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Three-dimensional structure of photosystem II determined by electron crystallography

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Introduction.

Photosystem two (PSII) is a multisubunit pigment-protein complex located within the thylakoid membrane of plants, algae and cyanobacteria. All the cofactors of PSII involved in electron transport are bound to the two structurally related and interconnecting proteins, known as the D1 and D2 subunits. Closely associated with the D1/D2 heterodimer are two chlorophyll binding proteins (CP), CP43 and CP47 (Rhee *et al*. 1998; Hankamer *et al.,* 1997b, 1999) and at least three extrinsic proteins which protect and optimise the water oxidase activity of the Mn cluster (33 kDa PsbO, 23 kDa PsbP, 17 kDa PsbQ for higher plants/green algae (Nield *et al*., 2000) and 33 kDa PsbO, 15 kDa PsbV, 11 kDa PsbU for cyanobacteria (Zouni *et al*., 2001)). These intrinsic and extrinsic proteins, together with a number of low molecular weight subunits including cytochrome b559, make up the core complex.

To determine the detailed mechanism by which PSII is able to drive the highly oxidising water splitting reaction it is necessary to elucidate its structure. Here we highlight and discuss features of the first 3D structure of the complete dimeric PSII core complex of higher plants at sufficient resolution to identify the positions of its transmembrane helices (Hankamer *et al.,* 2001a Hankamer *et al.,* 2001b). The structure builds upon the previously determined electron crystallographic structure of a sub-complex of PSII (Rhee *et al.,* 1998), a projection map of the core dimer (Hankamer *et al.,* 1999) and complements a recent X-ray structure derived for cyanobacterial PSII (Zouni *et al*., 2001).

Materials and Methods

Oxygen evolving PSII core dimers were isolated from spinach thylakoid membranes and used to produce two dimensional (2D) crystals (Morris *et al*., 1997; Hankamer *et al.*, 1999). Low dose images of frozen hydrated crystals were recorded in Philips CM200FEG (liquid N_2) and CM300FEG (liquid He) electron microscopes at tilt angles from 0^o to 35^o. MRC image analysis programs (Henderson *et al.,* 1986) together with locally developed software were used to process the data. α-carbon helical backbones were fitted into the map using the graphics program O (Jones *et al*., 1991) and molecular structure diagrams created using Molscript (Kraulis, 1991).

Results and Discussion.

The three dimensional (3D) map calculated from 23 merged tilted images is shown in Fig 1 viewed from the lumenal surface and sectioned to show the transmembrane domain. Densities corresponding either to individual or pairs of transmembrane helices are directly identified in the map and traced through the membrane plane and the helices are represented as cylinders.

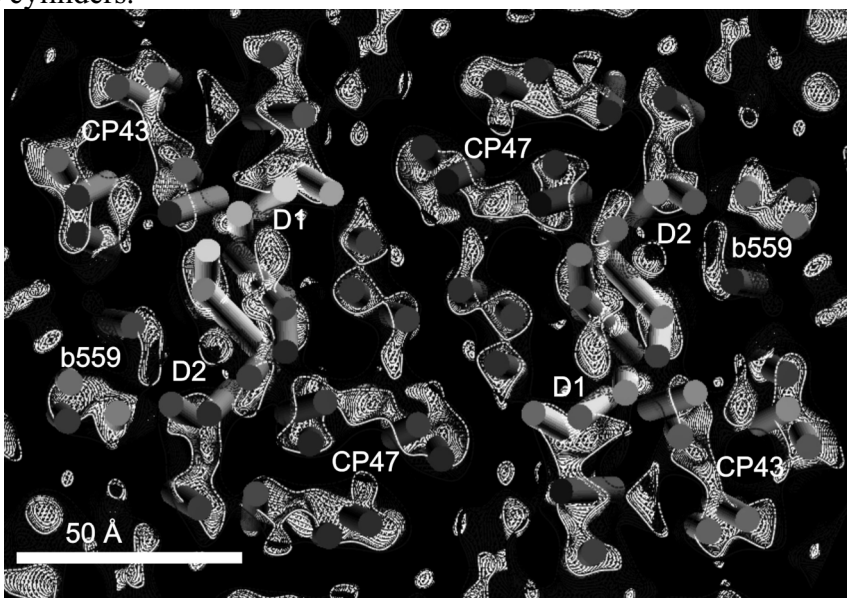

Fig 1. Transmembrane domain of the electron crystallographic 3D map of the spinach PSII core dimer viewed from lumenal surface. Helices built into the map are represented as cylinders and major subunits are labelled**.**

A number of the helices can be assigned to particular subunits on the basis of comparison with earlier electron crystallographic data obtained with the spinach PSII CP47 reaction centre subcomplex (CP47-RC) containing the D1/D2 proteins and CP47 (Rhee *et al*., 1998). As noted previously, the arrangement of the two sets of five transmembrane helices corresponding to the D1 and D2 subunits are arranged around a local pseudo-twofold axis in a similar manner to that of the L and M subunits of the reaction centre of purple bacteria (Michel and Deisenhofer, 1988). The six helices adjacent to the D2 proteins and close to the monomer-monomer interface, belong to CP47. Additional density within the six helical cluster can be attributed to chlorophyll molecules previously identified within the CP47 domain (Rhee *et al.,* 1998). An equivalent set of densities was found adjacent to the D1 protein, which are related to those of the transmembrane helices of CP47 by the local two-fold axis of the D1/D2 heterodimer. These densities accommodate three pairs of helices assigned to CP43. As in the case of CP47, additional density within the six helix bundle is assumed to be due to bound chlorophyll molecules. The assignment of the CP43 transmembrane helices was suggested previously based on a 2D projection map (Hankamer *et al*., 1999) but this is now supported by the 3D map in which the densities can be traced across the membrane. Two additional helices are attributed to the α- and β-subunits of cytochrome b559 (Zouni *et al*., 2001). The remaining transmembrane helices belong to the low molecular weight subunits of PSII and will be discussed below.

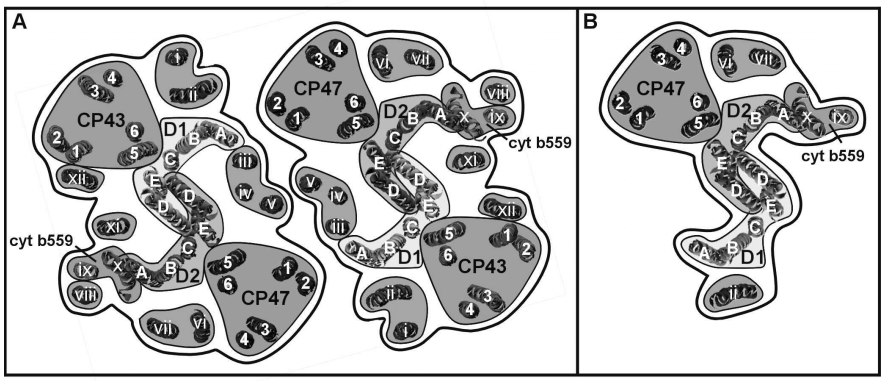

Fig 2 Helix organisation of the core dimer and CP47 RC complexes (A) Core dimer complex. (B) CP47 RC subcomplex

In addition to the 22 helices of the major subunits we have identified a further 12 transmembrane helices which we assign to low molecular weight subunits. These are shown diagrammatically in Fig 2A. For clarity these have been numbered from i – xii (Fig 2A) and can be compared with those present in the previously determined CP47-RC subcomplex (Fig 2B, Rhee *et al.,* 1998). To be noted, however, is that two helices previously assigned immediately adjacent to the B-helices of the D1 and D2 proteins have not been included. It is highly likely that the densities in these regions are due to chlorophyll molecules ligated to D1His198 and D2His198 rather than to transmembrane helices. Helices ix and x, are assigned to the apoproteins of cytochrome b559 based on the earlier electron microscopy study (Rhee, 1998) and the recent X-ray crystallographic structure of a cyanobacterial bacterial PSII core complex (Zouni *et al.,* 2001). Of the remaining 10 helices, three (iii, iv and v) are close to the two-fold axis of the dimer forming an interface between the two monomeric complexes. These helices are well defined in the map as can be seen in Fig. 1. Two further helices (i, ii) are identified adjacent to helix B of the D1 protein and two (vi, vii) close to helix B of the D2 protein. There also seem to be two additional helices (viii, xi) adjacent to cytochrome b559 and one other small subunit (xii) located near to helices 1 and 2 of CP43. The assignment of small subunits to specific PSII proteins is not possible at the resolution of the current map or indeed in the X-ray derived map of the cyanobacterial core complex (Zouni *et al.*, 2001). However, based on biochemical analysis on the monomeric and dimeric forms of the CP47-RC subcomplex (Zheleva *et al*., 1998) and a comparison with the structural model derived for the monomeric form of this subcomplex (Rhee *et al*., 1998) we suggest that two of the helices in the three helix cluster (iii, iv, v) at the monomer-monomer interface are likely to be PsbK and PsbL; since these proteins were present in the CP47-RC dimer but not in the monomer (Zheleva *et al*., 1998). On the other hand, PsbI, PsbW and $PsbT_c$ were identified to be present within the CP47 RC monomer (Zheleva *et al*., 1998) and therefore can be considered to correspond to helices ii, vi and vii. Given that PsbH and PsbX are not present in the monomeric or dimeric of CP47 RC but are components of the core dimer complex they may correspond to the extra helices observed in the new map (xiii, xi, xii) or possibly one of the helices of the interfacial cluster (iii, iv, v).

Recently, a 3.8Å structural model has been elucidated for PSII of the thermophilic cyanobacterium *Synechococcus elongatus* by X-ray diffraction analysis (Zouni *et al*., 2001). As in the case of the higher plant system, the cyanobacterial core complex was found to be dimeric. Moreover, the organisation of the transmembrane helices for the major subunits, D1, D2, CP43 and CP47 was very similar to those derived for the higher plant complex. The existence of the conserved features in such a diverse range of species reinforces the view that PSII of higher plants and cyanobacteria have a common evolutionary origin. Comparison of the two structures also provides strong evidence that the dimeric nature of the core is the main physiological form of PSII both of higher plants and cyanobacteria despite claims to the contrary (Holzenburg *et al.,* 1993). The similarities of the two structures are also carried

through at the level of the low molecular weight subunits. The position of cytochrome b559 and two adjacent small subunits (viii, xi) appear to be conserved as are the cluster of three helices (iii, iv and v) at the interface between the two monomers. Transmembrane helices vi and vii are also found in both structures. The differences are that the cyanobacterial system does not have helix i but instead has an additional cluster of three helices between cytochrome b559 and helices 1 and 2 of CP43 where only one helix (xii) was identified in the higher plant complex. It is possible that these differences relate to the difference in the light harvesting systems of the two types of photosynthetic organisms or alternatively represent adaptations linked to the thermal stability of *Synechococcus elongatus* PSII.

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