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Review

Structure, function and interactions of the PufX protein

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ABSTRACT

The PufX protein is an important component of the reaction centre–light-harvesting 1 (RC–LH1) complex of *Rhodobacter* species of purple photosynthetic bacteria. Early studies showed that removal of the PufX protein causes changes in the structure of the RC–LH1 complex that result in a loss of the capacity for photosynthetic growth, and that this loss can be overcome though further mutations that change the structure of the LH1 antenna. More recent studies have examined interactions of the PufX protein with other components of the RC–LH1 complex. This review considers our current understanding of the structure and function of the PufX protein, how this protein interacts with other components of the photosynthetic membrane, and its influence on the oligomeric state of the RC–LH1 complex and the larger-scale architecture of the photosynthetic membrane.

1. The purple bacterial photosystem

The photosystem in purple photosynthetic bacteria consists of a reaction centre (RC) and one or more types of light-harvesting (LH) antenna complex housed in a lipid bilayer membrane (for recent reviews, see [1–7]). The LH pigment-proteins are formed from oligomers of at least two types of polypeptide chain that encase multiple molecules of bacteriochlorophyll (BChl) and carotenoid. These pigments are responsible for capturing light energy in the form of a pigment excited electronic state, and funneling that energy to the RC to initiate photochemistry [8–11].

The simplest RCs are also formed from BChl, carotenoid and at least three polypeptide chains, together with two quinone molecules. The arrival of excited state energy in the RC triggers a membrane-spanning electron transfer reaction, the ultimate product of which is the double reduction and double protonation of a quinone to form quinol, and the oxidation of a water soluble redox protein — usually a *c*-type cytochrome (see for reviews [12–17]). The RC can therefore be thought of as a light-powered cytochrome *c*:quinone oxidoreductase. Light-driven electron transfer in the bacterium is completed by a partner quinol:cytochrome *c* oxidoreductase, the membrane-embedded cytochrome bc_1 complex, which oxidises quinol, reduces cytochrome *c* and operates a Q-cycle [18–20] (and see [21–24] for recent reviews). By arranging sites of quinone reduction/protonation and quinol oxidation/deprotonation on opposite sides of the membrane, light-powered cyclic electron transfer is linked to proton translocation across the membrane, forming the protonmotive force.

The RC from purple bacteria was the first membrane protein to be structurally characterised to a high-resolution by X-ray crystallography. The first structure to be described was that for the RC from *Blastochloris* (*Bl.*) *viridis* (formerly *Rhodopseudomonas* (*Rps.*) *viridis*) [25–27], followed shortly after by that for the RC from *Rhodobacter* (*Rb.*) *sphaeroides* [28–33]. An overview of the latter is shown in Fig. 1A and B. More recently a structure has been published for the RC from the moderate thermophile *Thermochromatium tepidum* [34,35]. These structures have aided the development of a detailed picture of the mechanism of light-powered charge separation, quinone reduction and cytochrome oxidation [14]. X-ray crystal structures have also been published for the so-called LH2 peripheral antenna complex from *Rps. acidophila* [36] and *Phaeospirillum* (*Psp.*) *molischianum* (formerly *Rhodospirillum* (*Rsp.*) *molischianum*) [37]. These show two concentric membrane-spanning cylinders of protein, each formed from multiple (nine or eight respectively) copies of α- and β-polypeptides, that encase rings of BChl and carotenoid cofactors. The structure of the LH2 from *Rps. acidophila* is shown in Fig. 1C.

In all characterised species of purple photosynthetic bacteria the RC is associated with an LH1 antenna pigment-protein, forming the so-called RC–LH1 core-complex. To date a high-resolution X-ray structure has not been forthcoming for such a core-complex, but a variety of data have established that the LH1 component forms a hollow cylindrical structure around the RC. In early low-resolution work, electron microscopy (EM) on membranes from *Bl. viridis* and *Ectothiorhodospira halochloris* showed a central RC surrounded by the LH1 antenna, the latter displaying a six or twelve fold symmetry [38–40]. Subsequent studies involving EM of aggregated LH1 complexes from *Rsp.*

Abbreviations: AFM, Atomic Force Microscopy; BChl, bacteriochlorophyll; *Bl.*, *Blastochloris*; EM, electron microscopy; LH, light-harvesting; LH1, core antenna complex; LH2, peripheral antenna complex; ORF, open reading frame; PDB, Protein Data Bank; PS^+, photosynthetic phenotype; PS^-, non-photosynthetic phenotype; *Psp.*, *Phaeospirillum*; *Rb.*, *Rhodobacter*; RC, reaction centre; *Rps.*, *Rhodopseudomonas*; *Rsp.*, *Rhodospirillum*

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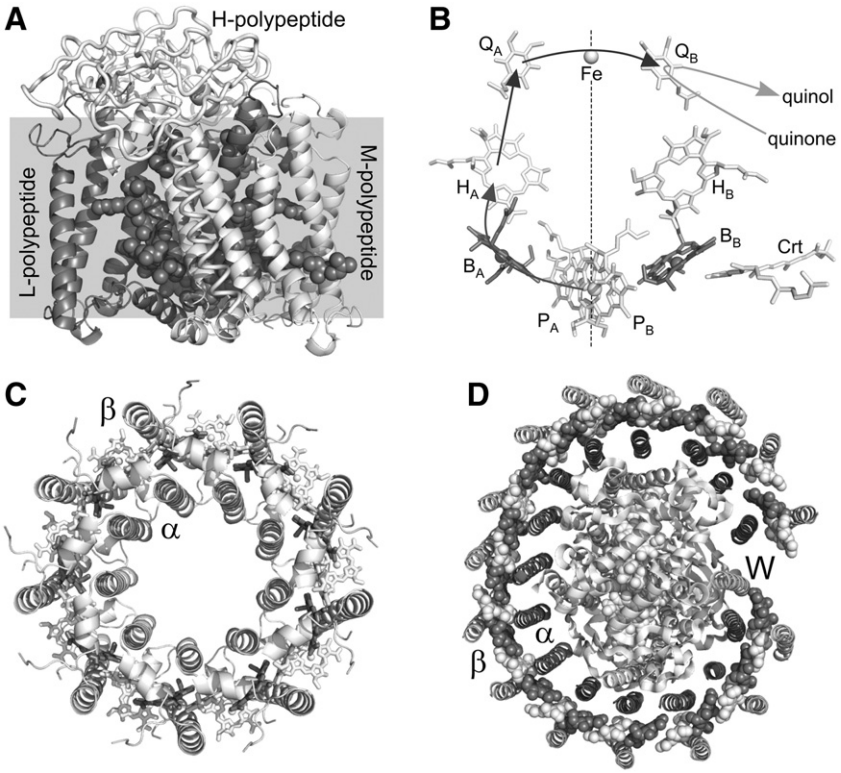

Fig. 1. Structures of photosynthetic proteins from the purple photosynthetic bacteria. (A) In the *Rb. sphaeroides* RC the ten cofactors (grey spheres) are held in place by a scaffold consisting of PufL (grey ribbons) and PufM (white ribbons). The H-polypeptide (white tube) has a single membrane-spanning helix and a cytoplasmic domain. The protein is embedded in the photosynthetic membrane (grey box). (B) The RC BChl, BPhe and ubiquinone cofactors (sticks) form two membrane-spanning branches. For clarity the hydrocarbon side chains have been removed and the B_A and B_B BChls are highlighted in grey. Mg atoms of BChl and non-heme Fe are shown as spheres. The dotted line shows the axis of two-fold symmetry and the black arrows show the route of electron transfer. The Q_B ubiquinone is dissociable (grey arrows). Panels A and B were prepared using structure 2BOZ [153]. (C) The LH2 complex from *Rps. acidophila* (structure 1NKZ [154]). Concentric cylinders of membrane-spanning helices of LH2 α and β polypeptides are shown as ribbons, and BChls are shown as sticks. (D) The RC–LH1 complex of *Rps. palustris* (structure 1PYH [52]) viewed from the periplasmic side of membrane. Central RC shown as in (A). Membrane-spanning helices of LH1 α (grey) and β polypeptides (white), and W-polypeptide (white) are shown as ribbons, and LH1 BChls are shown as spheres, alternating white and grey.

marina [41] and single particle analysis on individual RC–LH1 complexes from *Psp. molischianum* [42] reinforced this picture of LH1 forming a hollow cylinder around a central RC.

In 1995, Karrasch et al. reported structural information from EM at a resolution of 8.5 Å on 2-D crystals of the LH1 complex from *Rsp. rubrum* [43]. The density maps were interpreted as showing 16 α and β LH1 polypeptides arranged in concentric cylinders with intervening density attributed to a ring of 32 BChl molecules, this interpretation being guided by the X-ray crystal structures of the LH2 antenna [36,37]. The central cavity in this structure of LH1 was sufficiently large to accommodate a central RC. This basic architecture was further demonstrated in a number of EM studies using LH1 complexes from a variety of purple bacteria [44–47]. More recently, Atomic Force Microscopy (AFM) has been used to examine the topography of photosynthetic membranes from several species of purple photosynthetic bacteria. In those studies that achieved a sufficiently high-resolution it has been possible to count the number of pairs of α- and β-polypeptides that constitute each LH1 or LH2 complex, which present as ring-shaped structures in topographs (see [5,7] for recent reviews). Topographs of membranes from *Bl. viridis*, *Rsp. rubrum*, *Rsp. photometricum* and *Psp. molischianum* reveal LH1 as consisting of a continuous 16-member ring surrounding a central feature assigned as the RC [48–51]. In some cases it has been reported that LH1 forms an elliptical ring around the RC, matching the roughly elliptical cross-section of the RC in the membrane (e.g. see [46,48,49]).

To date the most detailed picture of an RC–LH1 core is a 4.8 Å resolution X-ray structure for the complex from *Rsp. palustris* [52]. In this structure, the LH1 cylinder has an elliptical cross-section and is formed by 15 α and β polypeptides that encase 30 BChls. A representation of this structure is shown in Fig. 1D. The LH1 cylinder is incomplete, with an additional transmembrane α-helix, termed W, being located near the gap between LH1 polypeptides. The structure of the *Rps. palustris* core-complex is therefore somewhat different from that described above for complexes from species such as *Bl. viridis* and *Rsp. rubrum*, where LH1 forms a closed 16-member ring around the RC. Scheuring et al. have recently examined photosynthetic membranes from *Rps. palustris* by AFM and also described the core-complex as consisting of a RC surrounded by an incomplete elliptical ring of 15 α/β pairs [53]. This is consistent with the X-ray crystal structure, although Scheuring et al. reported that the position of the gap in the LH1 ring was variable in relation to the long axis of the elliptical core-complex, in contrast to data from X-ray crystallography. As discussed below, it has been speculated that the W-polypeptide may play a similar role to the PufX protein that is found in *Rhodobacter* species of purple bacteria, and is the subject of the remainder of this review.

2. The PufX polypeptide

The L- and M-polypeptides of the RC and α- and β-polypeptides of the LH1 antenna are encoded by the *pufL*, *pufM*, *pufA* and *pufB* genes respectively. During sequencing of the *puf* operon in *Rb. capsulatus* it was noted that the gene sequence *pufBALM* was followed by an open reading frame (ORF) of 237 base pairs designated C2397 [54]. The location of this ORF 13 nucleotides downstream of the stop codon for *pufM*, and the existence of a hairpin transcription terminator downstream of C2397, led to the suggestion that C2397 was part of the *puf* operon. Analysis of mRNA transcripts subsequently showed this to be the case, and the ORF was renamed *pufX* [55,56]. Cloning and analysis