Minireview

The structural basis of light-harvesting in purple bacteria

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Abstract A typical purple bacterial photosynthetic unit consists of two types of light-harvesting complex (LH1 and LH2) together with a reaction centre. This short review presents a description of the structure of the LH2 complex from *Rhodopseudomonas acidophila*, which has recently been improved to a resolution of 2.0 Å [Papiz et al., J. Mol. Biol. 326 (2003) 1523–1538]. We show how this structure has helped to reveal the details of the various excitation energy transfer events in which it is involved.

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Key words: Bacterial photosynthesis; Bacteriochlorophyll; Carotenoid; Light-harvesting; *Rhodopseudomonas*

1. Introduction

Purple bacterial photosynthesis begins when a photon is absorbed by the light-harvesting apparatus. This excitation energy is then rapidly and efficiently transferred to the reaction centre (RC), where it is used to initiate photosynthetic electron transport and to 'drive' a transmembrane charge separation [2]. The purple bacterial photosynthetic units, that is the combination of antenna complexes with RCs, are located in intracytoplasmic membranes [3]. When these membranes are solubilised with a suitable non-denaturing detergent, such as lauryldimethylamine-*N*-oxide, sucrose density centrifugation resolves two pigment–protein complexes [4]. The less abundant, most dense band represents the light-harvesting complex 1 (LH1)–RC 'core' complex, while the most abundant, less dense band is the LH2 complex.

Both these complexes are constructed on the same molecular principle. A pair of small (5–7 kDa), very hydrophobic apoproteins (called α and β) bind bacteriochlorophyll *a* (Bchl*a*) and carotenoids non-covalently. These apoprotein pairs oligomerise to form the mature antenna complexes. Fig. 1 shows the absorption spectra of the LH2 and the LH1–RC 'core' complexes from *Rhodopseudomonas acidophila*. The Bchl*a* molecules in LH1 have a single strong near-infrared (NIR) Qy absorption band of ~875 nm, while LH2 has two strong Bchl*a* NIR bands at ~800 nm and ~850 nm. The position of the Qy absorption band of Bchl*a* is very sensitive to its environment [5]. The different Bchl*a* binding environments in these two types of antenna complex are functionally important because they create an energy gradient, which facilitates and 'directs' energy transfer from LH2 to LH1, and then on to the RC.

2. The structure of the LH2 complex from *Rps. acidophila*

The crystal structure of the LH2 complex from *Rps. acidophila* was first described in 1995 [6] to a resolution of 2.5 Å. This structure has now been improved, using cryocooling, to 2.0 Å [1]. As a result of this the crystallographic *R*-factor has been reduced from 0.205 to 0.173. The improved electron density in regions where disorder was present in the original 2.5 Å data has revealed the last five amino acids in the α-apoprotein. It has also been possible (as described below) to

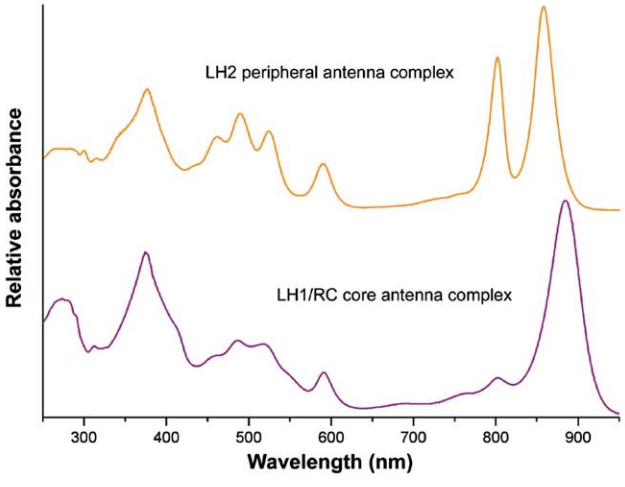

Fig. 1. The absorption spectra of the isolated LH2 (top) and LH1–RC (bottom) core complexes from *Rps. acidophila*. The absorption bands ~380 nm, 590 nm and 800–900 nm are the Bchl*a* Soret, Qx and Qy transitions, respectively. The peaks between 450 and 550 nm come from the carotenoids. The peaks at 800 and 760 nm in the LH1–RC 'core' complex are due to the RCs, not the LH1 component.

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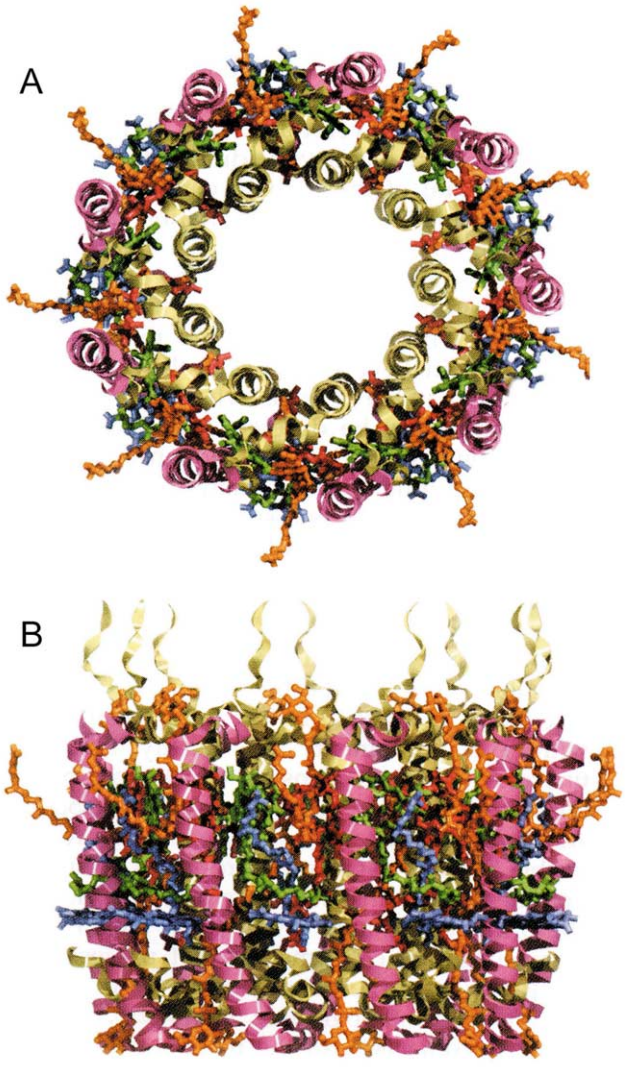

Fig. 2. A schematic representation of the overall structure of the LH2 complex from *Rps. acidophila*, based on the 2.0 Å data [1]. A: A view looking down onto the complex, as it would lie in the 'plane of the membrane'. B: A side view looking at the complex from 'within the membrane'. The α-chains are light green, the β-chains purple. The Bchl*a* molecules are α-bound 850 red, β-bound B850 green, B800 blue. The carotenoids are orange. The newly revealed C-terminal residues can be seen extending upwards in B. The presumed second carotenoid projects out from the complex.

correct the assignment of the ligand to the central Mg^{2+} atom in the bacteriochlorin rings of the B800-Bchl*a*s and to suggest the presence of a second carotenoid molecule per αβ apoprotein pair.

The LH2 complex is an $\alpha_a\beta_a$ circular nonamer (Fig. 2). The overall structure is cylindrical with the inner walls formed by a ring of nine α-apoprotein α-helices and the outer wall composed of a ring of nine β-apoprotein α-helices. All the pigments (except the second carotenoid) are arranged between the two walls of α-helices. The structure is closed top and bottom by the N- and C-termini of the apoprotein folding over and interacting with each other.

The Bchl*a* molecules are arranged into two groups. Towards the N-terminal (cytoplasmic) side of the complex there is a ring of nine monomeric Bchl*a* molecules, one per αβ-apoprotein pair. These absorb at 800 nm and are called the B800 molecules. The bacteriochlorin rings of these Bchl*a* molecules lie rather flat within the plane of the membrane and are separated centre to centre by 21.2 Å. The central Mg^{2+} ions are liganded to carboxyl-α-Met1 (in the original description this was thought to be an N-formyl group) (Fig. 3).

A second ring of 18 tightly coupled Bchl*a* molecules is located towards the periplasmic side of the complex (Fig. 4). These are the B850 Bchl*a* molecules. Their bacteriochlorin rings lie parallel to the transmembrane α-helices and their Mg^{2+} atoms have conserved histidine residues as the fifth ligand. Each α- and β-apoprotein has one of these histidines, therefore each αβ pair binds two B850 Bchl*a* molecules. The central Mg^{2+} atoms of these B850 Bchl*a*s are separated by 9.5 Å within one αβ pair, and by 8.8 Å from one αβ pair to the next. The original structural description of LH2 [6] identified a single well-defined carotenoid molecule (rhodopin-glucoside) per αβ pair and some partial electron density which could have been a disordered second carotenoid. The well-resolved one starts on the N-terminal side of the complex in one αβ-apoprotein pair, then passes in van der Waals contact past the edge of the B800–bacteriochlorin ring. It then proceeds to cross over into the next αβ-apoprotein pair and passes over the face of the α-apoprotein–band B850 bacteriochlorin ring (again in van der Waals contact). This carotenoid is in the all-*trans* configuration and effectively 'bolts' the neighbouring αβ-apoprotein pairs together. Indeed if there is no carotenoid then the LH2 complex is not assembled [7]. The improved 2.0 Å electron density suggests the presence of a second rhodopin-glucoside molecule, which lies along a groove on the outside of each αβ-apoprotein pair but that is probably only present with partial occupancy. In this case the sugar head group is clearly seen at the periplasmic side of the complex but the presumed conjugated portion of the molecule is only partially visible. Moreover it appears to be bent with a severe turn, which projects the molecule out of the groove towards the detergent micelle, which surrounds the hydrophobic core of the complex in the crystal [8]. Further work is required to be certain of the status of this second carotenoid molecule and to determine its in vivo configuration.

Soon after the structure of LH2 from *Rps. acidophila* was described, the structure of the LH2 complex from another purple bacterium, *Rhodospirillum molishianum*, was also determined [9]. It was very similar to that from *Rps. acidophila* except that it was an $\alpha_8\beta_8$ octamer. It is still not clear what structural features cause one to be a nonamer and the other an octamer, or indeed whether this difference has any significant functional consequences.

3. Functional studies on LH2

Unlike the chlorophyll-containing antenna complexes from plants and algae, the LH2 complexes from purple bacteria are particularly amenable to functional studies since all of the different pigment groups, seen in this structure, are well separated in the absorption spectrum. This spectral separation means that they can each be excited by light independently so that energy transfer from one group to another can be clearly visualised. This feature has led to LH2 being intensively studied by a variety of time-resolved spectroscopies and as a consequence all of their energy transfer reactions have been resolved from a few femtoseconds out to longer times (see [10] for a recent review).

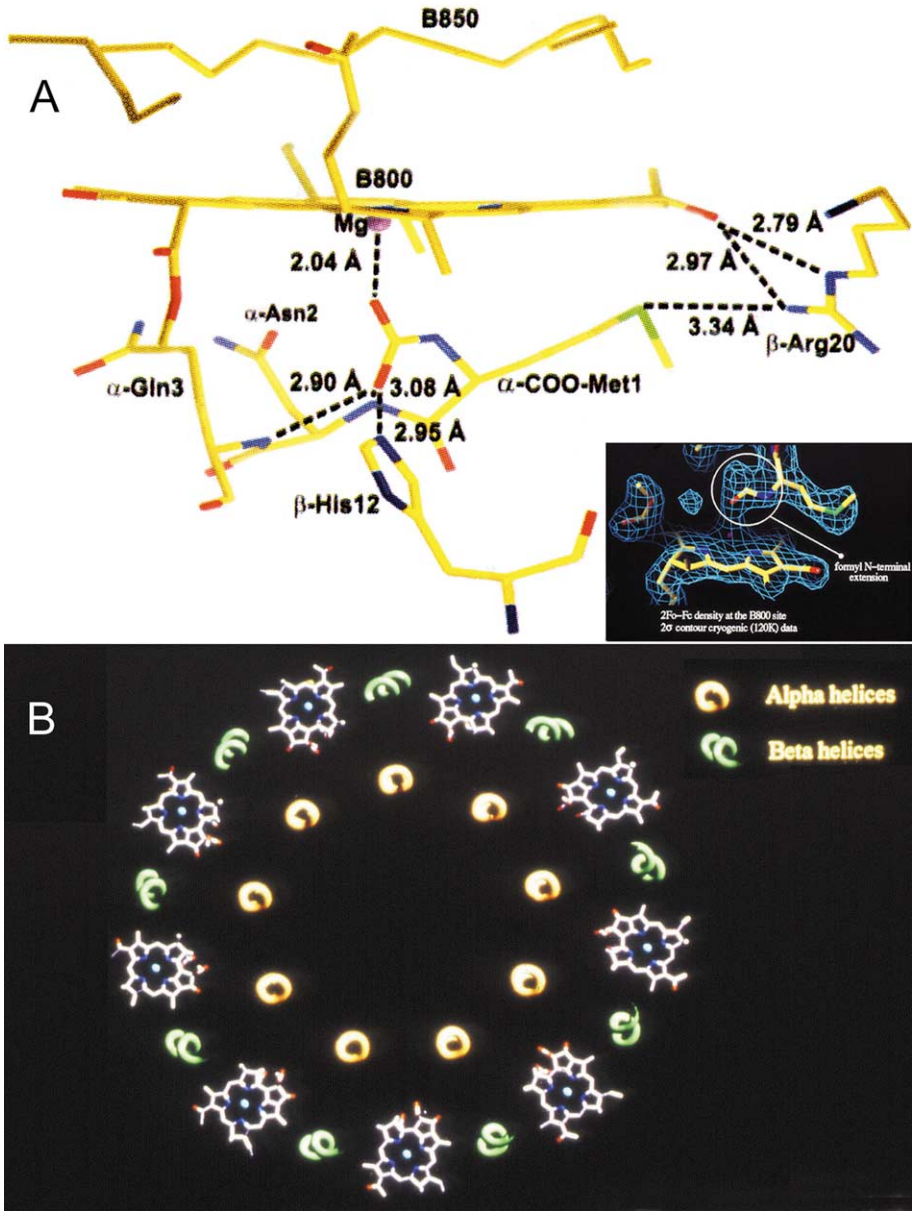

Fig. 3. The organisation of the B800 Bchl*a* molecules in the LH2 complex from *Rps. acidophila*. A: Two views of the binding pocket of the B800 Bchl*a* molecule, taken from the 2 Å data [1]. The small inset shows the electron density map, with the clear bifurcated density on the N-terminal extension of α-Met1. The main part of this picture shows the details of the hydrogen-bonding network surrounding the bacteriochlorin ring of the B800 Bchl*a* molecules. B: A lower resolution overview of the distribution and organisation of the nine B800 Bchl*a* molecules. Note that they are 'flat' within the plane of the membrane and rather peripherally arranged between the β-apoprotein α-helices.

Fig. 5 shows the organisation of the pigments in LH2, the distances between the different pigment groupings and the relative orientation of their major transition dipole moments. This provides the structural content in which the energy transfer reactions must be understood.

If the carotenoids in LH2 from *Rps. acidophila* are excited with a <20 fs excitation pulse then the rate constant for the energy transfer to the Bchl*a* molecules is 61 fs [11]. About two-thirds of this energy is transferred to the B850 molecules and one-third to the B800 molecules [12]. It then takes about 0.9 ps for the energy in the B800 Bchl*a* to be transferred to the B850 molecules [13]. The rate of this energy transfer reaction is remarkably temperature-independent and only slows down to 2–3 ps at 4 K [14]. This means that LH2 behaves rather like a 'solid-state' system, and there is no molecular motion needed during the energy transfer reactions.

Once the excitation energy reaches the B850 ring it is rapidly delocalised (the energy is shared among the tightly coupled Bchl*a*s). The extent of delocalisation is still controversial [15]. When the fluorescence properties of LH2 are investigated by single molecule spectroscopy the data have been interpreted in terms of rather full delocalisation of the energy over the whole B850 ring [16]. However, when the excited states within the B850 ring are interrogated kinetically (i.e. when whole ensembles of LH2 molecules are studied) the data appear to be better fitted by assuming that the excited state is shared over three to four Bchl*a* molecules [17]. Experimental and theoretical studies are still being actively pursued

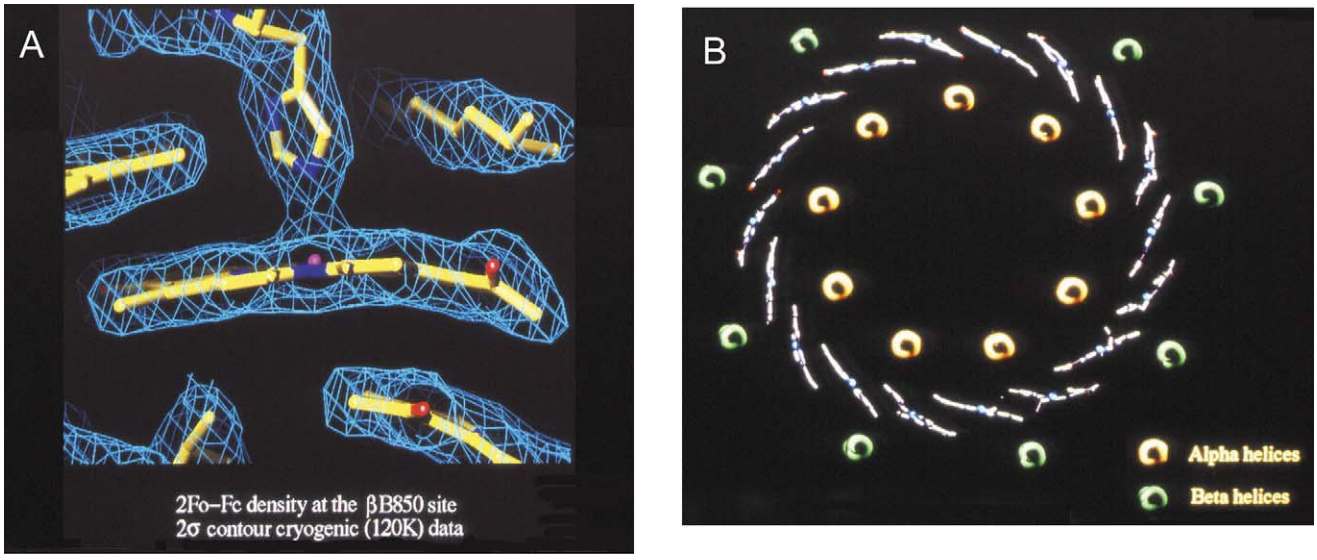

Fig. 4. The organisation of the B850 Bchl*a* molecules in the LH2 complex from *Rps. acidophila*. A: The electron density of a B850 Bchl*a* molecule taken from the 2.0 Å resolution data [1]. The bacteriochlorin ring is rather bent and the histidine residue, which is the fifth ligand to the central Mg^{2+} atom, is clearly seen. B: A lower resolution picture showing the overall distribution and organisation of the full complement of 18 B850 Bchl*a* molecules.

in this area to try to understand the detailed molecular mechanisms of these different energy transfer processes.

In the intact photosynthetic membrane LH2 transfers its excitation energy to LH1, from where is passes onto the RC. The overall time for 'trapping' of the energy by the RC is about 50 ps [3]. Since the excited singlet state lifetime for isolated monomeric Bchl*a* is about 1–2 ns the overall efficiency of light-harvesting in purple bacteria is high.

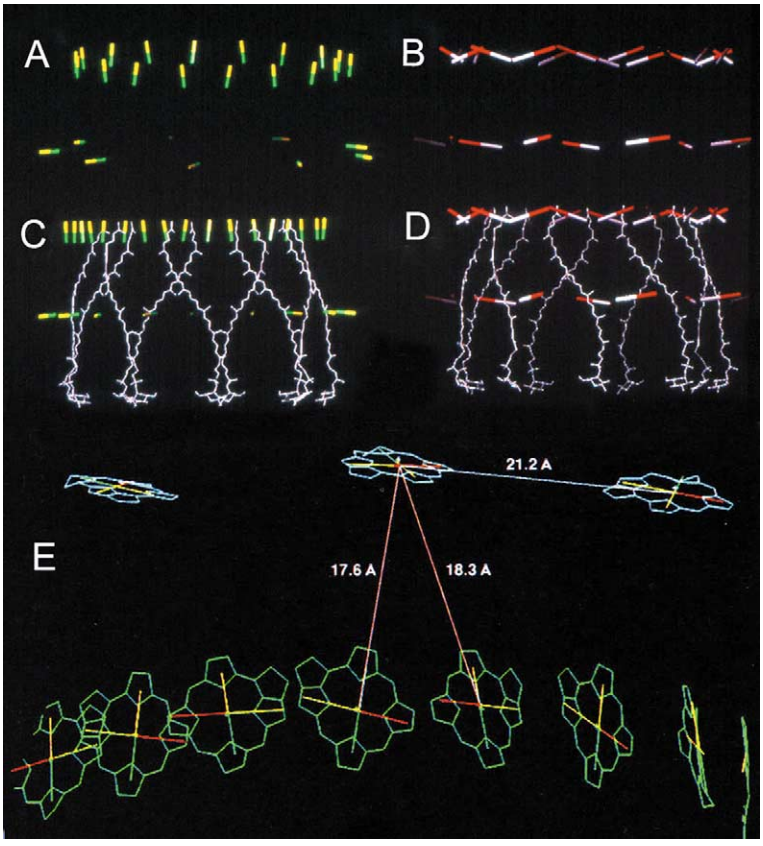

Fig. 5. The relative organisation of the different pigment groups in the LH2 complex from *Rps. acidophila*. A: The relative orientation of the Bchl*a* Qx transition dipole moments. B: The relative orientation of the Bchl*a* Qy transition moments. C,D: The orientation of the Bchl*a* Qx (C) and Qy (D) transition dipole moments relative to the long axis of the well-resolved carotenoid molecules. The major transition dipole moments of the carotenoid's excited singlet states (i.e. those important for energy transfer) lie parallel to the long axis of the carotenoid. E: The distances between the B800 and the B850 Bchl*a* molecules. This figure shows the structural framework which forms the basis for understanding the energy transfer reactions which occur in LH2.

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