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Structure and function of the photosynthetic reaction center from *Rhodopseudomonas viridis*

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Abstract - The structure of the photosynthetic reaction centre from the purple bacterium Rhodopseudomonas viridis has been solved by X-ray structure analysis. The pigment arrangement, the folding of the protein subunits and pigment-protein interactions are described. The relation to photosystem II reaction centers and evolutionary aspects are discussed.

INTRODUCTION

The earliest step in the conversion of light energy into energy usable by living cells is the absorption of light by photosynthetic pigments (chlorophylls, carotenoids, phycobilins), mainly in light-harvesting antenna complexes. The resulting excitation migrates among the antenna pigments until it is trapped in photosynthetic reaction centers. In the green photosynthetic bacteria, cyanobacteria, red algae and cryptophytes, the major light-harvesting complexes are water soluble pigment-protein complexes which are attached to the photosynthetic membranes, whereas they are complexes of pigments and integral membrane proteins in purple photosynthetic bacteria, green algae and higher plants. In the reaction centers which are always complexes of integral membrane proteins and pigments the excited state of the "primary electron donor" is deactivated by the transfer of an electron to an electron acceptor via intermediate carriers. It is one of the functions of the surrounding proteins to keep the electron donor, intermediate carriers and acceptors in a fixed geometry such that electron donor and acceptor are near opposite surfaces of the photosynthetic membrane. After transfer of an electron a major part of the energy of the absorbed light is stored in the form of difference in electric potentials across the membrane ("membrane potential") and redox energy in the form of the reduced electron acceptor. The electron acceptor in the reaction centers from purple photosynthetic bacteria and photosystem II of chloroplasts and cyanobacteria is a quinone molecule, whereas it is a non-heme iron sulfur protein in the reaction center from green photosynthetic bacteria and from photosystem I of chloroplasts and cyanobacteria (for review see ref. 1). The photooxidized primary electron donors can be re-reduced by cytochromes (mainly bacterial reaction centers), plastocyanin (photosystem I reaction centers), small molecules of sufficiently low redox potential, and - with the help of an additional water splitting complex - even by water in the case of photosystem II reaction centers.

The best known reaction centers are those from the purple photosynthetic bacteria (for review see ref.2, 3, 4). They are easy to isolate and relatively stable. Most of them contain three protein subunits which are called H (heavy), M (medium) amd L (light) subunits according to their apparent molecular weight as determined by sodium dodecylsulphate polyacrylamide gel electrophoresis. In addition, reaction centers from several purple photosynthetic bacteria, including Rps. viridis, contain a tightly bound cytochrome molecule, which re-reduces the photooxidized primary electron donor. The cytochrome subunit from the Rps. viridis reaction center contains four heme groups. Photosynthetic pigments are

four bacteriochlorophyll-bs, two bacteriopheophytin-bs, one menaquinone ("primary quinone" or "QA"), one non-heme-iron and one ubiquinone ("secondary quinone" or "QB"). The reaction centers from most of the other purple photosynthetic bacteria contain bacteriochlorophyll-a instead of bacteriochlorophyll-b, bacteriopheophytin-a instead of bacteriopheophytin-b, and the menaquinone is replaced by another ubiquinone.

The successful crystallization of the reaction center from Rps. viridis (ref. 10), yielding well ordered crystals in which the reaction centers retained their photochemical activity (ref. 13), was an important step towards the elucidation of its three-dimensional structure. X-ray analysis of these crystals allowed the calculation of an electron density map at 3 A resolution from which the arrangement of the chromophores could be determined (ref. 15). Subsequently the structure of the protein subunits (ref.16) and details of the pigment-chromophore interactions (ref. 17) were presented. Recently, reaction centers from Rb. sphaeroides could also be crystallized (ref. 11,12), and the photochemical activity of the crystalline reaction centers could be demonstrated (ref. 11, 14), and their crystal structure could be determined with the help of the known structure of the reaction center from Rps. viridis [ref. 18,19,20]. In the following we will review and discuss functional, structural and evolutionary aspects of the photosynthetic reaction centers from the purple photosynthetic bacteria.

PIGMENT ARRANGEMENT

There is good spectroscopic evidence (ref. 21, 22) that the arrangement of the photosynthetic pigments is the same in all species of the purple photosynthetic bacteria so far examined. Fig. 1 shows the arrangement of these pigments in the reaction center from Rps. viridis as determined by X-ray crystallography (ref. 15). The four heme groups (at the top of fig. 1) are related by a twofold local rotation axis which runs nearly perpendicular to the picture plane. The function of these hemes is to re-reduce the photooxidized primary electron donor which is a "dimer" of two non-covalently linked bacteriochlorophyll molecules ("special pair", just below the hemes in fig. 1). The existence of such a dimer had been postulated on the basis of EPR experiments (fig. 23), its detailed structure is now firmly established by the X-ray structure analysis. The ring systems of the two bacteriochlorophylls constituting the dimer are nearly parallel and they overlap with their pyrrole rings I. The plane to plane distance of these two bacteriochlorophylls is about 3.1 A. The ring systems of all the chlorine pigments are also related by a local twofold rotation axis. This diad (vertically in fig. 1) runs through the special pair on the periplasmic side of the membrane and through the non-heme iron on the cytoplasmic side of the membrane. Since the two "accessory" bacteriochlorophylls and the two bacteriopheophytins are also related by the local diad, two structurally equivalent branches are formed which could be used for electron transfer across the membrane. However, the symmetry is broken by the presence of only one quinone, and by the different arrangement of phytyl chains in both branches. The quinone must be the primary quinone (menaquinone) since the ubiquinone is lost during isolation and crystallization of the reaction centers (fig. 24). In addition, the two bacteriopheophytins are spectroscopically inequivalent absorbing light of different wavelengths. It is known that only the one absorbing light at longer wavelengths is involved in electron transfer. Comparison of absorbance spectra of crystals, taken with plane-polarized light (fig. 13) shows that the bacteriopheophytin absorbing at the longer wavelengths is the one closer to the quinone. These experiments establish clearly that only one way (the right hand one in fig. 1) is used for light driven electron transfer across the photosynthetic membrane.

The binding site of the secondary quinone could be determined by soaking quinones and competetive inhibitors (o-phenanthroline, terbutryn) into the crystals and subsequent difference Fourier analysis (ref. 16,17). These compounds bind into an empty pocket of the protein which is symmetry related (by the local diad) to the binding site of the primary quinone. Thus the electron has to be transferred from QA to QB parallel to the surface of the membrane. There is no evidence for participation of the non-heme-iron in this electron transfer, since it can be removed without drastic changes in the kinetics of this electron transfer (ref. 25).

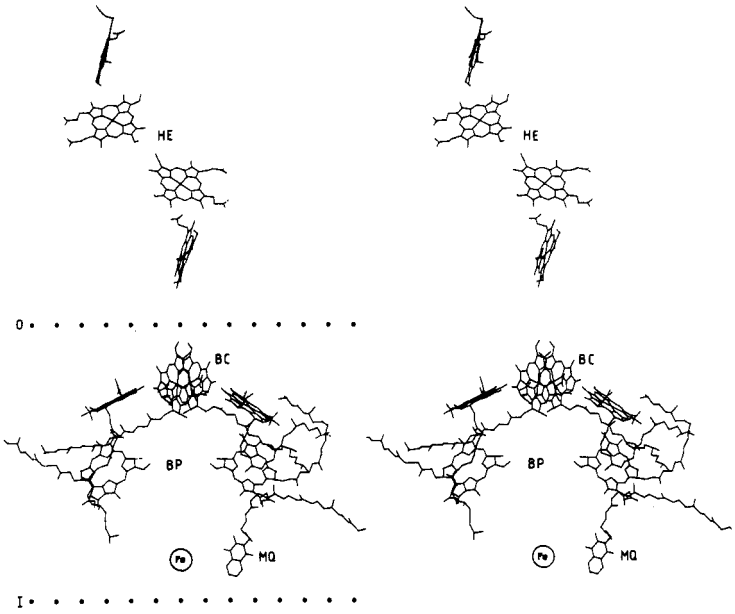

Figure 1 (stereo pair): Arrangement of the pigments in the photo-synthetic reaction center from Rps. viridis according to ref. 15, showing from top to bottom four heme groups (HE), four bacterio-chlorophylls (BC), two bacteriopheophytins (BP), one non-heme iron (Fe), and one menaquinone (MQ). The approximate twofold symmetry axis relating the photosynthetic pigments runs vertically in the plane of the picture. The approximate position of the periplasmic and cytoplasmic face of the photo-synthetic membrane is indicated by dotted lines.

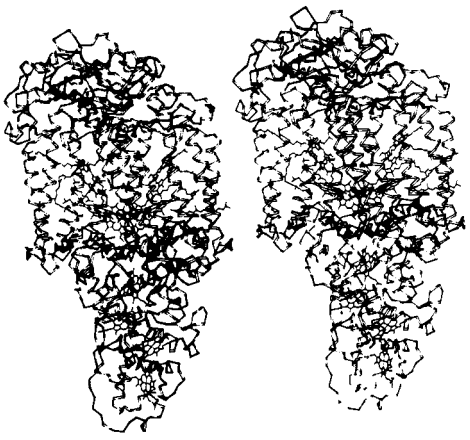

Figure 2 (stereo pair): Ribbon drawing of the polypeptide chains of the reaction center from Rps. viridis, together with the chromophore model (thin lines), showing the cytochrome (top), and the subunits L (middle left), M (middle right), and H (bottom, with the N-terminal helix extending from the cytochrome).

PRIMARY STRUCTURES

The sequences of the L, M and H subunits are published for Rb. capsulatus (ref. 7), Rps. viridis (ref. 8,9) and Rb. sphaeroides (ref. 6,26). The sequence homology of the H subunits is below 40 %. The sequence homology between the M subunits of Rps. viridis and Rb. capsulatus, as well as Rps. viridis and Rb. sphaeroides are close to 50 %, whereas they are close to 60 % between the L subunits. All L and M subunits from the three species possess sequence homologies in the order of 25-30 % indicating already a similar protein folding of both subunits and that they are derived from a common ancestor. Conserved between all L and M subunits are mainly glycines and prolines at the ends of helices and turns of the peptide chains, and the ligands to the pigments. Despite the low sequence homology the structure of all these reaction centers had to be very similar. This was born out for Rps. viridis and Rb. sphaeroides by the recent crystallographic analysis [ref. 16,20].

GENERAL ARCHITECTURE OF THE REACTION CENTER

Due to the X-ray structure analysis of the photosynthetic reaction center from Rps. viridis a detailed picture of the reaction center has emerged. Fig. 2 shows a drawing of the polypeptide chains, together with the chromophore model. The photosynthetic pigments are associated with the L and M subunits, as had already been shown by biochemical and spectroscopical experiments (ref. 2). They form the central part of the

reaction center. The L and M subunits possess five long membrane spanning helices which are related by the same twofold axis as the pigments. The structural differences between the L and M subunits are mainly at the amino-terminus on the cytoplasmic side (M has a longer aminoterminus), in the connection of the first and second transmembrane helix (M shows an insertion of 7 amino acids, which gives rise to a short helix parallel to the membrane), in the connection of the fourth and fifth membrane spanning helix (M possesses an additional loop providing glu M232 as a ligand to the non-heme-ferrous iron atom) and at the carboxy-terminus (the M subunit from Rps. viridis contains additional 17 amino acids, which are in contact with cytochrome subunit). The five transmembrane helices of the subunits L and M possess a remarkably open structure forming approximately half-cylinders. On both sides of the helical regions of the L and M subunits the polypeptide segments connecting the transmembrane helices and the terminal segments form flat surfaces perpendicular to the local diad. The cytochrome is bound to the surface close to the special pair on the periplasmic side. The H subunit possesses one membrane spanning helix close to its amino-terminus. Its carboxy-terminal domain is bound to the flat surface of the L-M complex on the cytoplasmic side. It is the only part of the reaction center with a significant amount of beta-sheet as secondary structure.

PIGMENT-PROTEIN INTERACTIONS

The photosynthetic pigments are bound into primarily hydrophobic pockets of the L and M subunits (ref. 17). The L and M subunits provide histidine residues as ligands to the magnesium atoms of the special pair bacteriochlorophylls (L173, M200 in Rps. viridis) and the accessory bacteriochlorophylls (L153, M180 in Rps. viridis). The Mg ions are five coordinated in agreement with recent resonance Raman data (ref. 27). The protein, besides being a scaffold for the pigments, must specifically interact with the pigments to suppress one of the two possible electron transport pathways. The choice of the pathway could be influenced already at the special pair, either by a deviation from the C2-symmetry, or by electrostatic effects due to polar amino acids in the immediate environment of the special pair. Charged amino acids are not found in the vicinity of the special pair. However, in Rps. viridis we find hydroxyl groups of three amino acid side chains (tyr M195, thr L248, tyr M208) close to the special pair. They are located towards the branch which is used for electron transfer. Two of them form hydrogen bonds with carbonyl oxygen atoms of the bacteriochlorophylls. On the side of the inactive branch we find only one polar side chain, his L168, in a position symmetry-related to tyr M195; his L168 also forms a hydrogen bond with the special pair. This asymmetric distribution of polar groups may cause an asymmetric electron distribution in the special pair. Sequence comparisons show that the hydrogen-bonding between special pair bacteriochlorophylls and protein must be different between Rps. viridis, Rb. sphaeroides and Rb. capsulatus. The accessory bacteriochlorophylls are not hydrogen-bonded to the protein. That bacteriopheophytin which is an intermediate electron acceptor forms a hydrogen-bond with a, most likely protonated, glutamyl residue (L104). This glutamic acid seems to be of crucial importance for the light driven electron transfer. It is conserved between all three bacterial species and the D1 protein from photosystem II reaction centers (see below).

Another interesting amino acid is trp M250 which forms part of the binding site of the primary quinone. It also touches that bacteriopheophytin which is involved in light-driven electron transfer thereby "bridging" the pheophytin and quinone. In the symmetry related position phe L216 is found whose side chain is too small to bridge the bacteriopheophytin and the secondary quinone. The ferrous non-heme-iron atom is found half-way in between the primary and secondary quinone binding sites. It is bound to four histidine residues (L190, L230, M217, M264) and one glutamic acid (M232 in Rps. viridis). The primary quinone is hydrogen bonded to the histidine M217 and the peptide nitrogen of alanine M258.

CONCLUSIONS ON THE STRUCTURE OF PHOTOSYSTEM II REACTION CENTERS

The X-ray structure analysis of the Rps. viridis reaction center shows clearly that the L and M subunits cooperate in a nearly symmetric manner to establish the primary electron donor (the special pair) and the electron accepting QA-Fe-QB complex. This feature, together with the sequence homologies of L and M subunits with the D1 and D2 proteins from photosystem II reaction centers and the herbicide binding to the D1 and L subunits has lead to the proposal that the core of photosystem II reaction centers is made up of the D1 and D2 proteins in a similar manner as L and M form the core of the reaction center from the purple bacteria (ref. 28).

The conservation of many important amino acids (ref.9,16) strongly favours our view that the reaction center core of photosystem II consists of the D1 and D2 subunit. The most important difference is the absence of the histidine ligands to the accessory bacteriochlorophylls in D1 and D2. This finding means that accessory bacteriochlorophylls are either absent, or bound to the protein in a different way. The sequence homology between D1 and D2 is higher than between L and M. In particular D2 compared to D1 does not show an insertion of seven amino acids between the fourth and the fifth membrane spanning helices which provided an additional loop with glu M232 as a ligand to the ferrous non-heme-iron atom. We consider hydrogen-carbonate as a likely candidate for being a ligand to the ferrous non-heme iron atom in photosystem II reaction centers instead of the glutamic acid in the reaction centers from the purple bacteria. This proposal may help to understand the pronounced effect of hydrogencarbonate on the electron accepting side of photosystem II reaction centers (for review see ref. 29). For a detailed recent discussion of the relation of photosystem II reaction centers and the reaction centers from purple photosynthetic bacteria see ref. 30.

The proteins CP47 and CP43 which are known to carry a considerable number of chlorophyll molecules function in our view as (energy transferring) antenna complexes surrounding D1 and D2 in the membrane.

EVOLUTIONARY ASPECTS

The basic symmetric arrangement of the pigments and the L and M subunits, and the need for both protein subunits to generate the special pair and the electron accepting quinone iron complex suggests that the reaction center from the purple bacteria evolved from a symmetric protein dimer containing two bacteriochlorophyll molecules forming a special pair, and possessing two equivalent pathways to transfer electrons to the cytoplasmic side of the membrane. Gene duplication and subsequent mutations should account for the origin of the asymmetric dimer. During evolution the asymmetric dimer, having switched off one of the electron transfer pathways, proved to be advantageous. A specialization of the two quinones into a more firmly bound QA and a loosely bound QB became possible. Having QA as an additional electron storing device may have been the most important advantage when replacing a symmetric reaction center by an asymmetric one during evolution.

The formation of a protein dimer may be the simplest way to give rise to chlorophyll dimer (special pair) as an efficient phototrap. Since photosystem I reaction centers most likely contain a dimer as primary electron donor, too, and apparantly contain two very similar proteins of 60 kD molecular weight (for reviews see 22), the motif of an asymmetric protein and pigment dimer may be repeated in photosystem I reaction centers.

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