The photosynthetic apparatus of *Rhodobacter sphaeroides*

André Verméglio and Pierre Joliot

The predominantly green color of the biosphere attests to the essential role of photosynthetic organisms on Earth. By this process, plants convert light energy into chemical energy to reduce carbon dioxide to organic matter such as carbohydrates. This capability is, however, not limited to plants. Certain bacteria are also able to perform this energy conversion for their growth and development. The molecular machinery involved in the initial steps is very similar in plant and bacterial photosynthesis, and purple bacteria are the model bacterial system for this process. One of the most often studied species is *Rhodobacter sphaeroides*. This Gram-negative bacterium of the Proteobacteria group is metabolically highly diverse. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus is composed of three multimeric transmembrane protein complexes: the antenna or light-harvesting complexes (LHCs), the reaction center (RC) and the cytochrome (cyt) bc$_1$ complex. The role of the LHCs, which non-covalently bind carotenoid and bacteriochlorophyll molecules, is to collect incident light. Like most purple bacteria, *R. sphaeroides* has two types of LHCs, termed LH1 and LH2. The amount of LH2 is modulated by several factors, such as light intensity and growth pressure, whereas the LH1 complement is synthesized in fixed stoichiometric amounts with the RC, forming the RC–LH1 core complex. The large amount of antenna pigments with respect to the RC (up to 100 bacteriochlorophyll molecules are present per RC) increases the cross section available for light capture.

When a photon is absorbed by the LHC, the excitation reaches the RC (where charge separation occurs) in less than 100 picoseconds (ps). At the RC, an electron is transferred from the excited primary donor, a bacteriochlorophyll dimer, to a molecule of bacteriopheophytin in 2–3 ps, and, subsequently, to the primary quinone (Q$_A$) in ~200 ps. These fast electron transfers stabilize the separated charges and ensure a quantum yield close to 1 (i.e. for almost every photon absorbed by the RC, one electron is transferred). During the few tens of microseconds following light excitation, the photosoxidized primary electron donor is reduced by the periplasmic protein cyt c$_2$ on the acceptor side, the electron is transferred from Q$_B$ to a second ubiquinone molecule (Q$_B$), forming an RC-bound semiquinone (Q$_B^\cdot$). Q$_B^\cdot$ undergoes a second reduction step during the next photochemical turnover of the RC. This doubly reduced Q$_B$ picks up two protons from the cytoplasmic space and is then released from the RC, joining the quinone pool. The electron-transfer cycle is completed by the oxidation of the quinol by cyt c$_2$ via the [Fe$_2$S$_2$] cluster of the Rieske protein, a reaction catalysed by the bc$_1$ complex, and the release of two protons into the periplasmic space.

**Photo-induced cyclic electron transfer**

Most of the photosynthetic machinery of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth. The photosynthetic apparatus of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth.
Deletion of pufX impairs turnover of the RC by strongly retarding the quinone exchange between this complex and the bc1 complex.

Structure–function issues in the photosynthetic apparatus
A major breakthrough in the elucidation of the molecular events of photosynthesis was the resolution of the structure of the RC of Blastochloris (formerly Rhodopseudomonas) viridis by X-ray diffraction. This pioneering work marked a new era in membrane-protein biochemistry. The subsequent description of the structure of the RCs of R. sphaeroides illustrated the high structural homology between RCs from different species. More recently, the 3-D structures of LH2s isolated from different photosynthetic bacteria and of the mitochondrial bc1 complex have been resolved. Despite the abundance of information available on the individual complexes, the characterization of their organization and assembly on a broader scale in the native membrane remains a challenge.

The efficient transfer of excitation (>90%) between the large number of LH2 pigments and the RCs necessitates close proximity of the different chromophores (<65 Å) and, therefore, a specific organization of these complexes in the membrane. By contrast, it is generally believed that no particular arrangement is required for electron transfer between the RCs and the bc1 complexes. However, analysis of the lateral distribution of membrane complexes by freeze-fracture electron microscopy indicates a unique spatial arrangement of the particles relative to one another, suggesting the existence of well-defined structural entities. The connection between these complexes is assumed to be mediated by random collisions between free, diffusive electron carriers such as quinone molecules in the membrane and cyt c2 in the periplasmic space. However, here we will summarize the biochemical, functional and structural data that indicate a high degree of organization of the electron-transfer chain in the native membrane of R. sphaeroides.

A ring structure of LH2s around the RC
Gentle detergent treatment of the inner membrane of photosynthetic bacteria can specifically extract LH2 complexes and RC–LH1 complexes. A clear picture of the close association of LH1 with the RC was provided by Miller’s early low-resolution electron microscopic analyses of native membranes of B. viridis. The RC–LH1 complexes of this bacterium, which has no LH2 and a low number of bc1 complexes, naturally form 2-D crystals. This work revealed that a ring of LH1 molecules surrounds a single RC. This organization of the complexes has been confirmed for several other species of photosynthetic bacteria. In particular, Walz and colleagues have recently obtained RC–LH1 crystals of sufficient quality to show that the RC is surrounded by 15–17 LH1s.

Fig. 1. Schematic representation of the photosynthetic electron-transfer chain in Rhodobacter sphaeroides. Bacteriochlorophyll molecules are represented by blue diamonds and bacteriopheophytin molecules by pink diamonds. Hemes are denoted by red diamonds. The red arrows represent excitation transfer and the black and blue arrows correspond to electron and proton transfer, respectively. The stoichiometry of electrons and protons corresponds to the absorption of two photons (hv) by the reaction center (RC). After absorption of a photon by the light-harvesting complexes (LH1 and LH2), the excitation reaches the reaction center, where charge separation occurs. An electron is transferred from the excited primary donor of the RC, a bacteriopheophytin dimer, to the secondary acceptor Qb via a molecule of bacteriopheophytin and the primary quinone, QA. After a second turnover, the doubly reduced QA picks up two protons from the cytoplasmic space. The quinol (QH2) formed is oxidized by cyt c2, a reaction catalyzed by the bc1 complex, which releases two protons to the periplasmic space. The cyclic electron transfer is completed by the reduction of the photosoxidized primary electron donor by cyt c2.
LH1 subunits. The closed structure of LH1 complexes, combined with the tight coupling of the bacteriochlorophyll molecules, ensures a rapid delocalization of the excited state and the possibility for energy to be transferred from any point of the ring. However, the packed organization of this structure raises the question of how the quinone-quinol transfer takes place between the RC acceptor side and the $bc_1$ complex during photoinduced electron transfer (Fig. 1).
Evidence for a supercomplex arrangement

A series of thermodynamic and kinetic studies has provided new insight into the organization of the components of the photosynthetic electron chain. A key observation was that the apparent equilibrium constants between the different reactants (RC, bc₁ complex and cyt c₁), measured during the photoxidation of these carriers, were much lower than those deduced from their midpoint potentials determined at equilibrium (in the presence of redox mediators) in the dark. This behavior can be explained as follows. In the photoxidation experiment, a rapid equilibrium is achieved at a local level, within specific domains or supercomplexes containing a small number of electron carriers, whereas equilibration at a macrolevel between these domains is a much slower process. Data analysis suggests that each supercomplex contains two RCs closely associated with one bc₁ complex and one cyt c₁ (Ref. 18), in agreement with the overall stoichiometry of these complexes in the chromatophore membrane. In other words, the close association of the RCs and the bc₁ complex results in the trapping of one molecule of cyt c₁ per RC dimer, hindering its diffusion into the periplasmic space.

Crofts et al. have, however, proposed an alternative interpretation of the observed kinetic and thermodynamic anomalies. They assume free diffusion of the electron transfer components in the aqueous phase, but heterogeneity in the stoichiometry of membrane complexes and cyt c₁ in the chromatophore population.

Another line of argument in favor of a supramolecular organization of components of the photosynthetic chain comes from the observation that the addition of a subsaturating concentration of myxothiazol, a specific inhibitor of the bc₁ complex, decreases the number of active bc₁ complexes but does not affect the rate of electron transfer for the uninhibited complexes. Moreover, the cyt c₁ connected with the inhibited bc₁ complexes remains photooxidized because it cannot interact with the uninhibited chains. This behavior implies that each photosynthetic chain acts as an isolated entity. Interestingly, Fernández-Velasco and Crofts observed different behavior using intact cells or isolated chromatophores. In isolated chromatophores, cyt c₁ appears to freely diffuse and interact with all the bc₁ complexes present in the vesicles. A possible explanation for this discrepancy could be the loss of supercomplex association during the preparation of the isolated chromatophores.

Supramolecular organization in native membranes

As well as the functional arguments, ultrastructural evidence of a supramolecular organization of the photosynthetic components has recently been obtained from freeze-fracture electron microscopy of tubular membranes from R. sphaeroides cells. These tubular membranes (100 nm in diameter and several hundred nm in length) correspond to regions of the intracytoplasmic membrane that do not contain LH₂ complexes. They are formed when the amount of LH₂ complexes is decreased, either by gene deletion or growth under anaerobic conditions. These tubular membranes contain all the membrane components of the photosynthetic apparatus, in the relative ratio of one RC-LH₁ complexes to two RCs and approximately 24 bacteriochlorophyll molecules per RC (Ref. 28). As a result of the well ordered arrangement of the particles in these membranes, electron micrographs of negatively
stained samples diffract up to 25 Å (Ref. 28). The calculated projection map of the upper face of these tubes is depicted in Fig. 4. The unit cell contains an elongated S-shaped supercomplex composed of two C-shaped structures with an external diameter of 112 Å, closely matching the dimeric arrangement in the freeze-fracture samples. Although a definitive interpretation of this projection map cannot be given at present, it is assumed that each C-shape corresponds to LH1 complexes partially surrounding one RC.

A dimeric association of RCs is in agreement with the results obtained by Francia et al. After detergent solubilization of *R. sphaeroides* chromatophores, they found two membrane complexes corresponding to monomeric and dimeric RC-LH1 complexes, in addition to isolated LH1 and LH2 complexes. Electron micrographs show that the dimeric RC-LH1 complexes comprise two intertwined rings of LH1 containing two RCs (Ref. 5). It was also shown that the PufX polypeptide is strictly required for the isolation of the dimeric RC-LH1 complexes. As low concentrations of the PufX polypeptide inhibit the in vitro oligomerization of LH1 complexes29, one possibility is that PufX plays an essential role both in the supramolecular arrangement of the photosynthetic apparatus and in the C-shaped structure of the LH1 complexes by restraining the formation of a closed ring. In this context, it is interesting that deletion of *pufX* induces a significant increase in the ratio of bacteriochlorophyll molecules to RCs, which might be related to the formation of a closed ring around the RC (Refs 30,31). The C-shaped geometry of the LH1 complexes would facilitate the diffusion of quinone molecules between the RCs and *bc1* complexes by creating a path between them. This is in contrast to other electron microscopic studies of monomeric purified RC-LH1 complexes, in which the RCs are surrounded by a closed LH1 ring.

Are these supercomplexes a common feature? Are these supercomplexes a general feature of the electron-transfer components of photosynthetic bacteria? The answer is almost certainly no. Even in the case of *R. sphaeroides*, the photosynthetic chains localized in the non-invaginated part of the cytoplasmic membrane do not appear to be organized in supercomplexes and share cyt *c*2 with the RCs and the cyt *bc1* complex in the invaginated part of the membrane limits its interaction with other complexes present in the cytoplasmic membrane or the periplasm, such as cytochrome oxidase or the denitrification enzymes; this favors photosynthetic activity over respiration or denitrification. Other photosynthetic bacteria, such as *B. viridis* and *Rhodospirillum rubrum*, possess a large excess of RCs over *bc1* complexes; the formation of supercomplexes of the *R. sphaeroides* type is, therefore, stoichiometrically restricted. However, in the case of *R. rubrum*, only one molecule of cyt *c*2 can bind to two RCs, showing that the dimeric association has also been observed in vivo for the RCs of photosystem II in green plants, which are highly homologous to the RCs of purple bacteria32.

Are other bioenergetic chains organized in supercomplexes? According to the chemiosmotic hypothesis, no specific supramolecular organization of membrane complexes is required, provided their coupling is completed by liposoluble and hydrosoluble diffusive carriers. Nevertheless, organization of electron transfer...
• Are membrane complexes of other bioenergetic chains organized in supercomplexes?
• What is the exact role of PufK?
• Are supercomplexes between photosystem II and b6f complexes present in the thylakoid membrane?
• Are the quinone molecules confined to the supercomplex?
• Is the open ring a general feature of light-harvesting complex I (LHI) in photosynthetic bacteria?

References

26 Hunter, C.N. et al. (1990) Biochemistry 29, 1439–1467
27 Junger, C. et al. (1999) EMBO J. 18, 534–542
38 Mckay-Hickey, K.E. et al. (1998) Biochemistry 37, 4730–4750

Questions for future research

• Is the open ring a general feature of light-harvesting complex I (LHI) in photosynthetic bacteria?

Acknowledgements

We wish to thank Anne Joost, Colette Jungs, Jérôme Lavergne, Jacqueline Olive, Jean-Luc Ranck, Jean-Louis Rigaud and Monique Sabaty for their collaborative work in the study of the photosynthetic apparatus of R. sphaeroides.

Carriers in supercomplexes allows a more efficient electron transfer, unimpeded by reactant diffusion, indeed, supramolecular associations have been observed for membrane complexes involved in the respiratory chain of a few bacteria[2,3]. A similar situation is encountered for soluble enzymes involved in cellular metabolism. Different enzymatic pathways, intermediates are transferred from one enzyme to another without complete equilibration with the surrounding medium[3]. This ‘metabolic channelling’ also implies the existence of specific organization in the form of multienzyme complexes. Formation and dissociation of supercomplexes could be a general and powerful way that bacteria have developed to adapt their bioenergetic processes efficiently to the available energy source.

References


Coming soon in Trends in Microbiology

• Multilocus sequence typing, by M.C. Enright and B.G. Spratt
• Pathogenic bacterial kinases and phosphatases injected into host cells, by R. Devinney, O. Steeie-Martiner and B.B. Finlay
• Mutation as an origin of genetic variability in Helicobacter pylori, by G. Wang, M.Z. Humayan and D.E. Taylor
• Serotype-converting bacteriophages and O-antigen modification in Shigella flexneri, by G.E. Allison and N.K. Verma

Don’t miss these and many more articles of interest; subscribe to Trends in Microbiology using the form bound in this issue.