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The first insight into the structure of the Photosystem II reaction centre complex at 6Å resolution determined by electron crystallography

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Abstract

Electron crystallography of two-dimensional crystals and electron cryo-microscopy is becoming an established method for determining the structure and function of a variety of membrane proteins that are proving difficult to crystallize in three dimensions. In this study this technique has been used to investigate the structure of a ~160 kDa reaction centre sub-core complex of photosystem II. Photosystem II is a photosynthetic membrane protein consisting of more than 25 subunits. It uses solar energy to split water releasing molecular oxygen into the atmosphere and creates electrochemical potential across the thylakoid membrane, which is eventually utilized to generate ATP and NADPH. Images were taken using Philips CM200 field emission gun electron microscope with an acceleration voltage of 200kV at liquid nitrogen temperature. In total, 79 images recorded at tilt angles ranging from 0 to 67 degree yielded amplitudes and phases for a three-dimensional map with an in-plane resolution of 6Å and 11.4Å in the third dimension. The map shows at least 23 transmembrane helices resolved in a monomeric complex, of which 18 were able to be assigned to the D1, D2, CP47, and cytochrome b559 alfa- and beta- subunits with their associated pigments that are active in electron transport (Rhee, 1998, Ph.D. thesis). The D1/D2 heterodimer is located in the central position within the complex and its helical scaffold is remarkably similar to that of the reaction centres not only in purple bacteria but also in plant photosystem I (PSI), indicating a common evolutionary origin of all types of reaction centre in photosynthetic organisms known today (Rhee et al. 1998). The structural homology is now extended to the inner antenna subunit, ascribed to CP47 in our map, where the 6 transmembrane helices show a striking structural similarity to the corresponding helices of the PSI reaction centre proteins.

The overall arrangement of the chlorophylls in the D1/D2 heterodimer, and in particular the distance between the central pair, is consistent with the weak exciton coupling of P680 that distinguishes this reaction centre from bacterial counterpart. The map in most progress towards high resolution structure will be presented and discussed.

Introduction

Alongside the broad photosynthesis research, considerable progress has been made over the last years in our understanding of the structure of the photosystem II (PSII) reaction centre. We reported the three-dimensional structure of the plant PSII subcomplex at 8Å resolution (Rhee *et al.* Nature, vol. 396, 283-286; see also the News & Views article in the same issue, p221-222) which gave us the clear insight into the subunit location as well as the evolutionary relationship between all types of photosynthetic reaction centres known today. Although we have yet to accomplish the atomic structure, the map obtained recently at 6Å resolution by electron crystallography allows us to have a closer look at the further structural implications. In the following, we shall introduce the emerging method of electron crystallography, summarize the structural details of PSII reaction centre complex, and discuss its possible functional roles in terms of the energy conversion as well as the biological assimilation to the surroundings.

Electron Crystallography

The use of *electrons* as an illuminating material has several potential advantages for the structural studies on biological objects. The major advantage arises from the fact that the amount of radiation damage for electrons is several hundred times less than for X-rays, thereby the specimen size required for imaging of radiation-sensitive materials, such as biological macromolecules, is correspondingly smaller. As well, no "phase problem" exists unlike X-ray crystallography, since the phases of the structure factors can be deduced from micrographs. The contrast recorded on microscopic images corresponds to the projection structure of a thin three-dimensional (3D) object, from which the structure factors (i.e. amplitudes and phases) can be extracted by using numerical Fourier analysis. In principle, the amplitude information is evaluated by electron

diffraction in combination with the data derived from images. The MRC image processing program suit has been developed for this purpose that is also capable of correcting the optic-dependent image defects, such as astigmatism, defocus, and spherical aberration. Because the Fourier transform of a two-dimensional projection of a 3D object is identical with the corresponding central section of the 3D transform of the object, the 3D transform can be built up plane by plane by using the transforms of different projected views of the object.

The 7Å structure of bacteriorhodopsin that Richard Henderson and Nigel Unwin at MRC, LMB, Cambridge, U.K. obtained in 1975 (Nature, vol. 257, 28-32), provided the first useful information about the structure of membrane proteins that are proving difficult to crystallize in three dimension. They pioneered high-resolution 3D reconstruction of tilted electron microscopic images using two-dimensional crystals. Subsequent technical and practical developments, such as high vacuum cryo-imaging technique and high voltage field-emission gun electron microscope, have led us to extend the potential of this method to determine a protein structure at atomic resolution.

The structure of PSII reaction centre complex at 6Å resolution

Photosystem II is a large, multisubunit membrane protein complex consisting of more than 25 subunits. It is responsible for the primary charge separation at P680, and it is the only protein complex in nature that is able to evolve molecular oxygen by oxidizing water. The plant reaction centre complex formed two-dimensional crystals in about 2 μm long, when the entire complex was solubilized by *n*-heptylthioglucoside. Biochemical evidence indicated that the crystals contain the D1, D2, CP47, cytochrome b559 α- and β- subunits with their associated pigments, and several small, presumably non-pigment binding proteins that span membrane once. The total molecular mass of the crystallized complex is ~160 kDa. It doesn't retain the oxygen evolving complex, but it can mediate energy as well as electron transfer. Images were taken at -182°C using Philips CM200 field emission gun electron microscope with an acceleration voltage of 200kV in EMBL (European Molecular Biology Laboratory). In total, 79 images recorded at tilt angles ranging from 0° to 67° yielded amplitudes and phases for a 3D map with an in-plane resolution of 6Å and 11.4Å in the third dimension (Table 1).

Table 1. Electron crystallographic data used for 3D structure determination of the PSII subcomplex

Two-dimensional crystals		
2-sided plane group	p22121	
Unit cell parameters	a=168.3Å, b=155.2Å, g=90°	
Thickness(Å)	70-80	
Phase determination from images		
No. of merged lattices	79	0-20°: 22 lattices
		20-30°: 16 lattices
		30-40°: 10 lattices
		40-50°: 19 lattices
		50-67°: 12 lattices
Maximum tilt angle	67°	
Resolution limit for merging	6.0Å	
No. of reflections merged	59456	
No. of independent phases	7193	
Overall weighted phase residual	29.9°	
In resolution range (Å): 200.0 – 14.0	23.2°	
14.0 – 10.0	30.5°	
10.0 – 8.2	38.2°	
8.2 – 7.0	41.8°	
7.0 – 6.0	48.7°	
Resolution in membrane plane	6.0Å	
Resolution in membrane normal	11.4Å	

The projection map at 8Å resolution which we initially published (Rhee et al. 1997) gives us the view of the crystallographic unit cell having 4 monomers in p22121 symmetry. The 3D map shows 23 elongated, 30 to 36Å long, rod-like densities in each monomeric complex which are characteristic of membrane-spanning α-helices at this resolution (Fig. 1). In the central position there are 10

helices which are arranged in near 2-fold symmetry around a local 2-fold axis. These are assigned to the D1/D2 heterodimer based on the predicted number of the transmembrane helices and on the comparison of the X-ray structure of the purple bacterial L and M subunits with respect to the amino acid sequence homology. The adjacent three pairwise helices are assigned to CP47 in terms of predictions that this protein has 6 transmembrane helices. The assignment of the D1 and D2 polypeptide is deduced from the mapping with respect to the bulkier sidechains and from the results of the cross-linking experiments that have been published elsewhere. Cofactors in the electron transfer chain in the D1/D2 complex occur in positions analogous to those in the bacterial reaction centre (bRC), but the distance between the chlorophylls corresponding to the bacterial special pair seems significantly larger and the configuration of the central pair chlorophylls in PSII appears asymmetrical. Most of the pigments associated with the CP47 are confined within the 6 helices. The electron density for the heme group of cytochrome (Cyt) b559, coordinated with the redox-active iron atom, is characterized in the map where the pair of helices contains the ellipsoid density that is located close to the stromal side as suggested with respect to the axial ligand of histidine residues. This region is assigned to Cyt b559. Cytochrome b559 seems to be α /β heterodimer in its native form, because the length and feature of the corresponding densities differ to one another.

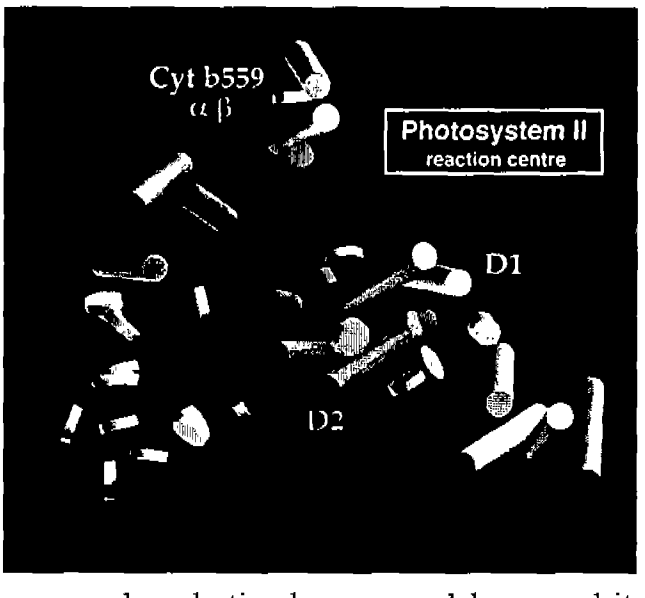

Fig. 1. Photosystem II reaction centre subunit location viewed from the lumen (adapted from *Rhee, 1998, Ph.D thesis*).

Altogether, 23 transmembrane helices are resolved in a monomeric complex of the photosystem II reaction centre, of which 18 were assigned to the D1 (yellow), D2 (orange), CP47 (red), and cytochrome (=Cyt) b559 (purple) with their associated pigments (chlorophyll: green, pheophytin: brown, and heme: white). The remaining 5, presumably non-pigment binding helices are coloured in blue. Cylinders represent α -helices, and disks represent tetrapyroles.

Discussion

One aspect of this study was a comparison of the structure of the photosynthetic reaction centres from purple bacteria, cyanobacterial photosystem I (PSI) and plant PSII (Fig. 2). This demonstrates unequivocally that there is a high degree of structural homology from bacteria to higher plants, on the basis of the helical scaffold and the associated pigments involved in the primary charge separation. All three reaction centres are composed each of two highly conserved subunits arranged around a pseudo-two-fold symmetry axis. The similarity between the purple bRC and PSII is more pronounced than that between PSII and PSI. This reinforces evidence from amino acid sequences that PSII is a direct evolutionary descendant of the reaction centre of purple photosynthetic bacteria, or of their common ancestor. Surprisingly, the six CP47 helices are strikingly similar to the structure of the first six helices of the PSI reaction centre proteins, which consequently provides important evolutionary clues.

A closer comparison, however, indicates unique structural features of the PSII that distinguish this oxygenic reaction centre from its bacterial counterpart. (1) The centre-to-centre distance of two chlorophylls analogous to that of the bacterial special pair appears to be ~12Å which is significantly larger than the corresponding distance of 7.6Å in purple bRC. They are non-parallel to one another with respect to the plane of the chlorins, and one of these central pair chlorophylls is likely to be tilted with respect to the membrane plane. Similar arrangement has been predicted by the experimental data of the P680 triplet axis, and is consistent with the weak exciton coupling observed in PSII reaction centre. One key profit of such pigments organization seems to be the high performance of facilitating kinetically efficient photochemical energy conversion in PSII. (2) The location of the heme in the Cyt b559 suggests that the heme group is likely to be involved in the cyclic electron transfer pathway in PSII. (3) Another notable structure is feasible in the helix 3 of D1/D2 near the lumenal end. Each L and M subunit has in this region a kink, brought about by their prolin residues. Primary sequence analysis indicates that, the corresponding prolins in D1/D2 are shifted by an insertion of two amino acid residues to the C-terminus. One of these two residues includes Yz161 in D1 and YD161 in D2. Yz 161 is the redox-active tyrosin that has been shown to mediate electron transfer between P680 and the Mn cluster. The map implies that roughly one helix turn above the kinks might be the positions of Yz161 and YD161.

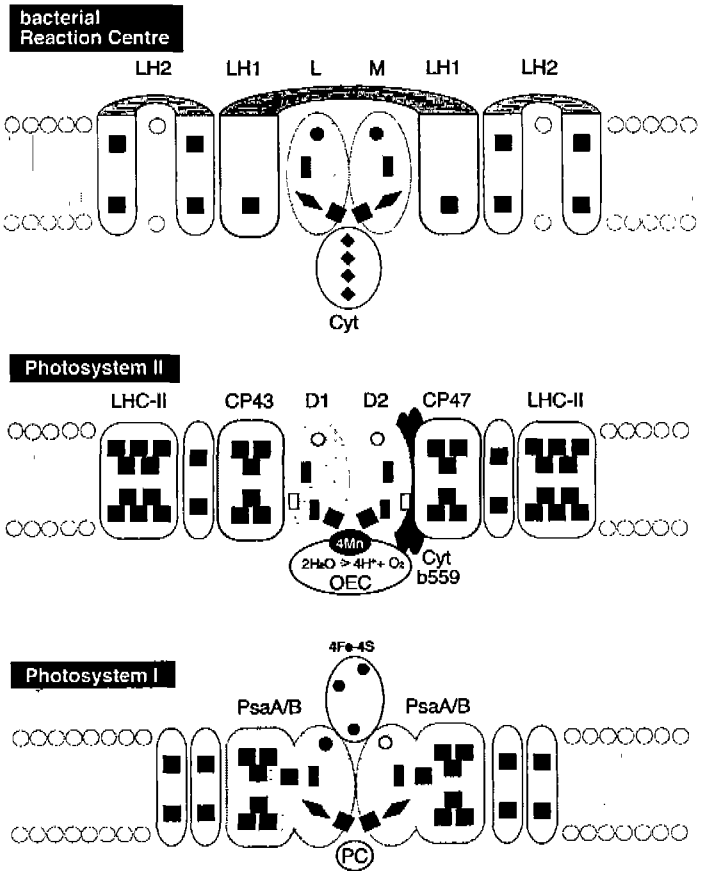

Fig. 2 Comparison of the three types of RCs found in photosynthetic organisms (adapted from *Rhee, 1998, Ph.D. thesis*). Top: bacterial RC. The L and M subunits are coloured in yellow and cofactors are in green. The outer light harvesting antenna proteins, LH2 (light green), transfer the light energy to the LH1 (red). Cyt (= Cytochrome). Centre: photosystem II. The cofactors (green) in electron transfer chain of the D1 and D2 proteins (yellow) seems to be arranged in a similar configuration like bRC, but the distance between the central pair seems to be significantly greater than that of the bacterial special pair. CP43 and CP47 (=Chlorophyll binding core antenna proteins), LHC-II (=Light-harvesting complex II). Mn (=Manganese cluster), OEC (=Oxygen evolving complex). Below: photosystem I. Each PsaA/B (=Reaction centre core protein) consists of a reaction centre core equivalent to D1 or D2, and a core antenna equivalent to CP43 or CP47. PC (=Plastocyanin), FeS (=Iron-sulfur clusters).

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