Volume 296, number 1, 29–32 FEBS 10571 January 1992

Analysis of the structure of photosystem I in cyanobacterial thylakoid membranes

Andreas Hefti[1], Robert C. Ford[2], Mette Miller[3], Raymond P. Cox[3] and Andreas Engel[1]

[1]*M.E. Müller Institute for High Resolution Electron Microscopy at the Biocentre, University of Basel, CH-4056 Basel, Switzerland,* [2]*Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD, UK* and [3]*Institute of Biochemistry, Odense University, Campusvej 55, 5230 Odense M, Denmark*

Received 28 October 1991; revised version received 13 November 1991

Using electron microscopy of negatively stained specimens, we have investigated the shape and degree of association of photosystem (PS) I complexes in cyanobacterial thylakoid membranes. When incubated at high concentrations of magnesium chloride (>0.15 M), the PSI complexes form small ordered arrays in the membrane composed of monomeric complexes in a P1 square lattice of dimensions $a=b=11$ nm. Averaged projections of the complex resemble those found for the purified PSI reaction centre after reconstitution (Ford, R.C, Hefti, A. and Engel, A. (1990) EMBO J. 9, 3067–3075). Some small differences in its shape are discussed, with particular reference to the differences in the polypeptide composition of the 2 preparations. We find that the complex remains in the monomeric form in the thylakoid membrane under all the conditions tested.

Electron microscopy; Membrane protein; Photosystem I; Photosynthesis; *Synechococcus* sp.

1. INTRODUCTION

Photosystem I (PSI) is a large membrane protein complex composed of more than 4 protein subunits; about 120 chlorophyll (Chl) *a* molecules; at least 10 carotenoid molecules and an electron transfer chain composed of Chl molecules, a phylloquinone and 3 iron sulphur centres [1]. It catalyses the later steps in the photosynthetic electron transfer system found in plant chloroplasts and cyanobacteria, being responsible for the photo-oxidation of a water soluble electron carrier, plastocyanin (or cytochrome c_{552}), and the photo-reduction of ferredoxin. Reduced ferredoxin can then be used in a variety of metabolic pathways including the fixation of carbon dioxide in the Calvin cycle. Previous structural studies of PSI have tended to concentrate on the detergent-solubilised reaction centre of PSI which, in its simplest form is composed of several protein subunits, the electron transfer chain, and about 70 light-harvesting Chl and carotenoid molecules [2–8]. The reaction centre purified from cyanobacteria exists in various oligomeric forms, the major ones being the trimeric and monomeric forms. Crystals of the trimeric reaction centre have been obtained and are currently the subject of X-ray crystallographic studies [9–11]. Recently we also obtained two-dimensionally ordered arrays of monomeric and trimeric reaction centres in reconstituted lipid bilayers [12] and showed their projections and surface relief reconstructions with a resolution of about 2 nm.

It is of importance to determine whether the PSI complexes in the cyanobacterial thylakoid membrane bear any resemblance to the highly purified and simpler reaction centres that we use for structural studies using electron microscopy and X-ray crystallography. The obvious difficulty in achieving this goal is that the native thylakoid membranes contain several protein complexes, as well as an extensive membrane-associated light-harvesting system contained in phycobilisomes. Thus, the identification of PSI complexes within this mixture seems, at first, very difficult to achieve.

To overcome this problem, we have used membranes that had been treated with a low detergent concentration to remove the PSII complexes [13] and were then extensively washed to remove most of the residual phycobiliproteins. The resulting membranes were highly enriched in PSI. We have examined the overall size and shape of the complex in these membranes and, in addition, we were able to obtain small 2D ordered arrays of the PS that have allowed us to analyse the complex in some detail by digital image processing.

2. MATERIALS AND METHODS

Membranes of the thermophilic cyanobacterium *Synechococcus* sp. OD 24 were obtained essentially as described in [14]. For electron microscopy, the membranes were washed twice in the incubation buffer, resuspended at about 1 mg Chl/ml and then incubated at room temperature for 60 min. Incubation buffer consisted of 20 mM MES, 5 mM $MgCl_2$, 15 mM NaCl, pH 6.3, plus additional $MgCl_2$ from 0–0.15 M, as detailed in the results section. The same procedure was

Correspondence address: R.C. Ford, Department of Biochemistry and Applied Molecular Biology, UMIST, PO Box 88, Manchester M60 1QD, UK.

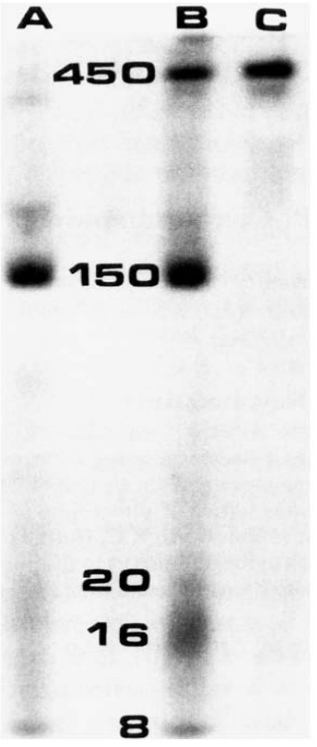

Fig. 1. SDS-PAGE of PSI-enriched thylakoid membranes washed with 0.15 M $MgCl_2$ (lane A) and 5 mM $MgCl_2$ (lane B); amounts equivalent to 3.5 and 4.9 μg Chl were loaded, respectively. Note that the yield of trimers is much higher at lower $MgCl_2$ concentrations. Purified trimeric reaction centre complexes were run in lane C (3.4 μg Chl was loaded). Apparent molecular weights of the various bands (in kDa) are shown on the gel.

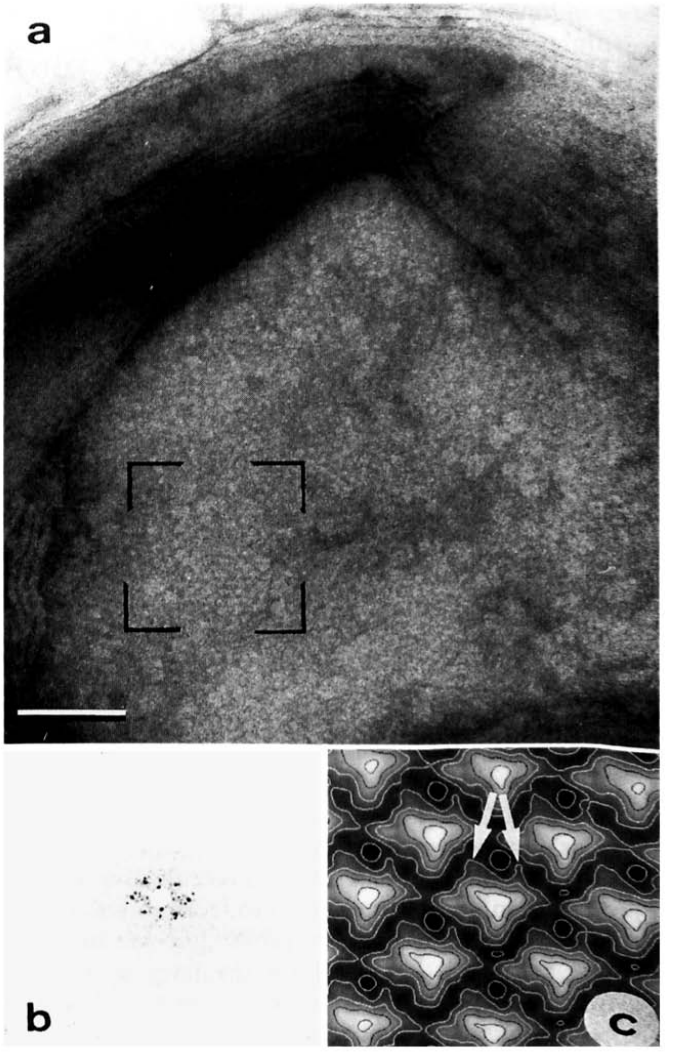

Fig. 2. PSI-enriched membrane fragments stained with uranyl formate. (a) At a concentration of 150 mM $MgCl_2$, small ordered arrays of particles were observed in the PSI-enriched membranes. The bar represents 100 nm. (b) The diffraction pattern from the boxed area in (a). (c) The unit cells have dimensions $a=b=11$ nm. The arrows point to protrusions that are not observed in the purified reaction centre.

employed prior to solubilisation of the membranes with dodecyl maltoside (1% w/v) at room temperature for 5 min. Assays for PSI enrichment were carried out as in [2]. Assays for the presence of PSII and the cytochrome complex were carried out by detection of the cytochrome b_{559} (for PSII) and the cytochromes f and b_{563} (for the b_6f complex), essentially as described in [15]. SDS-PAGE was carried out as described by Laemmli [16]; samples at a concentration of 0.5 mg Chl/ml were incubated with 1% (w/v) SDS, 20 mM Tris-HCl, pH 8.8, 10% (v/v) glycerol at 40°C for 5 min and then centrifuged at 16 000 × g for 1 min prior to loading onto the gel. Chl assays followed the method of Arnon [17]. Electron microscopy, negative staining and image analysis were carried out as described in [12].

3. RESULTS AND DISCUSSION

To characterise the PSI-enriched cyanobacterial membranes, we performed various assays for activity which are summarised in Table I. Although we only tested for activity of expected complexes, it seemed reasonable to suppose from the data that the membranes were largely composed of PSI. Using electron paramagnetic resonance spectroscopy, similar conclusions were drawn with membranes prepared from the cyanobacterium *Phormidium laminosum* (Rutherford, Setif and Ford, unpublished results). The average number of Chl molecules associated with PSI can be estimated from the measured Chl:P700 ratio.

It is possible to conclude from Table I that PSI has about 50% more Chl associated with it in the membrane than in the purified reaction centre. These data must be treated with caution, however, since several studies have shown that some detergents can change the extinction coefficient of P700 under some conditions, and this could exaggerate the actual difference between the Chl content of membrane-bound PSI and the purified PSI reaction centre [18].

SDS-PAGE under mildly denaturing conditions was used to further study the composition of the membranes and to compare it with purified reaction centres. The results are shown in Fig. 1. The overall staining pattern

is very similar for the PSI-enriched membranes (lanes A and B) and the purified reaction centre (lane C), with some additional bands present in the membrane sample at 20 kDa, 18–14 kDa (broad) and 8 kDa. Perhaps the least significant of these was the broad band at 18–14 kDa which was associated with a yellow colouration in the unstained gel. This band stained strongly at first, but then slowly faded in the destain, suggesting that it may not, in fact, be proteinaceous in nature, although this conjecture remains to be confirmed. The yellow colour was mainly due to carotenoid absorbing in the Soret region with a broad peak at about 430 nm. The identity of the other 2 bands on the gel also awaits further analysis, although there are several candidate PSI-associated polypeptides with molecular weights in the relevant region, notably the *psaF* (18 kDa) and *psaK* (8 kDa) gene products [8].

As discussed in a previous publication, for *Phormidium laminosum* PSI-enriched membranes, it is only the monomeric form of PSI that seems to exist in the native membranes [3]. Similarly, electron micrographs of negatively stained *Synechococcus* sp. membranes after incubation at low $MgCl_2$ concentrations showed areas packed with non-ordered elongated particles of maximum length 14 nm. No evidence for trimeric complexes could be found (data not shown). After incubation of the membranes at high concentrations of $MgCl_2$, small ordered arrays of the particles were observed, and it was again clear from the unit cell dimensions that these could not be formed from trimeric PSI complexes, but were rather, composed of monomeric units (Fig. 2). Fig. 2b shows the diffraction pattern produced from the area enclosed by the box in Fig. 2a. The diffraction pattern showed (2,1) orders indicating a spatial resolution of 5 nm. A correlation-averaged projection revealed complexes packed in a P1 square lattice with $a=b=11$ nm (Fig. 2c). The complex had an elongated form of maximum dimensions of 13×9 nm. In comparison, the $P2_1$ square lattices formed after reconstitution of the isolated reaction centre [4] have dimensions $a=b=14.5$ nm with 2 monomeric reaction centres in the unit cell. Maximum dimensions for the monomeric complex in this lattice are approximately 13×7 nm, slightly narrower than in Fig. 2c. The overall shape of PSI in the thylakoid membrane seems to be very similar to the reconstituted reaction centre, however its packing arrangement is different because the complexes must be unidirectional with respect to membrane plane in the native system. In both cases we observe a characteristic apex which subtends an angle of approximately 120 degrees (bottom side of the complex in Fig. 2c). The opposite side of the complex (top side of the complex in Fig. 2c) shows 2 protrusions (arrowed) which are absent from the corresponding images of the purified reaction centre. The reaction centre shows a bevelled outline in this region. These extra protrusions in the more native complex seem to account for its increased thickness compared to the reaction centre.

To answer the question of whether these additional features might correspond to proteins involved in binding the extra light-harvesting molecules present in PSI, or whether they might be proteins involved in mediating electron transfer to other electron carriers we must carry out further work, preferably with larger arrays. The use of antibodies directed against purified accessory proteins might provide a possible route to identify the location of these proteins.

In conclusion, we have been able to determine to what extent the purified PSI reaction centre resembles the complex in the thylakoid membrane. Although the membranes we must work with are not strictly 'native' since they have received a detergent pretreatment, albeit a very mild one, it is apparent, given the size of the membrane fragments and the orientation of the complex with respect to the membrane plane, that they are intact. Because the images obtained from the ordered arrays in the membranes are so similar to the images of the purified reaction centre, we can assign increased confidence to our structural investigations of the purified reaction centre. Further studies of the complex in the membrane should lead to the identification of the likely binding sites of accessory proteins to the reaction centre, increasing the value of the reaction centre structure when it emerges at high resolution.

Table I

Characteristics of the PSI-enriched membranes

Chl:P700 ratio		
chemical assay	+ 0.15 M $MgCl_2$	110
	+ 5 mM $MgCl_2$	111
photochemical assay	+ 0.15 M $MgCl_2$	103
	+ 5 mM $MgCl_2$	105
Chl:cytochrome b_6f		
chemical assay	+ 0.15 M $MgCl_2$	>2000
	+ 5 mM $MgCl_2$	>2000
Chl:PSII ratio		
chemical assay	+ 0.15 M $MgCl_2$	>4400
	+ 5 mM $MgCl_2$	>4400

Acknowledgements: We thank Professor U. Aebi for support and discussions. This work was supported by the Swiss National Foundation for Scientific Research Grant No. 31-25684.88 to A.E. and the UK Science and Engineering Council Grant No. G04271 to R.C.F.

REFERENCES

[1] Rutherford, A.W. and Heathcote, P. (1985) Photosynthesis Res. 6, 295–316.
[2] Ford, R.C. (1987) Biochim. Biophys. Acta 849, 16–24.
[3] Ford, R.C. and Holzenburg, A. (1988) EMBO J. 7, 2287–2293.
[4] Takahashi, Y. and Katoh, S. (1982) Arch. Biochem. Biophys. 219, 219–227.
[5] Boekema, E.J., Dekker, J.P., van Heel, M.G., Rogner, M. Saenger, W., Witt, I. and Witt, H.T. (1987) FEBS Lett. 283–286.
[6] Fish, L.E., Kuck, U. and Bogorad, L. (1985) J. Biol. Chem. 260, 1413–1421.

[7] Hoj, P.B., Svendsen, I., Scheller, H.V. and Moller, B.L. (1987) J. Biol. Chem. 262, 12676–12684.
[8] Bryant, D.A. (1991) in: Current Topics in Photosynthesis (J. Barber, ed.). The Photosystems: Structure, Function and Molecular Biology, vol. 11. Elsevier, Amsterdam, (in press).
[9] Ford, R.C., Pauptit, R.A. and Holzenburg, A. (1988) FEBS Lett. 238, 385–389.
[10] Witt, I., Witt, H.T., Di Fiore, D., Rogner, M., Hinrichs, W., Saenger, W., Granzin, J., Betzel, Ch. and Dauter, Z. (1988) Ber. Bunsenges. Phys. Chem. 92, 1503–1506.
[11] Shoham, G., Michaeli, D. and Nechustai, R. (1989) Physiol. Plant. 76, A82.
[12] Ford, R.C., Hefti, A. and Engel, A. (1990) EMBO J. 9, 3067–3075.
[13] Stewart, A.C. and Bendall, D.S. (1980) FEBS Lett. 107, 308–312.
[14] Miller, M., Pedersen, J.Z. and Cox, R.P. (1988) Biochim. Biophys. Acta 943, 501–510.
[15] Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
[16] Laemmli, U.K. (1970) Nature 227, 680–685.
[17] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
[18] Markwell, J.P., Thornber, J.P. and Skrdla, M.P. (1980) Biochim. Biophys. Acta 430, 389–394.