Domain organization of photosystem II in membranes of the cyanobacterium Synechocystis PCC6803 investigated by electron microscopy
Folea, I. Mihaela; Zhang, Pengpeng; Aro, Eva-Mari; Boekema, Egbert J.

Published in:
FEBS Letters

DOI:
10.1016/j.febslet.2008.04.044

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Domain organization of photosystem II in membranes of the cyanobacterium *Synechocystis* PCC6803 investigated by electron microscopy

I. Mihaela Folea\(^a\), Pengpeng Zhang\(^b\), Eva-Mari Aro\(^b\), Egbert J. Boekema\(^a,\)\(^*\)

\(^a\) Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

\(^b\) Department of Biology, University of Turku, FIN-20014 Turku, Finland

Received 10 April 2008; revised 18 April 2008; accepted 21 April 2008

Available online 6 May 2008

Edited by Richard Cogdell

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 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 LHClI 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 LHClI 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 LHClI 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 PSI 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 in the cyanobacterium *Synechocystis* PCC6803, may be important for preventing spill-over energy from PSII towards PSI.

\(^*\)Corresponding author. Fax: +31 50 3634800.

E-mail address: e.j.boekema@rug.nl (E.J. Boekema).

0014-5793/$34.00 © 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
2.2. Solubilization of membranes according to [19]. The unsolubilized material was discarded and the supernatant used for electron microscopy analysis. The suspension was stirred for 30 min using a small magnet stirrer, at 4°C and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to prepare double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].
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-
edge of the supramolecular organization of these photosynthetic complexes within the cyanobacterial membrane is also crucial.

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 PSII 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 PSII 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* BOS404 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 PSI 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 PSI rows at a distance, as in the study of [9]. It is not clear to what extent the PSII-phycobilisomes complexes will provide a steric 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 steric 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 bif complexes 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 possibility of excitation transfer via these sides towards a neighbouring PSI trimer or monomer will be very low or zero. This means that the PSII arrays could have the same function as in the green plants: to keep the excitation energy away from PSI. This is important 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 [3]. In this context it is also relevant to mention that in the experiments with *Synechocystis* 6803 on long term light adaptation [27], discussed above, it was found that PSI is the variable component in changes in the PSI to PSII stoichiometry. This could also implicate that the number and size of PSIII arrays are rather fixed and that during long-term changes in the light regime only the randomly ordered PSI complexes change in numbers.

The more rapid changes of membrane reorganization under state transitions remain to be solved. Three overall structural events seem or may take place at the same moments: (1) a rearrangement of the PSII rows or arrays into a more random organization; (2) a rearrangement of the phycobilisome positions from PSII towards PSI or vice versa; and (3) smaller changes in the PSIII and/or phycobilisome structure yet to be discovered. It is possible that a small membrane component, hypothesized in our model to connect the dimers in the rows and arrays, could play a role in array formation. Because of steric hindrance of the PSI extrinsic subunits it is not likely that the phycobilisomes have unlimited freedom to diffuse on
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 PSI 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 chance 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 variety of membrane complexes (black circumference, Fig. 4), but within an array they will have a lower chance to be close to PSI complexes.

Acknowledgements: We thank Dr. R. Kouril for model building, Dr. G.T. Oostergetel for help with electron microscopy, Dr. W. Keegstra for help with image processing and Dr. D. Schneider from Freiburg for discussion. Work was supported by the Netherlands organization for scientific research NWO.

References


