Photosynthesis

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Connecting Photosynthetic Light Harvesting and Charge Separation at Higher Detail**

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In 1985, the three-dimensional structure of a photosynthetic reaction center (RC) from the purple bacterium *Rhodopseudomonas viridis* marked a paradigm change.[1] Johann Deisenhofer, Robert Huber, and Hartmut Michel, who received the Nobel Prize in Chemistry for this work only three years later, not only had solved the very first crystal structure of a membrane-integral protein, but also shed light on the machinery that converts light energy into chemical energy during photosynthesis. This is essentially the only fundamental process to introduce energy into the living world. As we know today, the functional principles of light-driven charge separation found in the bacterial systems are fully retained in higher organisms, including the chloroplasts of higher plants.

At the heart of a reaction center, a "special pair" of bacteriochlorophyll (or chlorophyll in eukaryotes) molecules absorbs a photon, elevating an electron to an excited state. The "special" property of this arrangement lies within the ability to transfer the high-energy electron via a neighboring bacteriochlorophyll and an adjacent bacteriopheophytin (a demetalated chlorophyll) within 200 ps to a protein-bound quinone molecule, Q_A, where it still retains an energy of 0.8 eV out of the 1.4 eV gained through light absorption (Figure 1). From Q_A, the electron is then transferred via a bound Fe^{II} ion to an exchangeable ubiquinone molecule, Q_B, that dissociated from the complex to reach a cytochrome bc_1 quinol oxidase and generate a proton motive force across the bacterial membrane to drive ATP synthesis. While this transfer is achieved within 6 μs, the unproductive recombination and reduction of the special pair would at this point require more than 1 s. The remarkable efficiency and directionality of electron transfer in the RC is essential for photosynthesis to work, and its basis is the evolutionarily optimized, precise positioning of the cofactors that is almost impossible to mimic in the highly sought-after chemical catalysts for light-driven charge separation. Marcus theory has been extensively employed to rationalize the crucial processes of photosynthesis, indicating that the primary donor/acceptor arrangement represents the case of a Marcus-inverted region, where the activation energy for the forward reaction (charge separation) essentially disappears, while the back reaction (recombination) is highly disfavored.[3]

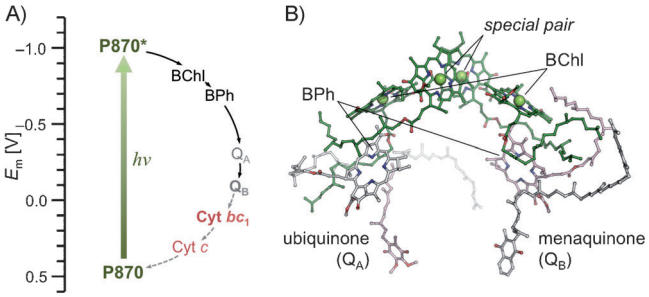

Figure 1. Photosynthesis in purple phototrophic bacteria. A) Cyclic electron transport in non-oxygenic photosynthesis. The cascade involves the photosystem, cytochrome bc_1, and a soluble cytochrome *c*.[2] B) The cofactor arrangement of the reaction center. Charge separation occurs at a "special pair" of bacteriocholorophylls, and the electron then is rapidly transferred via bacteriochlorophyll (BCh) and bacteriopheophytin (BPh) to a non-exchangeable quinone (Q_A). From here, transfer to a second, exchangeable quinone (Q_B) is slower, but still highly favored over recombination (structure from PDB-ID 1WMM).

As a drawback, productive charge separation will only result if the actual absorption of a photon occurs on the chlorophylls of the special pair. While the synthesis of the reaction center complex requires substantial energy, its effectively absorbing cross-section is very small. Phototrophic organisms therefore employ accessory protein components, the antenna or light-harvesting complexes (LHC) that use sets of cofactors to absorb photon energy and funnel it to a limited number of interspersed reaction centers. In cyanobacteria and the chloroplasts of eukaryotic cells this has given rise to the massive photosystem complexes I and II, while phototrophic bacteria employ far simpler components that lead to assemblies of striking efficiency and beauty (Figure 2). Light harvesting does *not* involve electron transfer, but instead photon energy is propagated as excitons, in a Förster-type mechanism of radiation-free resonance energy transfer. This assures maximum energy conservation, and allows conveying directionality by employing cofactors with different absorption maxima; but it requires a very dense packing of the participating chromophores, as the efficiency

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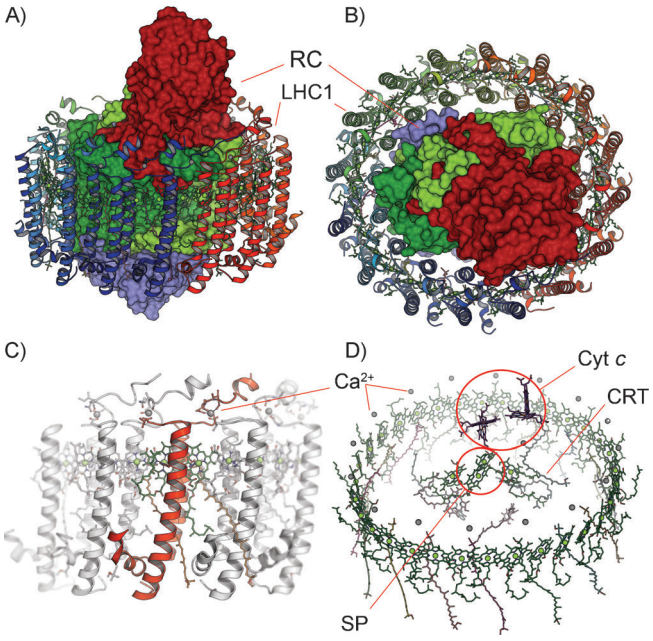

Figure 2. The LH1–RC complex form *Thermochromatium tepidum* (PDB-ID 3WMM). A) The light-harvesting system forms a ring of stoichiometry $\alpha_{16}\beta_{16}$ around a reaction center with subunits H (blue), L (dark green), and M (light green), and an extramembraneous tetraheme cytochrome domain, C (red). B) A top view of the complex shows the slight distortion of the LHC1 ring around the RC. C) The α and β peptides of a single monomer of the LHC1 ring form single membrane-spanning helices that coordinate two B915 cofactors and two molecules of spirilloxanthin (Spx). A Ca^{2+} ion links each αβ unit to the next. D) The ring of B915 and Spx cofactors surrounds the reaction center in the plane of the membrane. A single carotene (CRT) provides a possible exciton conduit from the LHC1 ring to the RC.

of resonance transfer decreases with the sixth power of the distance.

Purple non-sulfur bacteria conduct a cyclic process of photosynthesis that requires only a single reaction center and the cytochrome bc_1 complex and does not generate O_2 (Figure 1 A). The reaction centers are organized in globular invaginations of the cytoplasmic membrane and surrounded by light-harvesting complexes. Electron micrographs show that a ring-shaped LHC1 system completely surrounds the reaction center. A structure of an LHC1–RC complex from *Rhodopseudomonas palustris* was determined to a resolution of 4.8 Å in 2003, but the limited resolution did not allow for an analysis of molecular details.[4] Kunio Miki and co-workers now present the first detailed experimental structure of an LHC1–RC complex from the thermophilic bacterium *Thermochromatium tepidum* at a resolution of 3.0 Å.[5] The conformation of the RC is largely identical to the one observed in the absence of LHC1, with a membrane-integral arrangement of three peptide chains, H, L, and M, and a tetraheme cytochrome *c* domain protruding into the bacterial periplasm (Figure 2 A,B).[6] This cytochrome is reduced by a soluble ferredoxin and is able to transfer an electron to the special pair to initiate a new cycle of charge separation. In the new structure, both quinone binding sites are occupied, with a ubiquinone molecule bound to the Q_B site (Figure 1 B). The architecture of LHC1 is of striking simplicity and efficiency, consisting of two short peptide chains, α and β, that associate with two molecules of bacteriochlorophyll B915 and two copies of the carotenoid spirilloxanthin (Spx). This LCH1 protomer then forms a hexadecameric ring around the reaction center, linked and stabilized by Ca^{2+} ions between neighboring α- and β-subunits (Figure 2 C,D). This is at variance with the 2003 *R. palustris* structure, where only 15 LHC protomers associate and leave a gap that was filled by an additional peptide, the PufX-like chain W.[4] The occurrence of such a gap was remarkable in the light of the functionality of the LHC complex, where the 32 B915 cofactors are tightly packed, sufficiently close for direct π-stacking interactions and ideally spaced for efficient exciton transfer (Figure 2 B,D). The structure of an LHC thus is reminiscent of a storage ring, not unlike the electron storage rings at synchrotron sources, extending the lifetime of the excited state until the special pair becomes available, and allowing for efficient long-range energy transfer.

In contrast to the *R. palustris* structure, the storage ring in *T. tepidum* is fully closed (Figure 2 D), and the higher resolution now also identified a further cofactor, a carotene that is placed asymmetrically in the complex, where it might facilitate exciton transfer from the ring to the special pair. A problem that arises from a closed ring, however, is the fate of the electrons after charge separation, as the reduced ubiquinone Q_B must leave the RC to be oxidized at a cytochrome bc_1 complex. Miki and co-workers have identified small gaps between each of the monomers in the LHC1 ring and suggest these to allow passage of the quinone.[5] In practice, due to the asymmetric architecture of the RC this will only occur at few positions in the vicinity of the Q_B site in subunit L, and the crystal structure shows a remarkable cavity within the ring adjacent to this site (Figure 3 A). This cavity will of course not be empty, but is likely filled with phospholipid molecules that retain conformational flexibility and thus are disordered in a crystal structure.

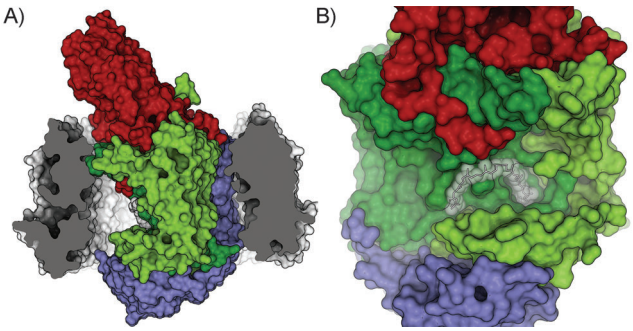

Figure 3. Quinone access and binding in the LH1–RC complex of *T. tepidum* (PDB-ID 3WMM). A) The closed LHC1 ring contains access channels for quinone (left). B) The Q_B in chain L (dark green) contains a bound ubiquinone molecule (white).

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