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Domain organization of photosystem II in membranes of the cyanobacterium *Synechocystis* PCC6803 investigated by electron microscopy

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Abstract The supramolecular organization of photosystem II (PSII) complexes in the photosynthetic membrane of the cyanobacterium *Synechocystis* 6803 was studied by electron microscopy. After mild detergent solubilization, crystalline PSII arrays were extracted in which dimeric PSII particles associate in multiple rows. Image processing of the arrays shows that the PSII dimers are tightly packed at distances of 12.2 and 16.7 nm. The domains are considered to be an important type of association for preventing either spill-over energy from PSII towards photosystem I (PSI) or direct energy flow from phycobilisomes to PSI, because the latter can only be at periphery of the arrays.

Keywords: Electron microscopy; Photosystem II; Single particle analysis; Membrane organization; *Synechocystis* 6803

1. Introduction

On a global scale the cyanobacteria and green plants are the most important groups of oxygenic photosynthetic organisms. Oxygenic photosynthesis is mediated by two large membrane-embedded photosystems, named photosystem I (PSI) and photosystem II (PSII). The green plant and cyanobacterial photosystems have several core subunits which are rather homologous, but the peripheral light-harvesting antenna proteins which are associated to PSI and PSII are very different in sequence and organization. Green plants have membrane-bound antenna proteins, belonging to the LHCII family. These proteins are lacking in most cyanobacteria. Instead, water-soluble phycobiliproteins are organized into giant (3–7 MDa) phycobilisome complexes, which are primarily associated to PSII, but also can serve excitation energy to PSI [1].

The organization of the photosynthetic thylakoid membrane of green plant chloroplasts is very special, because it consists of two main domains: the grana, which are stacks of thylakoids, and the stroma lamellae, which are unstacked thylakoids and connect the grana stacks. PSII and LHCII reside mainly in the grana membranes, while PSI and ATP synthase reside predominantly in the stroma (see [2] for a review). One of the main consequences of stacking is the physical separation of PSI and PSII. It has been argued [3] that the separation of the antenna systems of PSI and PSII is essential for efficient photosynthesis, because the kinetics of the trapping of excitation energy is much faster in PSI than in PSII, and a location of both antenna systems at short distances would lead to an uncontrolled flow of energy from PSII to PSI. The stacking not only prevents this spill-over of excitation energy, but it also provides the chloroplast the means to fine-regulate the light need for photosynthesis [4]. The stacking also provides PSII a very large functional antenna, in which excitation energy can flow within a thylakoid membrane and between two stacked membranes. In addition, it provides an easy means to adapt to low-light conditions, in which both the amount of LHCII and the extent of stacking have been shown to increase [5]. Within the membrane PSII is sometimes forming highly ordered semi-crystalline domains [2]. The cyanobacteria do not show a tight stacking of membranes, because of the presence of the phycobilisomes. There is no evidence for an extensive domain organization in cyanobacteria, but many electron microscopy studies have reported the presence of row-like associations of PSII dimers [6–9]. In one case small semi-crystalline PSII arrays were reported [10].

State transitions form a physiological adaptation mechanism that changes the interaction of the peripheral antenna proteins with the PSI and PSII core complexes. In cells adapted to state 1, phycobilisomes transfer energy primarily to PS II. In cells adapted to state 2 a significant proportion of phycobilisomes transfer energy to PS I instead. The main trigger for state transitions is reduction or oxidation of plastoquinone or a closely associated electron carrier. However, the signal transduction pathway that links a change in plastoquinone redox state to a change in the binding properties of phycobilisomes remains unknown [11]. On the larger scale level of the membrane the process is also not fully understood. It was found that in the cyanobacterial membrane large scale organization changes occur under state transition. It was found in *Synechocystis* 6803 and 6714 that under state 1 light the PSII complexes are arranged in row-like superstructures, whereas under state 2 light the orientation of PSII complexes was more random [9,12]. Based on this a “spill-over model” was proposed. It predicts that excess energy absorbed by pigments associated with PSII (either in the phycobilisome or the Chl a core antenna) spills over to PSI in state 2 (see [13] for an extensive discussion). The spill-over model considers that a change in the membrane organization of PSI and PSII, as observed by electron microscopy studies, is the main trigger for state transition.
of membranes

For preparing EM specimens, membranes from *Synechocystis* were solubilized with 2% digitonin at a 0.5 mg/ml final chlorophyll concentration. The suspension was stirred for 30 min using a small magnet stirrer, at 4°C and followed by 15 min centrifugation at 15,000 g. The unsolubilized material was discarded and the supernatant used for electron microscopy analysis.

2.3. Electron microscopy and single particle analysis

Samples were made from solubilized membranes by dilution in buffer with detergent and subsequent negative staining using 2% uranyl acetate on glow-discharged carbon-coated copper grids. Electron microscopy was performed on a Philips CM120 equipped with a LaB6 tip operating at 120 kV. The “GRACE” system for semi-automated specimen selection and data acquisition [20] was used to record 2048 × 2048 pixel images at 66850x calibrated magnification (0.375 nm) with a Gatan 4000 SP 4 K slow-scan CCD camera. A total of 50 PSII crystals and 500 single particle projections of PSII double dimers were collected from about 4000 images. Single particle analysis [21–23] was performed using Groningen Image Processing (“GRIP”) software packages on a PC cluster. Crystal fragments and single particles were repeatedly aligned and classified and finally the best 70% of the data set was taken for the final sums.

3. Results

Partial membrane solubilization and subsequent electron microscopy on negatively stained specimens revealed the projections of many single particles and small membrane fragments. Most of the membrane fragments consisted of rectangular-shaped crystalline arrays (Fig. 1). The most regular arrays were collected and submitted for image analysis. The arrays consist of several rows of dimeric PSII complexes, most of them comprised of two rows (left five examples, Fig. 1). The upper right frame displays an array that is part of a larger piece of membrane which is partly non-crystalline. In the lower right frame the crystal is built up of five rows of PSII dimers. The average 2D map, obtained by merging small crystal fragments from 50 crystals, shows that the arrays contain equidistant spaced PSII dimers. The spacing is 12.2 nm in the vertical direction (along the rows) and 16.7 nm between the rows, as indicated by the unit cell (blue box, Fig. 2A).

![Fig. 1. A gallery of PSII crystals, negatively stained with 2% uranyl acetate.](image-url)
Electron microscopy of solubilized membranes shows a large spectrum of single particle complexes, some of them easily recognizable by their particular shape. Among them, the most abundant were trimeric PSI, the ATP synthase and, NDH-1 complexes [18] and dimeric PSII. Moreover, double PSII dimers were found and a final 2D map was obtained from a sum of 500 projections (Fig. 2B). The features of the double dimer map appear to match very well with those of the dimers within the 2D arrays and with single dimers, analyzed previously [16,23]. The center-to-center distance between dimers in the double dimers is the same as in the crystals. But at the periphery the dimers are larger than the dimers in the crystals. The difference is caused by the detergent shell. Integral membrane proteins are only soluble as protein-detergent micelles and the detergent shell is not penetrated by the negative stain and thus significantly contributes to the total surface in projection (see also [16] for details). In the rectangular arrays PSII dimers are tightly packed in the lipid membrane, without detergent between them.

In order to see if the double PSII dimer has the same overall features as the singular dimers, the high-resolution X-ray structure [24] was fitted in the EM 2D map of the double dimer and the crystal (Fig. 3). The match is reasonably good, if we take in account that the periphery has the detergent contribution. At the interface, however, the EM map is larger in surface, indicated by red asterices (Fig. 3). It is possible that these sites contain subunits which are lacking in the purified dimers from which the X-ray structure was solved.

4. Discussion

The process of photosynthesis cannot be understood without a detailed knowledge of the structure of its single components. Detailed knowledge about the subunits and pigments of cyanobacterial PSI and PSII has been derived from high-resolution structures obtained by X-ray diffraction studies [24,25]. For insight of the interactions of PSI and PSII detailed knowl-
EM performed on partially purified *Synechocystis* 6803 membranes indicated that the only larger structures are PSII double dimers and the PSI crystalline arrays. A similar, but lower-resolution 2D map of double dimeric PSII particles was presented earlier for *Thermosynechococcus elongatus* [16], but the fact that the PSI is packed in well-ordered arrays is a novel finding. The double dimers are considered to be break-down products of rows of PSII dimers. We did not observe intact single rows, and just a few triple dimers were found. Apparently the rows break down more often in double dimers. The distance between the dimers was found to be 12.1 nm and the same spacing was found in freeze-fracture EM specimens before [7,8]. We measured a center-to-center distance between dimers in the double dimer of 12.8 nm. This distance is compatible but slightly smaller than the width of a phycobilisome, which is close to 15 nm for *Synechocystis* 6803 (A. A. Arteni, I. M. Folea, G. Ajlani, E.J. Boekema, unpublished results). This means that a 1:1 match of phycobilisomes on top of rows of PSI is possible as proposed before [7,13,26]. A good match is especially possible when the phycobilisomes make an angle with the PSI rows, as will be discussed below in our model.

The domains of multiple rows of PSII as observed in this study are unexpected, because most studies indicated only the presence of single rows of PSII, although small PSII arrays were found in the cyanobacterium *Synechocystis* BO8404 with a spacing of about 18 nm between the rows [10]. The distance between the rows is 16.7 nm whereas the length of a phycobilisome in this direction is about 6 nm. In a freeze-fracture EM study of *Synechocystis* 6803, the distance between the rows was 50 nm in the wild-type, 23 nm in a rod-less mutant and 16 nm in a PBS-less mutant [9]. The large size of the phycobilisome implies that in the PSII arrays only one third of the PSII dimer can be attached to a phycobilisome. It is not known, however, if the PSII were functionally attached to phycobilisomes because they are not present anymore. If fully present on PSII, the rows of phycobilisomes may keep the PSII rows at a distance, as in the study of [9]. It is not clear to what extend the PSII-phycobilisome complexes will provide a sterical hindrance for PSI complexes. At their cytoplasmic sides the PsaC-E extrinsic subunits are protruding about 4–5 nm from the membrane surface [25].

The issue of the sterical hindrance is also raising the questions what the function of the PSIII arrays might be and how the other membrane proteins are dispersed in the membrane. We present a model in Fig. 4 which shows the main membrane protein complexes on scale. In the model the ratio of PSI to PSII (compared as monomers) is 2:1; this is within the ratio of 3:0 found for *Synechocystis* 6803 grown under PSI light and 1.5:1 found for PSI light [27] and the monomer ratio of cytochrome *b/f* to PSI is 0.8:1, which is fairly constant under variable growth conditions [31]. The black rectangle, on top of a PSII dimer, indicates the size of a phycobilisome.

The edge of the supramolecular organization of these photosynthetic complexes within the cyanobacterial membrane is also crucial.

Fig. 4. A model for the organization of the photosynthetic membrane of the cyanobacterium *Synechocystis* PCC6803, densely packed with single and multi-subunit protein complexes [29]. PSII arrays consisting of several PSII rows (green) are surrounded by small numbers of randomly distributed PSII monomers and dimers (green), larger numbers of PSI complexes (trimers and some monomers; in blue), dimeric cytochrome *b/f* complexes (purple), monomeric ATP synthase (red), NDH-1 complexes (ochre) and various other types of membrane proteins, such as cytochrome oxdyase (grey). Small connecting masses (pink/green) between the PSI complexes are hypothesized to trigger and maintain dimer to dimer interactions. In the model the core PSI to PSII ratio is 2:1, which is within the ratio of 3:0 found for *Synechocystis* 6803 grown under PSI light and 1.5:1 found for PSII light [27] and the monomer ratio of cytochrome *b/f* to PSI is 0.8:1, which is fairly constant under variable growth conditions [31]. The black rectangle, on top of a PSII dimer, indicates the size of a phycobilisome.
the membrane surface, unless a kind of disconnection takes place. A refined spillover model of the cyanobacterial photosynthetic membrane was proposed [13]. It describes the redistribution of the Chl a excitation energy and the phycobilisome excitation energy. An important aspect of the model is the idea that the Chl a pools of CP43 and CP47 inside PSI are separated. Changes in the distribution of Chl a absorbed light are accomplished by a spillover mechanism characterized by excitation energy transfer from only one component of PSII (CP47) to PSI. Energy reaching the PSII reaction center from the phycobilisomes either directly or via CP43 would have a relatively low probability of visiting CP47 pigments. This energy would thus not be lost via spillover to PSI. In other words, the refined spillover model also implies that it matters how the PSI and PSII complexes are associated.

In our model of Figs. 3 and 4 the positions of CP43 (blue) and CP47 (purple) in the PSI double dimer and arrays are highlighted. It shows that in the supramolecular structures they are in close proximity. On the other hand, CP43 is not exposed at the periphery and neighbouring PSI trimers can not become in close connection, which means that there is no possibility for energy transfer from CP43 to PSI. This means that there is a large difference in the possibility of energy transfer between single dimers and PSI the PSII arrays and PSI. This indicates that the arrays might be relevant for preventing the spillover process. There is also a second explanation for the array formation possible, which is a lowering of direct energy transfer from phycobilisomes to PSI. Phycobilisomes attached to PSII dimers will be close to several other complexes (black circumference, Fig. 4), but within an array they will have a lower change to be close to PSI complexes.

This second possibility is realistic as well because there are some lines of evidence to suggest that spillover is not particularly prevalent in cyanobacteria specifically grown under physiological conditions [29].

In conclusion, we have shown in this paper that single particle averaging is able to detect the PSI superstructures without any purification step. Further work along these lines should focus on the cause of the array formation by membrane analysis of cyanobacteria lacking specific small PSII subunits. Second, it would be worth to see if the same arrays are present in other species, because cyanobacteria form a heterogeneous group of organisms with different types of overall membrane organisations [30]. Third, on the longer time scale it should be very relevant to directly establish the structure of the PSII-phycobilisome supercomplex.

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References


