membranes of green plants and algae. Its major task is to collect light energy and to use this for the reduction of plastoquinone, the oxidation of water and the formation of a transmembrane pH gradient. It consists of a central reaction center core complex and several peripheral light-harvesting pigment binding proteins belonging to the family of nuclear encoded Cab proteins. The core complex contains the reaction center proteins D1 and D2, cytochrome b_{559}, a number of very small proteins and two chlorophyll *a* binding proteins with apparent masses of 43 and 47 kDa (CP43 and CP47, respectively), which serve as an intrinsic antenna system [1]. The peripheral antenna is an unknown structure of several light-harvesting complex II (LHC II) proteins which together bind a large number of chlorophyll *a*, chlorophyll *b* and xanthophyll molecules. The LHC II proteins collect solar energy, and in addition are directly involved in a number of regulatory processes [2].

Several studies have suggested that in vivo the core complex has a dimeric structure with an anti-parallel organization of the monomers (reviewed in [3]). Our knowledge about the structure of the core complex has rapidly increased during the last years, in particular due to results from electron crystallography on two-dimensional crystals. The two-dimensional structure of a complex of D1, D2, CP47 and three smaller proteins has been determined at 8 Å resolution [4], and three-dimensional maps at lower resolution have also been reported (reviewed in [5]). The largest isolated particle which has been structurally characterized up to now is a supercomplex of a dimeric core surrounded by two copies of the CP26 and CP29 linker proteins and two trimers of LHC II, which presumably consist of the Lhcb1 and Lhcb2 proteins [6,7]. It has a rather rectangular shape in the membrane plane with dimensions (including the detergent layer) of about 300×155 Å [6]. Immunoblotting of isolated supercomplexes showed the absence of the minor antenna proteins CP24 and Lhcb3 [7]. The absence of these proteins in the supercomplex and the lower number of bound chlorophyll molecules per reaction center in the supercomplex than in the PS II membranes suggest that even larger complexes of PS II may exist in the thylakoid membranes.

In our search for larger complexes we decided to avoid all biochemical purification procedures, because the previously characterized supercomplex [6,7] appeared to be rather unstable in its detergent-isolated form and because potentially larger complexes could be even more fragile. We subjected PS II membranes to a very short and partial detergent treatment and analyzed the complete set of solubilized particles by electron microscopy (EM) and image analysis. With the detergent *n*-dodecyl-α,D-maltoside (α-DM) we found a relatively high number of large particles suitable for image analysis. The results indicate the presence of a large supercomplex with dimensions of about 320×220 Å containing a dimeric reaction center core surrounded by four trimeric LHC II complexes in two different types of binding sites.

2. Materials and methods

PS II membranes were isolated from spinach thylakoid membranes as described by Berthold et al. [8] with minor modifications as in [9]. For the rapid preparation of large complexes, we diluted the PS II membranes in 20 mM Bis-Tris (pH 6.5) and *n*-dodecyl-β,D-maltoside (β-DM) or α-DM at 4°C to reach final concentrations of 100 μg Chl/ml and 0.06% β-DM or α-DM. The suspension was stirred during 5 s, centrifuged during 3 min at 9000 rpm in an Eppendorf 5414 table centrifuge and pushed through a 0.45 μm filter to remove unsolubilized material, after which it was immediately used for further analysis. The composition of the solubilized material was checked by gel filtration chromatography using a Superdex 200 HR 10/30 column (Pharmacia) and diode array detection as described in [10]. In the mobile phase, however, 0.03% β-DM was replaced by 0.03% α-DM when α-DM was used as the solubilizing agent.

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For EM, the solubilized material was diluted four-fold in 20 mM Bis-Tris (pH 6.5)+0.03% α-DM, and prepared using the droplet method with 2% uranyl acetate as the negative stain on glow-discharged carbon-coated copper grids. Glow-discharged grids force molecules to attach with their flat sides on the film, thus preventing side views. During the staining procedure the grid was washed once with distilled water for several seconds to reduce detergent aggregation in the background. EM was performed with a Philips CM10 electron microscope using 100 kV at 52 000× magnification. Micrographs were digitized with a Kodak Eikonix Model 1412 CCD camera with a step size of 25 μm, corresponding to a pixel size of 4.85 Å at the specimen level. Image analysis was carried out using IMAGIC software. From 65 micrographs about 3300 top-view projections were selected for analysis, which included alignment of projections followed by multivariate statistical analysis in combination with classification, as used previously [6,11]. For the initial classification, we decomposed the data set into 50 classes, which is about the highest number that could be used given the size of the data set. Only five major groups of classes were found, each group representing one specific type of projection. They were further inspected for projections with the opposite handedness (for all types well under 10%) and homogeneity, by reclassifying subsets of projections. This resulted in the finding of a sixth type of projection, present in small numbers. From the six groups the 'best' 60–80% of the particles belonging to classes with well-preserved features were summed, with the cross-correlation coefficient in the aligned step as a quality parameter for eventual summing. The resolution of the final images was estimated by using the Fourier correlation method [12].

3. Results

The original aim of this work was to look for larger associations of PS II and LHC II than those described before [6]. We subjected PS II membranes to a short treatment of detergent in quantities less than required to completely solubilize the membranes. With the gel filtration techniques developed and applied for PS II RC complexes in [10], we found with β-DM as detergent several fractions with clearly different A_{470}/A_{435} ratios (Fig. 1A), indicating the presence several types of complexes with very different Chl b/Chl a ratios. The complex that eluted after 19.5 min is present in the largest amount, has the highest A_{470}/A_{435} ratio of 0.8 (and thus the highest content of Chl b), and is therefore readily interpreted as the trimeric LHC II complex. The complexes eluting at about 22.5 min may be interpreted as monomeric LHC complexes. At shorter times of about 17.5 and 16 min two complexes were eluted with a very low A_{470}/A_{435} ratio of 0.35, which can be attrib-

uted to monomeric and dimeric PS II core complexes, respectively. At 14 min an even larger complex was found with an intermediate A_{470}/A_{435} ratio of 0.55, suggestive of a supercomplex of PS II and LHC II, as expected from earlier work [6]. The relative amounts of various fractions suggest that the amount of supercomplex is in fact rather low.

With α-DM as solubilizing agent completely different chromatograms were observed (Fig. 1B). In analogy with the situation with β-DM a pronounced peak due to trimeric LHC II was seen at 19.5 min, but the amount of monomeric LHC complexes at about 22.5 min was lower, and at 16–17 min the A_{470}/A_{435} ratio did not approach 0.35, suggesting fewer free PS II core complexes. With α-DM a relatively strong peak was observed at 14 min with again a A_{470}/A_{435} ratio of about 0.55, but with α-DM a unique and rather intense peak arose at 13 min with an even higher A_{470}/A_{435} ratio of about 0.64. These data suggest that with α-DM supramolecular organizations of the PS II core and the various LHC II complexes are much better preserved than with β-DM and that with α-DM even larger supercomplexes may be expected than with β-DM.

When the α-DM fragmented membranes were negatively stained (Fig. 2), complexes of various size could be observed. Among them were many small (< 100 Å) triangular-shaped particles, possible mainly trimeric LHC II complexes. However, larger complexes were also clearly depicted. The rectangular-shaped supercomplexes [6] could be recognized directly, despite the fact that no biochemical purification step had been carried out. Since the particles were not obscured by a high background, which could have arisen in case of excess lipid structures, they were very suitable for image analysis. To be certain of not overlooking specific aggregates, we selected all particles with the size of a fragmented supercomplex (see Fig. 3c in [6]) or larger. A total of 3300 projections were collected, aligned, treated with multivariate statistical analysis and classified. The classification showed that the supercomplex, first described in [6], and two of its fragments comprised about half of the data set (Fig. 3D–F). The projections of the supercomplex (Fig. 3D) and of its main fragment (Fig. 3F) have a slightly more rectangular appearance than those reported in [6]. It is possible that during the purification procedure of the complexes analyzed in [6] a relatively small protein in the
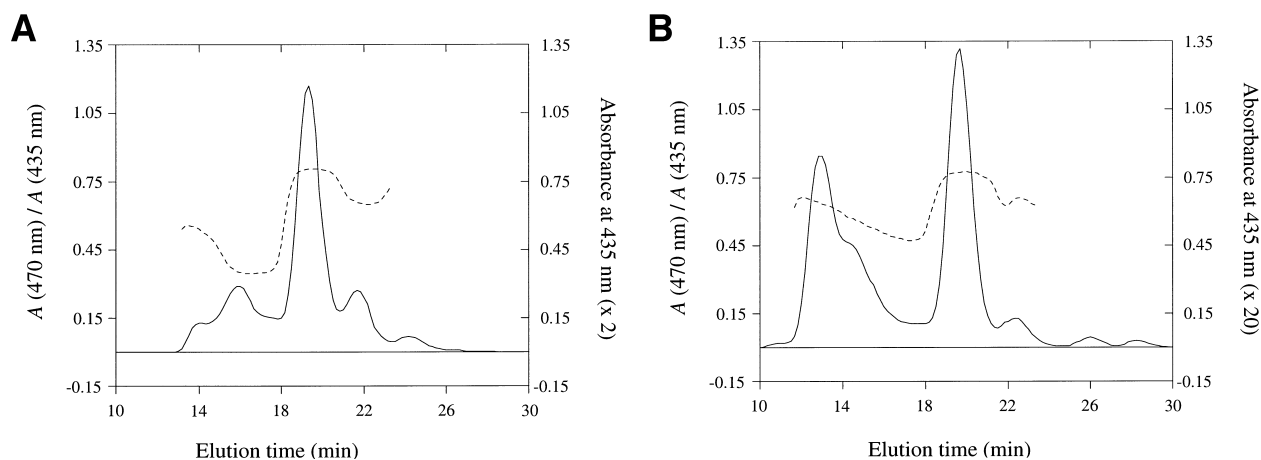

Fig. 1. FPLC gel filtration chromatograms (full lines) recorded at 435 nm of PS II membranes disrupted with β-DM (A) or α-DM (B). Both chromatograms are plotted together with the A_{470}/A_{435} ratio (dashed lines). The flow rate was 35 ml/min.
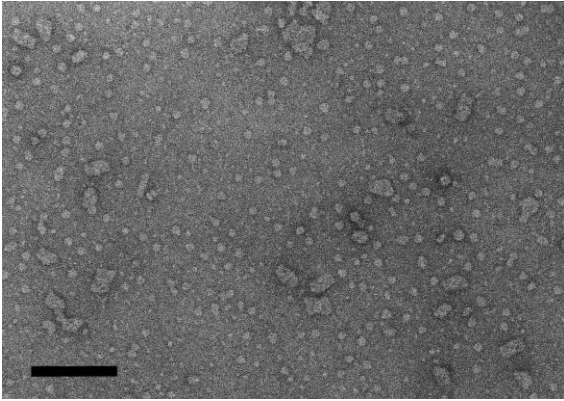
Fig. 2. Electron micrograph showing top-view projections of PS II complexes of α-DM-fragmented PS II membranes. Bar represents 1000 Å.

picted in Fig. 3E, which seems to lack a mass in the upper left corner of the complex.

The most surprising result from the image analysis is that three other characteristic types of projections could be revealed (Fig. 3A–C). These projections were less abundant (together they comprise about 20% of the dataset), but they all show a new and very characteristic feature, i.e. a large extra mass in the lower left and/or upper right corner of the complex. The largest structure is shown in Fig. 3A, which has the extra mass on both sides of the complex. The length of this particle is the same as that of the supercomplex (Fig. 3D), but the width at the upper and lower parts increased to about 220 Å. The total width is about 300 Å, the same as the length of the complex. The shape resembles that of a parallelogram with dimensions of about 320 and 220 Å. There were no other types of particles present in significant numbers.

4. Discussion

lower right and upper left corners was removed from the complexes. This view is strengthened by the projection de-

The averaged two-dimensional projections of the various particles presented in Fig. 3 all have at least 24 Å resolution,
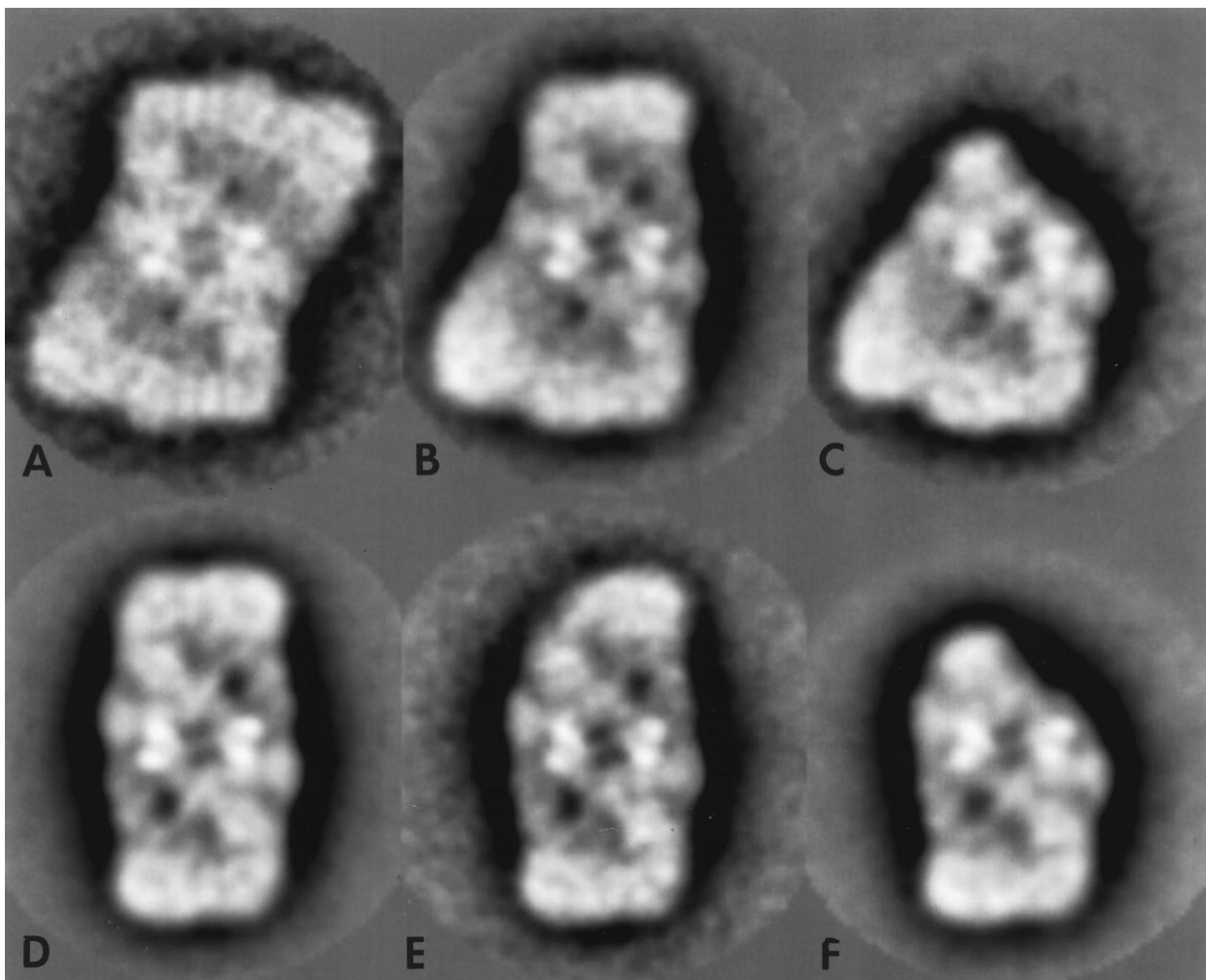

Fig. 3. Average projections of the six largest and most abundant types of supercomplexes found in a dataset of 3321 projections. The projections represent $C_2S_2M_2$ (A), C_2S_2M (B), C_2SM (C), C_2S_2 (D,E) and C_2S (F) types of supercomplexes. Notes: after sorting out by classification all types of particles were independently processed. On the images of A and D a two-fold rotational symmetry was imposed afterwards to further decrease the noise. The number of summed images is: 30 (A), 225 (B), 125 (C), 260 (D), 120 (E) and 350 (F).
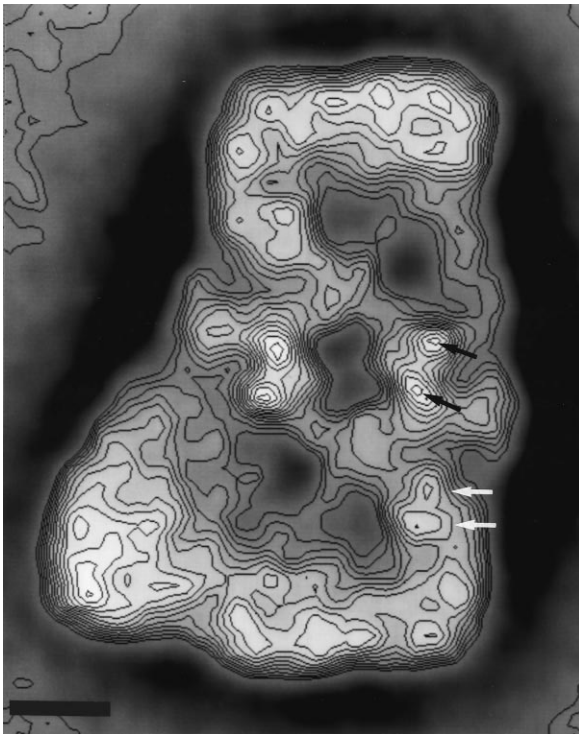
Fig. 4. The C_2S_2M type of PS II supercomplex contoured with equidistant levels. Black arrows indicate the two highest densities, which are about 24 Å separated and tentatively assigned as the CP47 subunit (upper arrow) and the 33 kDa extrinsic subunit (lower arrow, see [5]). White arrows indicate a 17 Å spacing, seen in the density profile of an area assigned to the CP43 subunit [5]. Bar represents 50 Å.

being the distance between the two strongest densities of the central core part (see also Fig. 4), assigned as the extrinsic loop of CP47 and the 33 kDa subunit [5]. The images comprising the largest number of particle projections (Fig. 3B,D) even have structural details at 17 Å. The well-preserved detail in the negatively stained preparations facilitates close comparison between the various particles and it appears that they are all dimeric complexes of PS II cores, surrounded by a smaller or larger peripheral antenna. The features of the complex of Fig. 3D are highly similar with those of previously published projection maps [5–7] but have a slightly higher resolution. The fragments in Fig. 3E,F were also seen previously. The other complexes, however, have not been observed before. The full dimeric complex (Fig. 3A) is substantially larger than the supercomplex, and in its fragments (Fig. 3B,C) the features of the extra mass are better resolved. They reveal that the dimeric complex of Fig. 3A has space to accommodate a dimeric reaction center core complex plus four trimeric and six monomeric LHC II complexes, although the exact position of these components remains to be established.

Our report is the first to show that a completely detergent-disrupted photosynthetic membrane can be analyzed by electron microscopy and image analysis without extensive biochemical purification. The disadvantage of this method is obviously that details of the protein composition of the various fractions cannot be given. However, due to the diode array-assisted chromatography technique (Fig. 1B) we know that the largest fractions at 13 min are characterized by a higher

A_{470}/A_{435} ratio than the slightly smaller fractions at 14–15 min, and thus we have good reasons to assume that the extra masses in Fig. 3A–C are enriched in Chl b and thus can be due to one trimeric LHC II complex. Perhaps an additional monomeric complex (CP24?) is present as well but this is not certain. The advantages of the method are that it is very quick, that therefore the probability of getting artificial aggregates decreases considerably [13] and that it may even be used to investigate possible ultrastructural reorganizations, such as those caused by state transitions or qE quenching [2,14].

The results described above suggest a second possible type of binding site of trimeric LHC II. In order to facilitate the discussion on the various complexes we propose short abbreviations for the different types of complexes. We designate the original supercomplex (Fig. 3D) C_2S_2, in which C represents a monomeric core complex and S the 'strongly' bound LHC II trimer in the position as reported in [6]. Consequently, the fragment in Fig. 3F is designated C_2S. The large complex in Fig. 3A could then be called $C_2S_2M_2$, in which M is 'moderately' bound trimeric LHC II. Consequently, the complexes in Fig. 3B,C should then be designated C_2S_2M and C_2SM, respectively. The fact that in the complete dataset of large particles more complexes with S-type than with M-type LHC II proteins are found indicates that at least in α-DM the S-type LHC II complexes are bound more strongly. This should certainly be true for β-DM, because with this detergent no significant amounts of M-type LHC II complexes were found in the supercores [6] and the largest particles in the gel filtration experiment are missing.

The detection of a second type of trimeric LHC II in the supercomplexes is not totally unexpected. In most models of the structural organization of the thylakoid membrane six or eight trimeric LHC II complexes surround a dimeric PS II core [2,15,16]. The relatively small amounts of PS II core and monomeric LHC complexes and the large amount of trimeric LHC II in the chromatogram presented in Fig. 1B may indicate that indeed additional trimeric LHC II is present in the membranes. Further work has to show whether or not there is a specific location of these complexes.

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