Structure and Function of the Reaction Centre – Light Harvesting 1 Core Complexes from Purple Photosynthetic Bacteria

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Summary

Reaction centre-light harvesting 1 core complex is a fundamental unit in photosynthetic bacterium. It is the place where light energy is collected and used to power photosynthetic redox reaction, leading to the synthesis of ATP ultimately. The reaction centre is surrounded by elliptical LH1 complex. The subunit of the LH1 ring is a heterodimer of α-, β-polypeptide pair, to which pigment molecules, BChl *a* or BChl *b* and carotenoid are non-covalently bonded. There are at least three different types of the RC-LH1 core complexes found in photosynthetic bacteria so far. The core complex from *Rps. palustris* is a monomer. Its LH1 ring consists of 15 pairs of α/β-polypeptide with an extra protein 'W' located between two α-polypeptides, forming an incomplete ring. The gap of the LH1 ring was proposed as a gate to facilitate quinone/quinol exchange between reaction centre and cytochrome *bc*1 complex. A dimeric core complex was found in PufX-containing species, such as *Rba. sphaerides*. Two RCs are associated by 28 α/β-apoprotein pairs and two pufX proteins, forming an S-shaped RC-LH1-PufX core complex. The pufX protein causes incomplete LH1 ring and dimerization of the core complex. Monomeric RC-LH1 from *Tch. tepidum* has a complete elliptical LH1 ring that is composed of 16 pair α/β-apoprotein pairs without pufX-like protein. Sixteen Ca^{2+} are coordinated on C-terminal region of the α/β-polypeptide to stabilize the core complex and cause BChl *a* Qy absorption redshift to 915 nm. Carotenoid, spirilloxthanin contacts with α/β-apoproteins intimately to form an inter subunit interaction within the core complex, providing a further stability of the complex.

Keywords

Photosynthesis • Photosynthetic bacteria • Reaction center • Light harvesting 1 core complex • RCLH1 • Carotenoid • Bacteriochlorophyll

Contents

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2.1 Introduction

Purple photosynthetic bacteria refer to a unique group of microorganism that use sun light as their energy source. The light energy is absorbed by pigment molecules, such as bacteriochlorophyll (BChl) and carotenoids (Car), which are non-covalently bound to so-called light harvesting complexes. In general, there are two major classes of light harvesting complex in the purple photosynthetic bacteria, light harvesting complex 1 (LH1) and light harvesting complex 2 (LH2). The LH2, sometime called peripheral antenna complex, is composed of oligomer of two short polypeptides (α and β) with associated pigments, Car and BChl *a* or BChl *b*. The α/β-polypeptide pair, therefore, is a building block of this cylinder-like complex (Koepke et al. 1996; McDermott et al. 1995). The building block of the LH1 is constructed similar as molecular architecture as that in the LH2 complexes. The LH1 complex is always intimately interacted with the reaction centre (RC) in a fixed stoichiometry. The term of core complex usually refers to the combination of the RC and the light harvesting complex 1 in the purple photosynthetic bacteria. A short name, such as RC-LH1 or RC-LH1-PufX is often used in literatures. As its name implies that the photosynthetic core complex or RC-LH1 is the central part of bacterial photosynthesis. This chapter will focus on the recent development on structural determination of the RC-LH1 core complexes from the purple photosynthetic bacteria by starting with basic background of bacterial photosynthesis and building blocks of the core complex. For the readers who are interesting in general works on the purple photosynthetic bacteria, two recent books edited by Blankenship, R. E. and Hunter, C. N. provide more detailed and comprehensive information on the bacterial photosynthesis. (Blankenship 2014; Hunter et al. 2009).

2.2 The Purple Photosynthetic Bacteria

The purple photosynthetic bacteria, much like the name suggested, are a group of dark coloured bacteria, with different morphologies such as rod, spirilla, cocci or vibrios, which can convert light energy to chemical energy to maintain their metabolism. The pigment molecules that are involved in the light absorption are usually BChl *a* or BChl *b* and carotenoids. It is these pigments that give the purple bacteria such gorgeous colours, from purple, red to green, depending on the amount and type of different carotenoids in an individual purple bacterium.

The purple photosynthetic bacteria can be divided into two groups, i.e., purple non-sulphur bacteria and purple sulphur bacteria according to their tolerance and utilization of sulphide (Imhoff et al. 1984). The purple sulphur bacteria use sulphur or sulphide, such as hydrogen sulphide H_2S as an electron donor for carbon dioxide reduction in its respiration, while the purple non-sulphur bacteria use organic electron-donor, such as succinate or malate instead. Usually, sulphide is toxic for the purple non-sulphur bacteria although the most species of the purple non-sulphur bacteria can still grow at low level of sulphide (<0.5 mM). Different from higher plant or cyanobacteria photosynthesis, photosynthesis in the purple bacteria does not give off oxygen, and it only occurs under anoxic conditions, which is called as anoxygenic photosynthesis. Therefore, an environment having abundance of oxygen hinders their photosynthetic growing. That is why they are typically found in hot sulphuric spring (for purple sulphur bacteria especially) or stagnant water.

The purple non-sulphur bacteria, such as *Rhodobacter* (*Rba.*) *sphaeroides*, *Rhodopseu domonas* (*Rps.*) *palustris, Blastochloris (Blc.) virids* can grow photoheterotrophically or even photoautotrophically and chemoheterotrophs in darkness as well. It is therefore relatively easy to grow them in laboratory conditions. Having such

versatile metabolisms, the purple non-sulphur bacteria become the most intensively studied species for the bacterial photosynthesis.

Growing conditions for the purple sulphur bacteria, in the other hand, is relatively stricter than that in the purple non-sulphur bacteria. Firstly, they need sulphide as electron donor. That is why the large population of the purple sulphur bacteria species are found in hot springs containing sulphide. Secondly, many of them prefer high illuminated condition, implying they grow phototrophically in nature. Thirdly, some species are isolated from extreme growing conditions, such as halophilic, high or low temperature, acidic, alkaline etc. These extremophilic purple bacteria provide us chances to study photosynthesis under harsh conditions related to molecular adaptation, protein stability etc. A high thermo-stability of the core complex from a thermophilic purple sulphur bacterium, *Thermochromatium* (*Tch.*) *tepidum*, leading to a successful 3.0 Å resolution 3D structure determination of the RC-LH1 core complex, is a good example (Niwa et al. 2014; Suzuki et al. 2007).

2.3 Mechanism of Photosynthesis in the Purple Photosynthetic Bacteria

Photosynthesis is one of the most crucial reactions taking place on the Earth. By converting light energy from the Sun to chemically useful form that is used to fuel the organisms' activities, it provides almost all foods, energies we need alone with oxygen we breathe. Although the concept of photosynthesis is commonly related to oxygenic higher plant, much of milestone results revealing the mechanism of the photosynthesis in nature come from the purple photosynthetic bacteria due to their relatively simpler photosynthetic system (Cogdell et al. 2006).

In the purple bacteria, photosynthesis takes place in bacterial cell membrane, which is located near the surface of the cell. The major protein complexes involved in the reaction chain are embedded in lipid bilayer. These include peripheral light harvesting antenna complexes, such as LH2; RC-LH1; cytochrome *bc*1 complex and ATP synthase. Water soluble cytochrome *c*2 and quinone/quinol are needed to complete proton and electron cycles in the reaction chain. A schematic arrangement of all required components is shown in Fig. 2.1. Photosynthetic reaction starts from absorption of incident light photons by antenna system, e.g., LH2 (Vangrondelle et al. 1994). The energy absorbed by LH2 complexes is then rapidly transferred to LH1 in ~5 ps. Accepted excitation energy both by transferred from the LH2 and absorbed by LH1 itself is stored in LH1 ring by delocalization in ~80 fs. Finally, the energy is delivered to the RC special pair of BChl *a* (B870) in a relatively longer time constant of ~35 ps due to a longer distance between LH1 BChl *a* (B875) and RC B870. Subsequently, the RC special pair B870 is excited. When the excited B870 returns to its stale ground state, it releases an electron to bacteriophephytin (BPhe) *via* accessory BChl *a*. This electron travels continuously down to ubiquinone site (Q_B), where ubiquinone is reduced. By the second cycle of electron transferring, the ubiquinone is fully reduced to ubiquinol (QH_2). In the meantime, two protons are taken from cytoplasmic side. After fully reduction, QH_2 molecule is released from the RC Q_B site to quinone pool toward cytochrome *bc*1 complex, where quinol is oxidised to quinone by releasing two protons to periplasmic side and two electrons to a mobile electron carrier cytochrome *c*2 that brings electrons back to the RC special pair, completing the electron cycle. Successive electron cycle companied with proton translocation from cytoplasmic side to periplasmic side leads to the formation of electric potential, proton motive force (pmf), across the membrane. It is this pmf that is used to power a variety of energy-requiring biological reactions in cells, for example, the synthesis of adenosine triphosphate (ATP), which is the most commonly used as "energy currency" of cells. Companied the synthesis of ATP, protons are pumped back to cytoplasm across the membrane, completing the proton cycle in the process of photosynthesis.

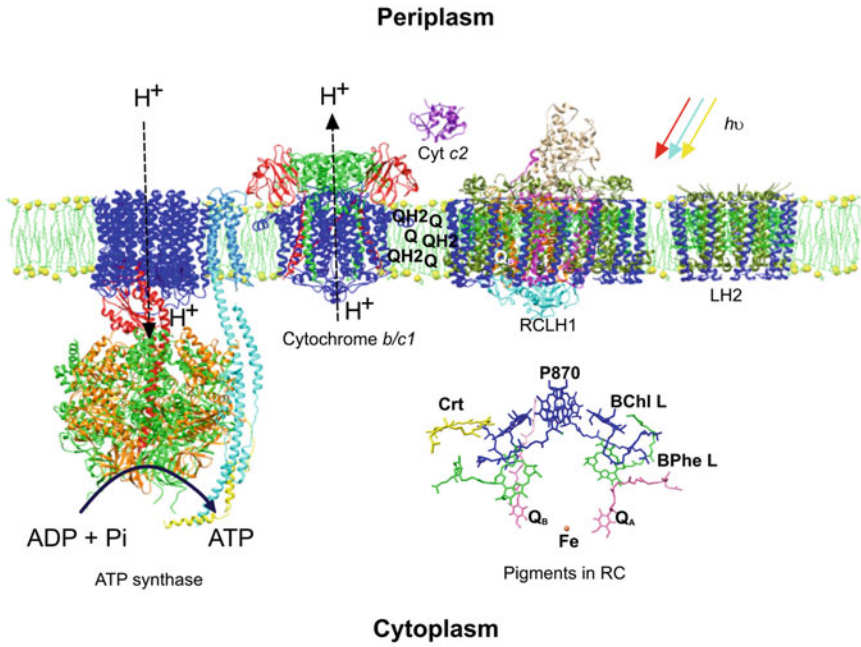

Fig. 2.1 Schematic arrangement of major protein complexes involved in the bacterial photosynthesis. On right side is LH2 of *Rps. acidophila* (McDermott et al. 1995) that consists of nine α/β subunits, forming a cylinder-like complex embedded in lipid bilayer. Next to the LH2 is RC-LH1 core complex from *Tch. Tepidum* (Niwa et al. 2014). RC, composed of subunit H in cyan, M in magenta, L in orange and C in dark khaki, is surrounded by 16 α/β LH1 subunits with α-polypeptide in olive drab and β-polypeptide in medium blue. Pigment organization in the RC is shown just below the RC-LH1 core complex. 3D crystal structure of cytochrome *bc*1 complex from *Rba. sphaeroides* was used (Esser et al. 2008). This dimeric complex comprises cytochrome *b* in blue, cytochrome *c*1 in green and Rieske Fe-S protein in red. On left side is an ATP synthase from *E. coli*. (Rastogi and Girvin 1999). A water-soluble protein, cytochrome *c*2 (Paddock et al. 2005) and a putative quinone pool are shown as well

2.4 Building Block of the Core Complex of Purple Photosynthetic Bacteria

2.4.1 Pigment Molecules

The RC-LH1 core complex plays an important role in the process of bacterial photosynthesis. The LH1 complex not only transfers excitation energy from LH2 complexes to RC but also absorbs light energy alone. Actually, in some of non-sulphur purple bacteria, such as *Rhodospirillum (Rsp.) rubrum* and *Blc. virids*, there are no peripheral antenna LH2 complexes at all, and the LH2 deletion mutant of *Rba. sphaeroides* can still grow photosynthetically. Strong absorbance caused by pigment molecules in the core complex in visible and near infrared regions ensure that they still have enough absorbed energy to maintain cell's biological processes.

Figure 2.2 shows the absorption spectra of four different RC-LH1 core complexes purified from (A) *Rba. sphaeroides*, (B) *Rps. palustris*, (C) *Tch. tepidum* and (D) *Blc. virids* respectively. The spectra show that the major pigment molecules in the core complexes from the purple bacteria are BChl *a* or BChl *b* and carotenoids. Absorbance between ~425 and 550 nm is caused by carotenoids. BChl *a* possesses a strong Qy absorbance band in the near infrared region, ~875 nm (B875). The Qy can red-shift depending on different molecular environments. For example, the Qy band of the BChl *a* in the RC-LH1 core complex of *Tch. tepidum* is red-shifted to 915 nm (B915). In the case of BChl *b*-containing

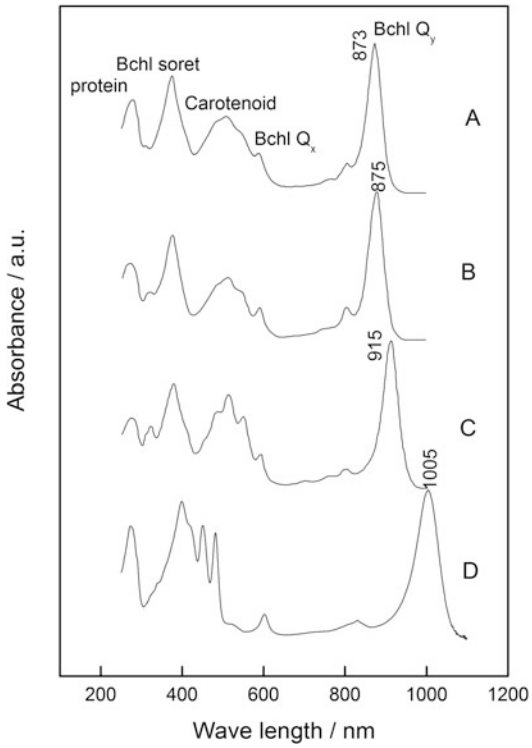

Fig. 2.2 Absorption spectra of purified RC-LH1 core complexes from (*A*) *Rba. sphaeroides*; (*B*) *Rps. palustric*; (*C*) *Tch. Tepidum* and (*D*) *Blc. virids,* which contains BChl *b*. Peaks at ~280 nm are ascribed to protein; Peaks at 376 nm are Soret band of BChl *a* in the LH1, which is overlapped with the Soret band of BChl *a* of special pair, accessory BChl *a* and Bphe in the RC; 425–550 nm absorption peaks are attributed to carotenoids; BChl *a* Qx band appears at ~590 nm; Qy bands of Bphe, accessory BChl *a* and special pair BChl *a* are located at 752, 800 and 863 nm; The strongest absorbance band in near infrared region is Qy band of BChl *a*. The Soret, Qx and Qy bands of BChl *b* in the LH1 complex of *Blc. virids* are red-shifted

core complex, the Qy is red-shifted further to ~1005 nm, e.g., in *Blc. virids*. Bchl *a*/*b* Soret band appears at ~376/398 nm and Qx band at ~590/600 nm.

Two types of bacteriochlorophyll occur in the purple bacterial core complexes, either BChl *a* or BChl *b* depending on different species. Both of them are a substituted tetrapyrrole. The tetrapyrrole ring is stabilised by a central magnesium atom that coordinates with four nitrogen atoms in the tetrapyrrole ring. A highly hydrophobic C20 alcohol tail, either phytol or geranylgerniol is esterified to an acid side chain of the tetrapyrrole ring. The alternative arrangement of C–C and C = C bonds in the tetrapyrrole ring of BChl *a* and BChl *b* decides that they are strong and efficient photoreceptor molecules. In organic solution, such as diethyl ether, BChl *a* has a maximum absorption (Qy) at ~771 nm, and BChl *b* at ~796 nm. Once associated into the core complexes, however, their Qy absorption red-shift to ~875 nm (e.g. in *Rba. sphaeroides*) and ~1005 nm (e.g. in *Blc. virids*) (Jay et al. 1984) in purified core complexes respectively. The difference between BChl *a* and BChl *b* is very small indeed. Actually, they share the same biosynthetic pathway until a branch-point at 8-vinyl-Chlide *a*. Tsukatani and co-workers found that chlorophyllide *a* oxidoreductase (COR) from BChl *b*-producing species, such as *Blc. visids*, use 8-vinyl-Chlide *a* to synthesize 8-ethylidene group on BChlide g, a precursor to final product of BChl *b*. The COR enzyme from BChl *a*-producing species, such as *Rba. capsulatus*, in the other hand, catalyses 8-vinyl reduction, leading to BChl *a* as its final product in its biosynthetic pathway (Tsukatani et al. 2013). Therefore, it only has one bond different between BChl *a* and BChl *b* on C8 position. In the former case, an ethyl group is attached to C8. In the later case, however, an ethylidene group is connected to C8. It is this difference that provides BChl *b* a wider carbon-carbon conjugated region than BChl *a*, resulting in further red-shift, which makes BChl *b* containing species to utilise infrared light energy in longer wavelength. Figure 2.3 shows their structural difference and absorption spectra in organic solvent.

The second major photoreceptor pigment in the purple bacteria is carotenoid. Generally speaking, carotenoids in photosynthetic bacteria possess three main functions. Light harvesting, light protection and light harvesting complex structural stability (Cogdell and Frank 1987; Frank and Cogdell 1996; Polivka and Frank 2010). By strong absorbing light in the blue-green spectral region (450–550 nm), where BChls have weaker absorbance, and transferring absorbed energy fast and efficiently to BChls, they actually act as an important energy donor in the photosynthesis. By dissipation excess amount of light exposure in antenna

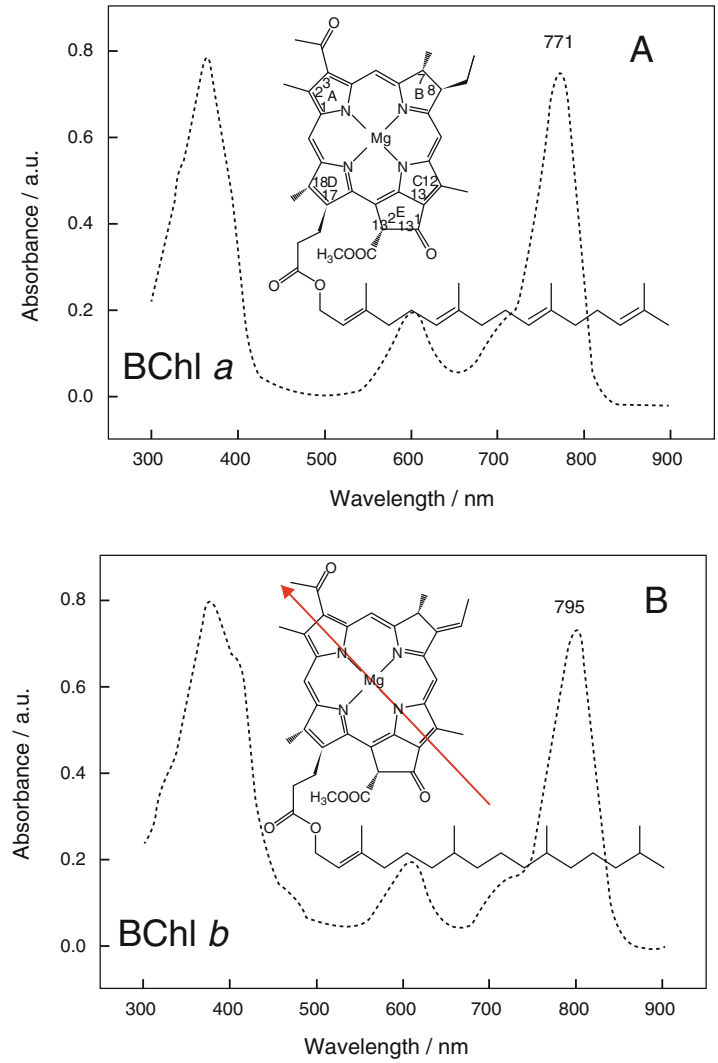

Fig. 2.3 Chemical structures of BChl *a* and BChl *b*. (**a**) BChl *a*. IUPAC nomenclature is used for the numbering of the carbon atoms and the rings. C20 hydrophobic geranylgernyl tail is attached. Absorption spectra of BChl *a*/b in diethyl ether are shown in dashed lines. (**b**) BChl *b* with Phytyl tail. Qy transition dipole is indicated with a *red arrowed line*

complexes, they also act as a photo-protector. Finally, by interacting with polypeptides in the antenna complexes, the carotenoid molecules stabilize the structure of the light harvesting complexes (Davis et al. 1995; Fiedor and Scheer 2005; Karrasch et al. 1995; Niwa et al. 2014).

Molecular structure of the carotenoid can be considered as a result from joining together of eight isoprene units. Four of the units connected in head-to-tail manner form a half carbon skeleton of the carotenoid molecule, and two halves of this carbon skeletons join together in reverse way to form a whole C40 prototype carotenoid, which looks central symmetry. By structural modification, such as hydrogenation, dehydrogenation, cyclization, hydration, methylation etc. on half or two halves of the prototype, a huge number of different carotenoids can be synthesised. In reality, each structural modification is controlled by an individual enzyme, which is encoded by a gene in the bacterium cell. In *Rba. sphaeroids*, for example, the carotenoid genes (*crt*) are clustered within a 9.1 kb region of the chromosome and mapped to a region with the 45.7 kb photosynthetic gene cluster by transposon mutagenesis (Coomber et al. 1990).

Similar to BChl molecules, all carotenoid molecules possess a conjugated structure. The length of conjugated double bonds and various function groups attached decide the colour of individual carotenoid molecule. Generally speaking, the greater of the number of conjugated double bond, the further red-shift of maximum absorption wavelength (λmax) (Khoo et al. 2011). As a result, the colour of carotenoids found in the purple bacteria vary from yellow, orange to deep red due to the different molecular structure they have.

There are more than 50 different carotenoids found in the purple bacteria. Most of their chemical structures are quite distinct from those found in higher plant. They are synthesised in the purple bacteria cell following two different biosynthetic pathways, i.e., spirilloxanthin pathway (normal spirilloxanthin, unusual spirilloxanthin, spheroidene and cartenal pathways) and Okenone pathway (okenone, and R. g.-keto carotenoid pathways) (Takaichi 1999). Usually, almost all carotenoids in a biosynthetic pathway, whatever final product or its precursors, can be detected in purified protein complexes, indicating that all carotenoids have ability to bound light harvesting complexes. The preference binding of carotenoid to the light harvesting complexes, however, exists in nature (Qian et al. 2001). By artificial reconstitution or mutagenesis modification of the carotenoid biosynthesis pathway, selected carotenoid can be inserted into light harvesting complexes (Akahane et al. 2004; Chi et al. 2015). The carotenoids listed in Fig. 2.4 are some commonly found in the purple photosynthetic bacteria.

2.4.2 LH1 Subunit, α/β-Polypeptide Pair

Bacterial light harvesting 1 complex is consisted of two polypeptides called α and β, which forms a heterodimer. The heterodimer, together with associated carotenoids and BChls consists a subunit of the LH1 complexes called as α/β-polypeptide pair or even shortly as α/β pair. Early researches on diverse purple bacteria revealed abundant structural information on the α and β polypeptides (Brunisholz and Zuber 1992; Zuber 1985; Zuber and Cogdell 1995). (1) They have relative small molecular weight, ~5.5 kDa, corresponding 40–70 amino acid residues. (2) All of them possess a central hydrophobic core that is long enough to cross the photosynthetic membrane once as a single transmembrane α-helix, with both polar N- and C-termini. (3) A histidine residue is reserved, which was proposed as a ligand to the central Mg atom of the BChl. Amino acid sequences of α-, β-polypeptides from seven different purple bacteria are aligned against the His residue. This is shown in Fig. 2.4.

Reversible dissociation of LH1 complex to its subunit, α/β pair, which has a maximum absorbance at ~820 nm (B820) revealed more structural details of the subunit. To dissociate LH1 ring to its subunit, the carotenoid involved in the LH1 ring need to be removed first by either extraction or blocking its biosynthetic pathway genetically because the stabilization of the LH1 from carotenoids is so strong that no any suitable detergent can dissociate it (Loach and Parkes-Loach 1995; Miller et al. 1987). By the use of detergent such as n-octyl β-D-glucopyranoside (β-OG), and careful control dissociation conditions, LH1 ring subunit, B820, can be obtained from a variety of species (Chang et al. 1990b; Heller and Loach 1990; Jirsakova and Reiss-Husson 1993; Kerfeld et al. 1994; Meckenstock et al. 1992; Miller et al. 1987; Parkes-Loach et al. 1994). The B820 has shown that it has a composition of $\alpha_1\beta_1BChl_2$ (Chang et al. 1990a; Loach and Parkes-Loach 1995; Miller et al. 1987). If carotenoid molecules were added back, the B820 subunit can be re-associated to LH1 ring (Davis et al. 1995; Fiedor and Scheer 2005; Karrasch et al. 1995). Further reversible dissociation of B820 subunit to fundamental components and association back to B820 using a series of BChl analogues revealed that C3 acetyl and $C13^2$ carbonyl groups are required to form subunit and LH1 complexes (Davis et al. 1996; Parkes-Loach et al. 1990). Site-directed mutagenesis in the LH1 complex of *Rba. sphaeroides* showed that C3 acetyl group of BChl *a* interact with Trp

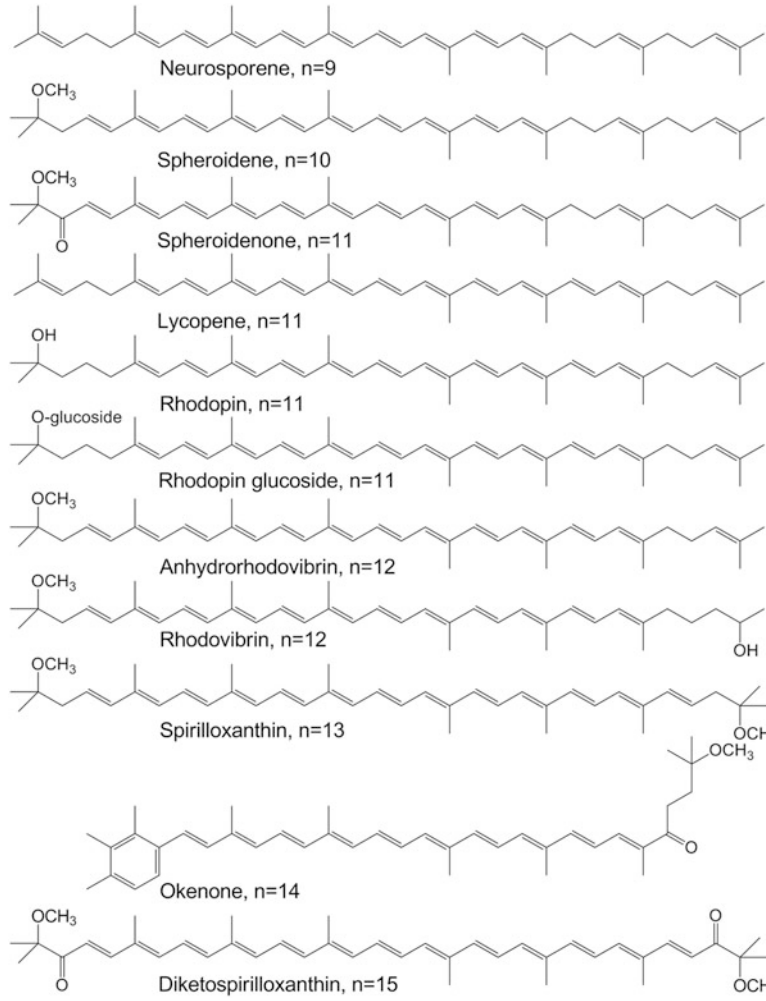

Fig. 2.4 Selected carotenoids found in the purple photosynthetic bacteria. The number of *n* refers to the number of conjugated double band

residues, one with αTrp + 11 and another with βTrp + 9 (Davis et al. 1997; Kehoe et al. 1998; Olsen et al. 1994; Sturgis et al. 1997). The role of His0 acting as an electron donor to coordinate central Mg atom of the BChl *a* was proved by mutagenesis work. Mutation to other amino acid residues resulted in no or very low level of LH1 expression (Olsen et al. 1997).

According to mutation experimental data on *Rba. sphaeroides* LH1 complex, a cross-hydrogen bonding via His0 residue was proposed as well (Olsen et al. 1997). In addition to providing ligand to BChl *a*, His0 residue in one polypeptide may also form a hydrogen bond with the $C13^1$ keto group of BChl *a* that coordinates with the His0 in other polypeptide, to stabilize B820 and LH1 complex (Olsen et al. 1997). NMR determination of BChl *a* in the B820 subunit of *Rsp. rubrum* revealed the relative position of two BChl *a* molecules (Wang et al. 2002). They form a dimer with overlap of ring C and ring E, which is very similar to the B850 BChl *a* in the LH2 crystal structures of *Phs. molischianum* (Koepke et al. 1996) *and Rps. acidophila* (McDermott et al. 1995). All experimental data from reversible dissociation, site-direct mutagenesis, NMR, spectroscopy, electron microscopy etc. on the LH1 subunit build up a clear framework of the LH1 complex before its high resolution 3D structure available.

2.4.3 Reaction Centre

As described above, RC is the place where a light-induce charge separation across membrane takes place. The basic components in a simplest RC include three polypeptide chains associated with pigment molecules. A high resolution 3D structure of the RC from *Blc. virids* became available in 1985 as the first membrane protein structure solved by X-ray crystallography (Deisenhofer et al. 1985; Deisenhofer and Michel 1989). Except for three polypeptides called H (heavy), M (medium) and L (light) based on their apparent molecular weight as determined by electrophoresis, the RC from *Blc. virids* contains a subunit C (cytochrome). L and M subunits both have five membrane-spanning helices, forming a core of the complex by associating four BChl *b*, two Bphe *b*, one non-heam iron, two quinones and one 15-*cis*-carotenoid. The segment polypeptides connecting transmembrane helices form a flat surface parallel to membrane surface. H subunit has two distinctive parts: a single membrane spanning helix on N-terminal side, and a globular domain on C-terminal, which attaches M and L subunit on cytoplasmic side. On the opposite side, M and L subunit are attached by the cytochrome. The total height from tip of the cytochrome to tip of the H subunit is ~130 Å. Importantly, it has an elliptical cross section with long and short axis of ~70 and ~30 Å respectively. Later, we will see that it is this ellipse that decides the overall shape of the RC-LH1 complexes. Shortly after the RC of *Blc. virids*, 3D structure of the RC from *Rba. sphaeroides* were published (Allen and Holmes 1986; Chang et al. 1986). Overall, two structures have been shown to be very similar each other except that there is no cytochrome attached in the RC of *Rba. sphaeroides* and it associates BChl *a* molecules instead of BChl *b*. The third 3D high resolution RC from a purple sulphur bacteria *Thc. tepidum* showed a similar architecture compared with the RC from *Blc. virids*. It has a C subunit, but use BChl *a* and BPhe *a* as its major pigments (Nogi et al. 2000). These structures showing in Fig. 2.5 help people to understand how photosynthetic bacteria convert light energy to proton motive force by light powered charge separation, redox reactions of quinone/quinol and cytochrome movement etc.

2.4.4 PufX Protein

With basic building blocks, i.e., B820, RC and pigment molecules, the core complex RC-LH1

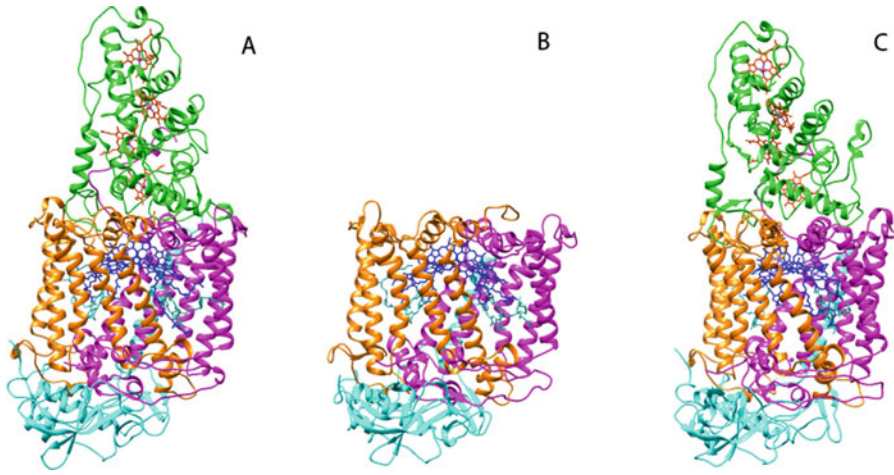

Fig. 2.5 3D structural models of RC from purple photosynthetic bacteria. (**a**) *Blc. Virids*; (**b**) *Rba. sphaeroides*; (**c**) *Tch. tepidum*. Subunits are coloured as follows: RC–H in *cyan*, RC–M in *magenta*, RC–L in *orange*, RC–C in *green*, hem in *red* and BChl in *blue*. Orientations of (**b**) and (**c**) are aligned against (**a**) with cytoplasmic side on *bottom* and periplasmic side on *top*

can be reconstituted (Bustamante and Loach 1994), although the assembly mechanism of the RC-LH1 core complexes in vivo and in vitro could be different from each other not only on the assembly procedure (Pugh et al. 1998) but also on different building blocks. In *Rba. sphaeroides*, for example, an extra polypeptide called PufX was found in its core complex, which was shown to be important for the photosynthetic growth of the cell and molecular architecture of the core complex(Holden-Dye et al. 2008).

PufX is a small transmembrane polypeptide encoded by *PufX* gene that is located at the position downstream of the *pufM* gene, and was first found in *Rba. capsulatus* and *Rba. sphaeroides* (DeHoff et al. 1988; Youvan et al. 1984a, b; Zhu et al. 1986). Later, this gene was known in other *Rhodobacter* genus, including species of *blusticus*, *veldkampi* and *azotoformans* (Tsukatani et al. 2004). The *pufX* encodes a polypeptide that consists of 78 and 82 amino acids in *Rba. sphaeroides* and *Rba. capsulatus* respectively (Lee et al. 1989). The analysis of the isolated mature PufX polypeptides has shown that they are processed at C- and N-termini. Met on N-termini encoded by *pufX* gene are not presented in both species, 12 and 8 amino acids on C-termini were removed as well respectively (Parkes-Loach et al. 2001). Intriguingly, amino acid alignment of PufX polypeptides from both species showed a very low degree of identity being between 23 and 29% depending on the alignment details (Fulcher et al. 1998; Lilburn et al. 1992). This little homology is also shown in all other *Rhodobacter* species (Tsukatani et al. 2004), implying that some polypeptides could be pufX-like proteins that have low identity compared with pufX but act as it. The W protein found in the core complex of *Rps. palustris* is a good example (Roszak et al. 2003).

The *pufX* gene was shown to be essential for photosynthetic growth in *Rba. sphaeroides* because *pufX* gene deleted mutants failed to grow photosynthetically (Barz et al. 1995a, b; Farchaus and Oesterhelt 1989; Lilburn and Beatty 1992; Lilburn et al. 1992). By deleting LH1 gene or reducing size of LH1, however, photosynthetic growth can be restored (McGlynn et al. 1994, 1996). It was suggested, therefore, that instead of directly facilitating cyclic electron transfer between RC and the cytochrome *bc*1 complex, pufX protein provides a gate to allow the qunione/quinol to pass through the LH1 helixes (Cogdell et al. 1996; Parkes-Loach et al. 2001). It was demonstrated that the pufX protein is co-purified with RC-LH1 core complex, leading to a conclusion that the pufX is a component of the core complex(Recchia et al. 1998), with a 1:1 stoichiometry of pufX/RC in the core complex(Francia et al. 1999). Two independent determined solution structures of the PufX from *Rba. sphaeroides* by NMR spectroscopy confirmed that the PufX has a single transmembrane α-helix of 34 amino acid residues, which is much longer than typical transmembrane α-helix (Tunnicliffe et al. 2006; Wang et al. 2007). However, the conformation of the α-helix they provided is different. One is approximately straight (Wang et al. 2007), resulting in protruding of the α-helix out of membrane a couple of turns at both the C- and N-terminal end. The other is bended (Tunnicliffe et al. 2006), permitting the α-helix to accommodate in membrane. This conformation difference could reflect the flexibility of this α-helix hinged by Gly residue and imply structural function of the PufX.

It is clear now that PufX affects the assembly of the core complexes. Electron microscopy image analysis on negatively stained tubular membrane isolated from a mutant *Rba. sphaeroide* lacking of LH2 antenna showed a dimeric structure of the core complex. Two RCs, each of which is associated by an incomplete C-shaped LH1 ring join together to form an S-shaped dimer (Jungas et al. 1999). Deletion of *pufX* gene results in the formation of monomer RC-LH1 core complex, in which the RC is enclosed by a complete LH1 ring (Siebert et al. 2004). In the later section, we will see more detailed structural description of this core complex.

2.5 Structural Diversity of the RC-LH1 Complexes

The light harvesting core complex from photosynthetic bacteria was first visualized in *Blc. virids* membrane by the use of electron microscopy (Miller 1979, 1982). This BChl *b*-containing core complex is shown rather regular arrangement in membrane due to the lack of LH2 complexes. Image synthesis using Furrier Transform on this well ordered membrane revealed its subunit structural details of the core complex. Central density protruded on both cytoplasmic and periplasmic sides was attributed to RC and the density surrounding RC, with a diameter of 110 Å, was interpreted as LH1. Similar characteristic feature was observed in the core complex from *Phs. molischianum* (Boonstra et al. 1994) and *Rhodobium (Rbi.) marinum* (Meckenstock et al. 1992) using electron microscopy. However, the resolution of ~20–30 Å provided by negatively stained EM was not high enough to resolve subunits in the LH1 ring. The precise number of subunit in the LH1 ring was not known unambiguously until in 1995. Karrasch and co-workers reconstituted a LH1 complex of *Rsp. rubrum* from its building block, B820. 2D crystal of the reconstituted LH1 was applied for electron crystallography using cryo-EM. Image processing resulted in an 8.5 Å resolution projection map, which is good enough to show individual electron density caused by single transmembrane helix. Sixteen B820 subunits or α/β-apoprotein pairs form a closed LH1 ring with a hole in the middle which can just hold a RC (Karrasch et al. 1995). An 8.5 Å cryo-EM projection structure of the core complex purified from wild type *Rsp. rubrum* showed a same number of α/β subunit of the LH1 ring surrounding one RC in middle (Jamieson et al. 2002). Two different shapes of the LH1 ring, circle and ellipse, reflect the fact that the LH1 ring is quite flexible (Jamieson et al. 2002; Qian et al. 2003). The model of RC-LH1 being a RC enclosed by a closed LH1 ring also was observed by atomic force microscopy in 2D crystals or photosynthetic membranes (Scheuring and Sturgis 2009). Recent published 3.0 Å resolution X-ray crystal structure of the RC-LH1 core complex from *Tcl. tepidum* confirmed the model unambiguously, i.e., an elliptical LH1 ring consisting of 16 α/β subunits encloses one RC in the middle to form a monomeric RC-LH1 core complex.

An exceptional monomeric RC-LH1 complex was found in *Rps. palustis*. Its 4.8 Å resolution X-ray crystal structure showed that the existence of an extra small polypeptide called W causes an incomplete LH1 ring which only consists of 15 α/β subunits (Roszak et al. 2003). Their structural details will be described in later sections.

As mentioned above, the PufX protein affects the assembly of the core complexes: the PufX protein hinders the completion of the LH1 ring and facilitates the dimerization of the core complex. During the past decade, there have been controversial interpretations on the structure of the dimeric core complex RC-LH1-PufX (Bullough et al. 2008; Cogdell et al. 2006; Holden-Dye et al. 2008). Initially, two halves of dimeric core complex found in tubular membrane of *Rba. sphaeroides* was proposed being associated by a cytochrome *bc1* complex (Jungas et al. 1999). However, cytochrome *bc*1 complex cannot be detected in purified tubular membrane, ruling out the existence of the cytochrome *bc*1 complex in the dimeric core complex(Siebert et al. 2004). By measuring the length of LH1 arc on its projection map from 2D crystal of the dimer core complex, 24 α/β subunits of the LH1 from *Rba. sphaeroides* was estimated, and a density in the centre of the dimer was attributed to a pair of pufX based on 26 Å resolution projection map (Scheuring et al. 2004a). An 8.5 Å projection map of the same complex, however, showed 28 density pairs which were assigned as α/β subunits. An extra density between RC and LH1 and near the LH1 gap was attributed to PufX (Qian et al. 2005). 28.4 ± 2.2 BChl *a* molecules per RC obtained from quantitative pigment analysis of purified dimeric core complex supported the assignment of 14 α/β subunits per RC. Furthermore, the orientation of the RCs in the dimeric core complex was determined by

comparing its projection map with the simulated RC projection map at 8.5 Å derived from 3D X-ray structure of the RC from *Rba. sphaeroides*. This produced an orientation of the planes of the special pair BChl *a* porphyrin rings relative to the long axis of the dimer is ~17.5°, a good agreement with the orientation determined by LD measurements (Frese et al. 2000). Based on AFM topographs, a model of the dimeric pufX containing core complex from *Rba. blasticus* was presented (Scheuring et al. 2005). The model contains 26 α/β pairs, a dimer pufX in centre of the core, and an orientation of special pair relative to long axis is ~ − 40.0°. PufX protein was also identified in an alkaliphilic non-sulphur purple bacterium *Rhodobaca* (*Rca.*) *bogoriensis* (Milford et al. 2000), a sole example that PufX is found out of *Rhodobacter* genus. A 13 Å projection map of dimeric core was interpreted in a different way—two C-shaped LH1 ring, which compose of 13 α/β pairs and two PufXs, surround the RCs. They form a dimer through an interface of two LH1 subunits (Semchonok et al. 2012).

Obviously, 3D structures of the core complex, with resolution high enough to resolve transmembrane helix at least, are absolutely necessary for an unambiguously interoperation. By the time of this writing, only three X-ray crystal structures of the core complex from the purple bacteria are available in protein data bank (PDB). Their structural features will be described in the next section in the order of their publication time.

2.5.1 X-Ray Crystal Structure of the Core Complex from *Rps. palustris*

The crystal structure of the core complex from the purple bacterium *Rps. palustris* was determined at 4.8 Å resolution by X-ray crystallography. The phase was obtained by molecular replacement with the RC of *Rba. sphaeroides* (McAuley et al. 1999). The model of this RC-LH1 core complex is shown in Fig. 2.6. An elliptical LH1 ring has a dimension of ~110 Å by ~95 Å measured as centre to centre distance of opposite β-helices. The inner α-apoprotein elliptical ring with a long axis of ~78 Å matches with overall shape of the RC very well, so that the LH1 subunits appears to be wrapped around the RC. These features are similar with that seen in EM projection maps and AFM topographs of the RC-LH1 core complexes (Jamieson et al. 2002; Scheuring et al. 2006, 2004b). However, the LH1 ring is interrupted by a transmenbrane helix 'W', which is located between α polypeptides, resulting in a gap in the LH1 ring. This W protein, having a molecular weight of ~11.0 kDa, has been suggested as an analogous protein to the PufX although very little is known about it, even including its amino acid sequence. Nevertheless, the unique orientation of the W protein respect to the RC provided us a possible way to understand how ubiquinone (Q_B)/ubiquinol (Q_BH_2) shuttle between RC and cytochrome *bc*1 complexes. Located on the opposite of RC-H single transmembrane helix, and near the gap of LH1 ring, the W protein is suggested as a part of gate through which Q_BH_2 can be released from its binding side to membrane lipid phase outside of the RC. It is also likely that W plays a key role for assembly of the core complex. It should be emphasized that at 4.8 Å resolution, it is impossible to resolve individual amino acid and pigment molecules in the core complex. To fully understand structure and function of this core complex, a higher resolution 3D structural data and more biochemical measurements are absolutely necessary.

2.5.2 X-Ray Crystal Structure of the Core Complex from *Rba. sphaeroides*

It is clear that the core complex in *Rba. sphaeroides* takes dimeric form, i.e., two monomeric core complexes, each of which consists of one RC surrounded by an incomplete C-shaped LH1, are associated together to form a bended S-shaped dimer (Ashby et al. 1987; Bahatyrova et al. 2004; Jungas et al. 1999; Qian et al. 2008, 2005; Scheuring et al. 2004a; Siebert et al. 2004;

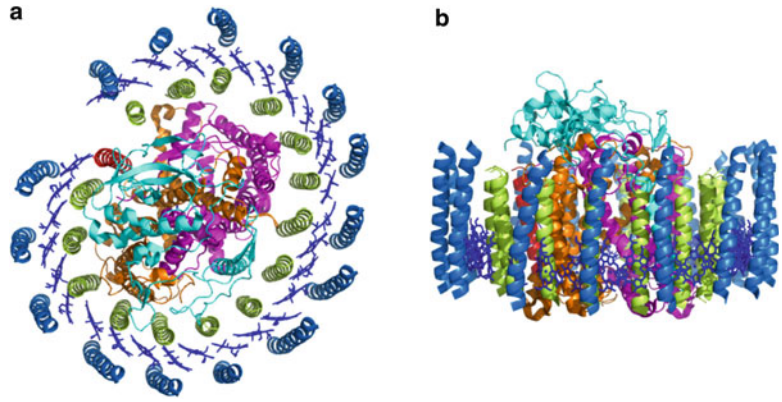

Fig. 2.6 3D structure of the RC-LH1 core complex from *Rps. palustris* at 4.8 Å resolution (**a**) *Top view* from cytoplasmic side, β-apoprotein in *blue*, α-apoprotein in *green*, RC-H in *cyan*, RC-M in *magenta*, RC-L in *orange* and W in *red*. A pair of BChl *a* (in *blue* stick) without phytal tail was modelled into the complex based on the density between α- and β-helices. For the purpose of clarity, pigment molecules in the RC are not shown (**b**) *Side view* by rotating (**a**) 90° so that cytoplasmic side is on *top*. Only transmembrane part of the polypeptides of the LH1 is modelled using ploy alanine

Westerhuis et al. 2002). However, controversies are never stopped during the last decade on the issues, such as stoichiometry of the pufX, size of LH1 ring, orientation of RC, conformation of LH1 polypeptides, and location of the pufX. An 8.0 Å X-ray crystal structure of the core complex from *Rba. sphaeroides*, which clearly shows the interaction among individual polypeptides provides us clearer insight into the structural-function relationship of the complex (Qian et al. 2013).

Overall Structure Having a molecular weight of ~521 kDa, the dimeric core complex of *Rba. sphaeroides* is composed of 64 polypeptides. Each half of the dimer consists of a PufX and a RC surrounded by 14 LH1 α/β subunits, with two BChl *a* molecules sandwiched between α- and β-transmembrane helices. Two halves of the complex are associated together through the PufX protein, which intimately interacts with RC-H subunit in one half monomer and 14th β-helix of the other half monomer, forming a S-shaped LH1 ring if viewed from periplasmic side. Two halves of monomer inclines each other ~11 degrees against periplasmic side, forming a V-shaped molecule if viewed parallel to the membrane surface. Its 3D view is showed in Fig. 2.7.

Polypeptide Interactions Involved the PufX It has long been suggested that the PufX plays a key role for dimerization of the RC-LH1-PufX core complex. The 3D X-ray crystal model of the core complex shows three important interactions that involve the PufX protein (see Fig. 2.8).

The 3D model of the dimeric complex shows that orientation of LH1 α/β subunits, among α2/β2 to α14/β14 are quite similar, being nearly straight transmembrane part, but α1/β1 is significant different from the others. Figure 2.8a shows that PufX contacts with the N-termini of LH1 α1/β1 on the cytoplasmic side of the membrane forming a α1/β1/pufX cluster. In this cluster, the bended confirmation of the PufX polypeptide chain can be superimposed onto its minimized averaged solution structure determined by NMR (Tunnicliffe et al. 2006) with an RMS deviation of 2.08 Å. Distinct bended confirmation of β1 polypeptide was observed in the cluster as well, reflecting a flexibility of the β polypeptide, which could possess different confirmations depending on different environments. The PufX polypeptide also intimately contacts with extrinsic domain of the RC-H subunit, with PufX N-terminal residues ~Asn 8 and RC-H C-terminal residues ~244–247 (see Fig. 2.8b). It is likely that this interaction forms a start point of the LH1 complex, with

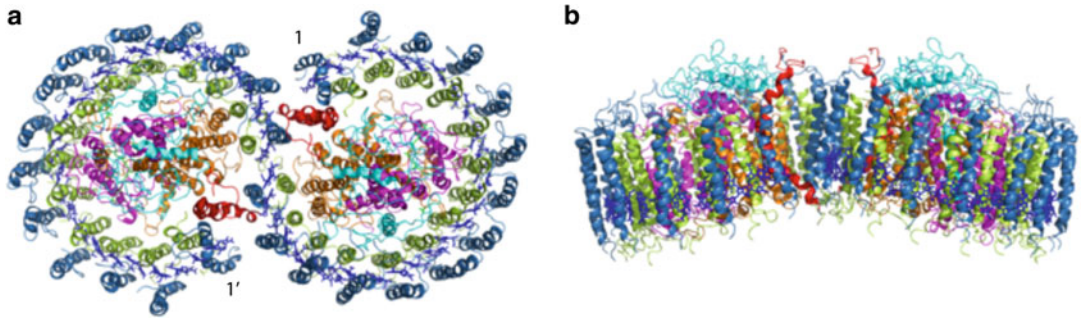

Fig. 2.7 The structure of dimeric core complex from *Rba. sphaeroides* (**a**) *Top view* from cytoplasmic side. PufX protein is coloured in *red*. For clarity, only α1/β1 and α1′/β1′ pairs are labelled (**b**) *Side view* parallel to the membrane surface with cytoplasmic side on *top*

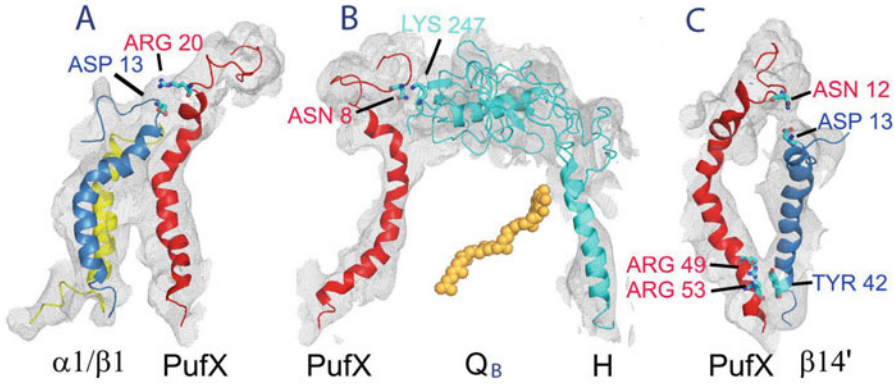

Fig. 2.8 PufX interactions that stabilize the dimer core complex (**a**) *Side view* of the interaction of the pufX with the α1/β1 pair on the cytoplasmic side. PufX in *red*, β1 in *blue* and α1 in *yellow* (**b**) Interaction of pufX with RC-H on the cytoplasmic side of the membrane. RC-H in *cyan*, ubiquinone in *brown* (**c**) Interactions of the pufX on one half of the dimer with β14′ on the other half of the dimer

which the rest of LH1 subunits can follow. Given relative bigger volume of this cluster compared with normal α/β pair, this is also likely a point that stops encirclement of the LH1 ring. The third interaction comes from PufX and β14′ polypeptide both on the N- and C-termini extrinsic regions (see Fig. 2.8c). It was suggested that these interactions are crucial for dimerization of the core complex. The model predicts that Asn12 of the PufX on the one half of the dimer is close to Asp13 of LH1 β14′ on the other half. This explains why the truncation of 12 or more amino acids from N-terminus of the PufX stops dimerization of the core complex (Francia et al. 2002; Ratcliffe et al. 2011). C-terminal truncation of the PufX also affects dimerization, suggesting this part is involved protein-protein interactions. The model provides such evidence showing that PufX C-terminal from residues 49 contacts with β14′ C-terminal residues from 38. Unfortunately, 8.0 Å resolution structural data cannot provide precise contact information. However, alternation of PufX Arg49 and Arg53 to Leu or even only PufX Gly52 to Leu abolishes the formation of the dimer, indicating that all alternation of C-terminal confirmation of the

PufX will likely change its interaction with β14′, and affect dimerization of the core complex (Ng 2008).

Quinone Channel To allow the turnover of RC photochemistry, a shuttle of quinol/quinone between RC Q_B binding side and quinone pool out of the complex through LH1 barrier is needed. A continuous 3D volume enclosed by the LH1 ring inside of the complex was found. This volume, as a putative quinone channel, has two gates. One at LH1 ring gap, allowing Q/QH_2 to be in and out of the complex directly, and the other at the interface of the dimer, allowing Q/QH_2 migrate between two halves of monomer possibly. By the use of this channel, it is possible for the migration of Q/QH_2 within spherical membrane or even through long tubular membrane that is densely packed with dimeric core complexes. A 3D model of the quinone channel can be viewed on youtube website (https://www.youtube.com/watch?v=vsaYyjfNGmI)

It should bear in mind that only with massive different experimental data support the 3D X-ray structure of the core complex of *Rba. sphaeroides* at 8.0 Å resolution can be solved. These data include electron microscopy (Qian et al. 2005, 2008), NMR (Conroy et al. 2000; Ratcliffe et al. 2011; Tunnicliffe et al. 2006), available high resolution structures of RC (Ermler et al. 1994) and LH2(Koepke et al. 1996; McDermott et al. 1995), and site-direct mutagenesis (Olsen et al. 1994, 1997; Sturgis et al. 1997). At this resolution, individual amino acid, carotenoid and phytyl tails of BChl *a* cannot be resolved from its electron density map. Therefore, we still need to wait for a higher resolution data for the precise description of the structure-function relationship of the complex.

2.5.3 X-Ray Crystal Structure of Core Complex from *Tch. tepidum*

Thermochromatium tepidum is a thermophilic purple sulphur photosynthetic bacterium found originally from a hot spring in Yellowstone National Park. Growing anaerobically at optimum temperature between 48 and 58 C, its BChl *a*–containing RC-LH1 core complex presents a unique optical property, a maximum absorbance at 915 nm (B915). The 3D X-ray crystal structure of the core complex from *Tch. tepidum* was solved to 3.0 Å. This is the first core complex structure showing at near-atomic resolution level so far. It provides us more details to understand its molecular mechanism involved in primary photosynthetic reactions.

Overall Structure The core complex of *Tch. tepidum* is composed of a LH1, a RC, a cytochrome and 80 cofactors with a molecular weight ~ 380 kDa. Sixteen LH1 α/β subunits form a double complete elliptical LH1 ring surrounding the RC which has four subunits L, H, M and C, a cytochrome attached on periplasmic side of the RC. The major and minor axis length of outer elliptical LH1 ring is 105 Å and 96 Å, and 82 Å and 73 Å for inner LH1 elliptical ring were measured. Each α/β heterodimer subunit associates two BChl *a* molecules and one carotenoid molecule, spirilloxanthin. No PufX-like protein has been found in the core complex of *Tch. tepidum*. Figure 2.9 shows its 3D structure.

Protein-Protein Interactions in the LH1 The structure of the LH1 subunit α/β pair is very similar to those in LH2 complexes, especially to that in *Phs. molischianum* LH2(Koepke et al. 1996). Each α/β sandwiches two BChl *a* molecules and one spirilloxanthin. In C-terminal region, a Ca^{2+} ion was identified. Figure 2.10 shows their relative position in LH1 ring. Central Mg atom of the β-B915 is coordinated by β-His 36; its C3-acetyl group forms a hydrogen bond with β-Trp 45; its carbonyl oxygen of the phytyl ester group is hydrogen bonded to α-Gln 28 and β-Trp 28. Similarly, α-His 36 coordinates the central Mg atom of α-B915; its C3-acetyl group forms a H-bond with α-Trp 46. No H-bonds were found on C13-keto groups of the B915. All of these intra-subunit interactions not only fix BChl *a* molecules onto

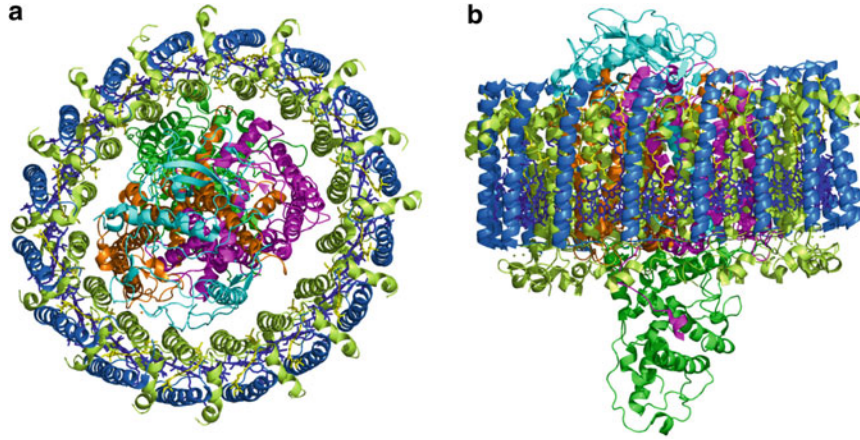

Fig. 2.9 The structure of RC-LH1 core complex of *Tch. tepidum* (**a**) *Top view* from cytoplasmic side. Colour codes are same as in Fig. 2.7, except for cytochrome in *green* (**b**) *Side view* parallel to the membrane surface with cytoplasmic side on *top*

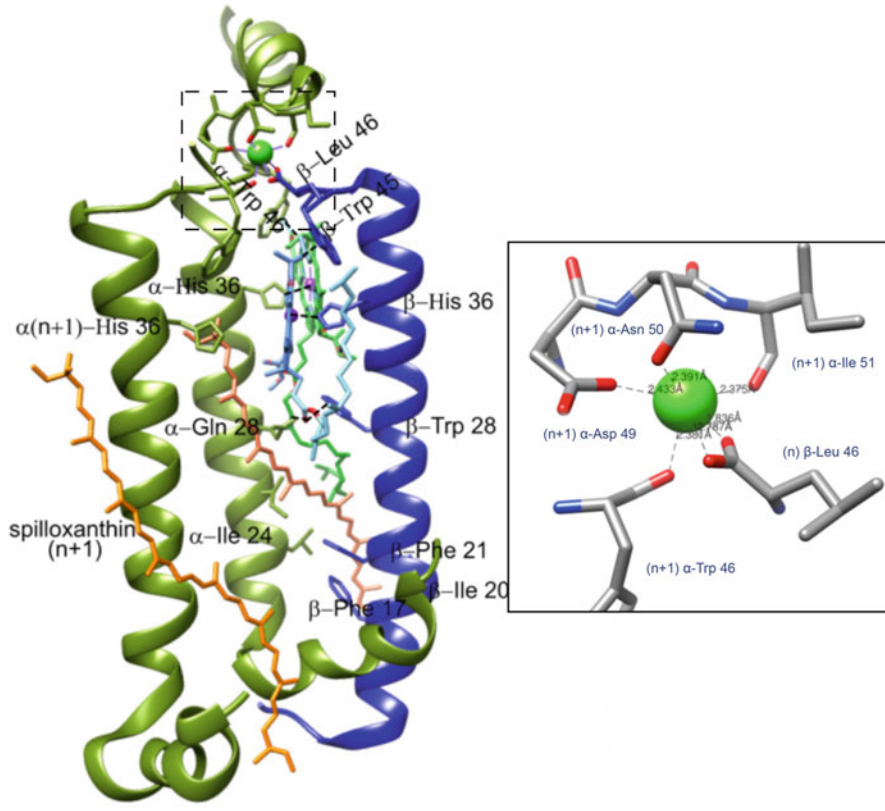

Fig. 2.10 Intra- and inter-subunit interactions in the LH1 ring. α-polypeptides in *olive green*, β-polypeptide in *blue*, spirilloxanthin in *brown*, β-B915 in *cyan*, α-B915 in *green*, Mg^{2+} in *purple* and Ca^{2+} in *bright green*. Insert is an enlargement of the area covered by a *dashed square*

the scaffold of α/β-polypeptide in a correct orientation but also keep α/β-polypeptides together to stabilizes LH1 subunits.

The structure also reveals two important inter-subunit interactions that strengthen stability of the LH1 ring further. Sixteen Ca^{2+} binding sites were identified in the C-terminal regions of α/β-apoproteins. Six coordinated bonds can assign to a Ca^{2+}, four of them from C-terminal of α-polypeptide (α_{n+1}-Trp46, α_{n+1}-Asp 49, α_{n+1}-Asn 50, α_{n+1}-Ile 51) and two others from its neighbouring β-polypeptide Leu 46's carboxyl group, forming an inter-subunit linker within the LH1 ring. It is suggested that these linkages give the RC-LH1 of *Tch. tepidum* an extra thermo-stability and cause BChl *a* Qy red--shift. It should note that under current resolution the number of coordinated bond to each Ca^{2+} ion and corresponding bond lengths vary slightly within the LH1 ring. A recent 1.9 Å resolution structure of the core complex provides more details of the coordinations (unpublished personal communication).

All-*trans* spirilloxanthin curves through the α/β pair at approximately 30° to the membrane normal. In addition to intimate interactions with α/β polypeptides and BChl *a* tails, one of the methoxy groups of the spirilloxthanin is in close proximity to the upstream neighbouring (n + 1) α-His 36, and the other methoxy group to the downstream neighbouring N-termini of (n−1) α/β-polypeptides, forming another inter-subunit linker. It has long been known that carotenoid has a function to stabilize light harvesting complexes. This structure for the first time shows such molecular mechanism clearly.

Putative channels for Q/QH_2 shuttling between RC and cytochrome *bc*1 complex are located on the cytoplasmic side of the transmembrane region between adjacent α/β pairs. Given the intrinsic flexibility of the LH1 ring the Q/QH_2 channel with an averaged dimension that approximately equals to the head of ubiquinone could let Q/QH_2 pass through the apparent LH1 barrier. The structure confirms the conclusions from previous electron microscopic study and molecular dynamic simulation that proposed a quinone diffusion mode through LH1 ring by LH1 'breathing' movement (Aird et al. 2007; Jamieson et al. 2002).

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