REVIEW

STUDY ON THE STRUCTURAL BASIS OF PERIPHERAL LIGHT HARVESTING COMPLEXES (LH2) IN PURPLE NON-SULPHUR PHOTOSYNTHETIC BACTERIA

Tatas H.P. Brotosudarmo[1,2,]*, and Richard J. Cogdell[1]

[1]*Division of Biochemistry & Cell Biology, University of Glasgow*
Biomedical Research Building, 126 University Place, G12 8TA, United Kingdom

[b] *Ma Chung Research Center for Photosynthetic Pigments, Ma Chung University*
Jl. Villa Puncak Tidar N/01, Malang 65151, Indonesia

Received May 20, 2010; Accepted August 26, 2010

ABSTRACT

Photosynthesis provides an example of a natural process that has been optimized during evolution to harness solar energy efficiently and safely, and finally to use it to produce a carbon-based fuel. Initially, solar energy is captured by the light harvesting pigment-protein complexes. In purple bacteria these antenna complexes are constructed on a rather simple modular basis. Light absorbed by these antenna complexes is funnelled downhill to reaction centres, where light drives a trans-membrane redox reaction. The light harvesting proteins not only provide the scaffolding that correctly positions the bacteriochlorophyll a and carotenoid pigments for optimal energy transfer but also creates an environment that can modulate the wavelength at which different bacteriochlorophyll molecules absorb light thereby creating the energy funnel. How these proteins can modulate the absorption spectra of the bacteriochlorophylls will be discussed in this review.

Keywords: *photosynthesis, peripheral light harvesting complex, H-bonding, energy transfer*

INTRODUCTION

In the early processes of photosynthesis, sunlight is absorbed and this excitation energy is then funnelled to the reaction centre (RC), where the primary charge separation takes place (Fig. 1A) [1-2]. The initial absorption of solar energy occurs in light harvesting pigment-protein complexes that surround the reaction centres (Fig. 1) [3-5]. In principle, photosynthesis could have evolved just with RCs. However, this would have meant that except under very high intensity light there would be a relatively long time-gap between two photons reaching the same RC. This would have cause a major problem because several of the redox reactions that take place within the RCs require multiple one-electron turnovers. If the RCs had to wait too long between the arrivals of the consecutive photons then back reactions would become favourable. In this case the whole charge-separation process would become inefficient. One of the important function of a LH system is to increase cross-sectional area for photon capture in order to supply the RCs with sufficient numbers of photons, so that the forward electron-transfer reactions take place frequently enough and the back reactions are reduced to a minimum [2].

In the purple bacteria there are two types of light harvesting (LH) complexes, the peripheral (LH2) and the core (LH1). Both LH complexes contain a pair of small (5-7 kDa) transmembrane polypeptides, called α and β, that oligomerise to form the intact native complexes. The N-termini of these apoproteins are located at the cytoplasmic surface of the photosynthetic membrane and

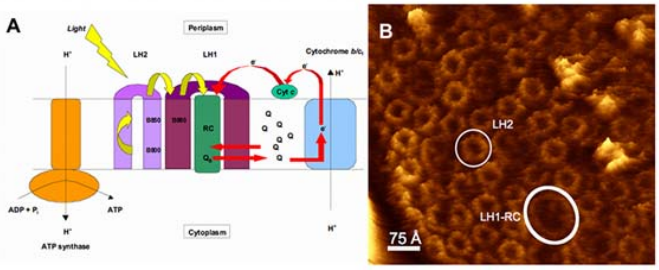

Fig 1. A schematic diagram of the photosynthetic membrane of a typical purple bacterium (A). The major integral membrane protein involved in the light reaction of photosynthesis are displayed. The yellow arrows show energy transfer and the red arrows the redox reactions involved their simple cyclic electron transport pathway. The reaction centre (RC) reduces the secondary electron acceptor (ubiquinone, Q_B) which has to pass through the LH1 complex in order to deliver its reducing equivalents to cyclic electron pathway. (B) An image of the photosynthetic membrane from *Phs. molischianum* taken by atomic force microscopy (AFM) [52] (kindly provided by Dr. Simon Scheuring). The peripheral light harvesting (LH2) and the core (LH1-RC) complex are indicated in circles.

* Corresponding author. Tel/Fax : 0044-141-330 7264/3779
Email address : tatashardo.brotosudarmo@glasgow.gla.ac.uk

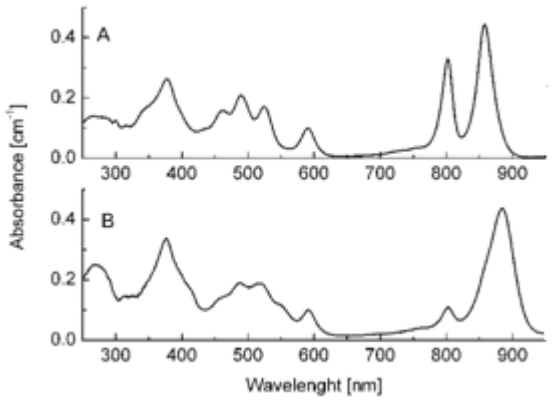

Fig 2. Room temperature absorption spectra of LH2 and LH1 complexes from *Rhodopseudomonas acidophila* 10050.

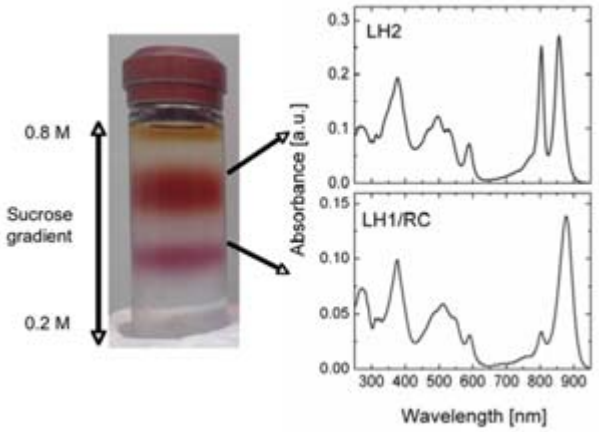

Fig 3. First step of isolation and purification of LH2 and LH1-RC complexes from *Rps. palustris*. The photosynthetic membrane was solubilized by 1% (v/v) LDAO (Fluka Biochemicals). Then the solubilized materials were gently layered onto the top of the sucrose gradients. After high-speed centrifugation (149,000 g at 4 °C) for 16 h the two major LH complexes were separated. The two complexes have different colour, the LH2 is red (middle) and the LH1-RC (core) is pink (bottom). The low-density cellular materials and denatured complex are yellow (top). The absorption spectra of LH2 and LH1-RC (core) complexes were controlled and the complexes were collected from the tubes.

the C-termini at the periplasmic side. These polypeptides non-covalently bind bacteriochlorophyll *a* (Bchl *a*) and carotenoid (Car) pigments, which function to absorb the solar energy.

Fig. 2 shows the absorption spectrum of LH2 and LH1 complexes from *Rhodopseudomonas acidophila* 10050 at room temperature. The Bchl *a* molecules in the LH1 complex have a strong near infrared (NIR) Q_y absorption band at ~880 nm, while the LH2 has two strong Q_y transition band at ~800 and ~850 nm. The position of Q_y transition band of Bchl *a* molecules is very sensitive to its environment [6], such as aggregation, local pigment-protein interaction, central metal ions and axial binding, macrocycle deformation as well as hydrogen binding. Different binding environment of Bchl *a* molecules in these two complexes is functionally important as it creates energy gradient and facilitate the energy transfer from LH2 to LH1 and down to RC. The question of how the binding pigment-protein interaction modulate the absorption spectra of Bchl *a* molecule for optimal energy transfer will be addressed in this review.

GETTING THE STRUCTURE OF LH2

Isolation and purification

A detailed understanding of the function of proteins requires knowledge of their three-dimensional structure. X-ray crystallography has been recognized to be an important tool in resolving detailed structural information of proteins. Working with membrane proteins such as LH2 complexes requires an appropriate detergent in order to maintain the membrane protein in solution. In this case, the photosynthetic membrane has to be solubilized by addition of the detergent (e.g. lauryldimethylamine N-oxide, LDAO) prior to isolation and purification. One of the most joyful things about purifying LH2 antenna complexes is that they have such beautiful array of colours, ranging from brown and pinks to deep reds and purple. Also their spectral integrity can be monitored easily by eye, making the purification procedure much easier that for colourless protein. Ones the cells are broken by mechanical disruption in a French pressure cell, the membrane pellets are re-suspended in 20 mM Tris-HCl, pH 8.0, their concentration is adjusted to an OD_{850} of 50 cm^{-1} and they are solubilized in 1% (v/v) LDAO at room temperature. Any un-solubilized materials can be removed by low-speed centrifugation. The first step of the isolation and purification of LH2 and LH1-RC is described in Fig. 3. To ensure the high purity, the LH2 complexes were loaded onto a DE52 cellulose anion exchanger column. The purity of the LH2 complex can be monitored by measuring the ratio of the absorption maximum at ~850 nm band to that at~280 nm band [7]. The Q_y transition bands of Bchl *a* are at ~800-900 nm, when the Bchl *a*s are bound to their apoprotein. Therefore any reduction in the ratio of A850/A280 indicates either a decreased bound Bchl *a*, from denatured complexes, or contamination of extraneous protein. At this stage, the LH2 complex is ready to be crystallized.

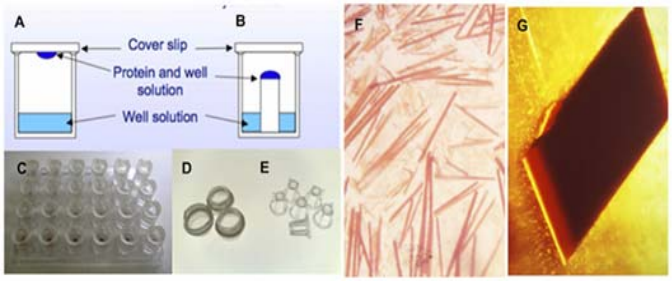

Fig 4. Typical apparatus used in vapour diffusion method for the crystallisation of LH2 complexes. The vapour diffusion method is the most widely used and it is proven to be very successful in crystallizing proteins (see statistics presented by http://www.mpdb.ul.ie/index.asp). There are two common methods in vapour diffusion, hanging drops (A) and sitting drops (B). For hanging drops (A), the protein droplet hangs from the lid (D), whereas for the case of sitting drops (B), the protein droplet sits in the bridge well (E). Both entail a droplet containing purified protein, buffer, and precipitant being allowed to equilibrate with a larger reservoir containing similar buffers and precipitants in higher concentrations. Initially, the droplet of protein solution contains a lower concentration of precipitant than required to induce precipitation. As water vaporises from the drop to the reservoir, the precipitant concentration increases to a level optimal for crystallisation. The vapour diffusion plate from EasyXtal (QIAGEN) (C) offers 24-well plates easier setup for hanging or sitting drop. (E) Bridges are needed for sitting drop. (F) The first crystal of LH2 from *Rps. acidophila* 10050 was successfully grew in 1983. (G) The optimized crystal of LH2 complex of *Rps. acidophila* strain 10050. The crystal was grown from phosphate in the presence of benzamidine hydrochloride and β-octyl-glucoside. It is ~1 mm across in its longest dimension. (Crystal images are courtesy of Prof. R.J. Cogdell FRS).

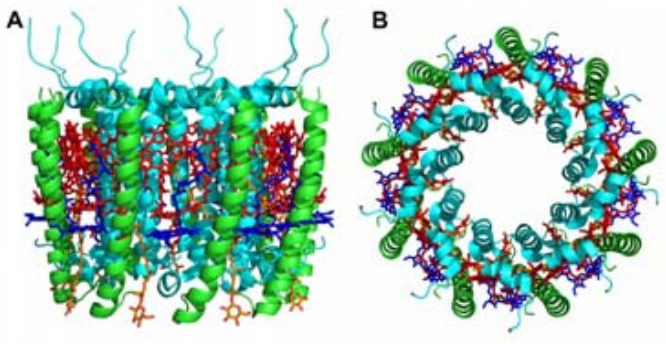

Fig 5. The high resolution (2.0 Å) structure of LH2 complex from *Rps. acidophila* 10050 (PDB: 1NKZ) [16]. Front (A) and side (B) views of the LH2 complex showing nine-membered circular module of α- (cyan) and β- (green) polypeptides with B800 Bchl (blue), B850 Bchl (red) and the carotenoid (orange)

Crystallisation

Generally proteins crystallize when they are gradually induced to precipitate. This is usually achieved by equilibration with precipitant, e.g. ammonium sulphate or polyethylene glycol, which influence the solubility of the protein yet do not denature them. During the process of crystallization of membrane proteins, very often the precipitant reacts with the detergent and causes the detergent to phase separate. When phase separation happens the membrane protein usually denatures in the oily detergent phase and crystals will never be formed. The problem was then overcome when Michel [8-9] and Garavito [10] independently discovered that addition of specific small molecules, e.g. heptane-1,2,3-triol, benzamidine hydrochloride, etc. [11] could alter the phase boundaries. This small amphiphile shifts the phase separation point to be above the critical precipitation point, so that the crystallization becomes possible.

The first success of crystallizing LH2 was achieved by Richard J. Cogdell group in early 1980s [12]. Unfortunately, these crystals looked very beautiful under microscope but diffracted X-rays only to about 12 Å (Fig. 4F). The reason of this poor disorder was likely to be the variation in lipid content [13]. An optimisation on the purification protocols was then improved by washing the LH2 complex in β-octylglucoside, and set up the crystallisation in this detergent. Large crystals grew and diffraction to 3.2 Å can be achieved in the mid-1990s (Fig. 4G).

STRUCTURE OF LH2 AND THE ENERGY TRANSFER

The high-resolution X-ray structure of the LH2 complexes [14-16] from *Rhodopseudomonas (Rps.) acidophila* strain 10050 and low-light adapted strain 7050 show a remarkable symmetry in the arrangement of the pigments embedded in the protein matrix (Fig. 5). These complexes are modular. Each module consists of a protein heterodimer (αβ), which binds three Bchl *a* pigments and one carotenoid molecule. Nine such modules, αβ-polypeptides, are arranged circularly to form the single LH2 complex. The α-polypeptide is located inside the ring and the β-polypeptide is on the outside (Fig. 5). Inside the protein matrix the bacteriochlorin rings of the Bchl *a* molecule are organised in two ways (Fig. 5). Nine monomeric Bchl *a* molecules have their bacteriochlorin rings oriented parallel to the plane of the membrane (blue in Fig. 5), and absorb the light with absorption maximum (λ_{max}) at ~800 nm. They are called B800 Bchl *a*s. These monomeric B800 Bchl *a*s are separated by 2.1 nm from

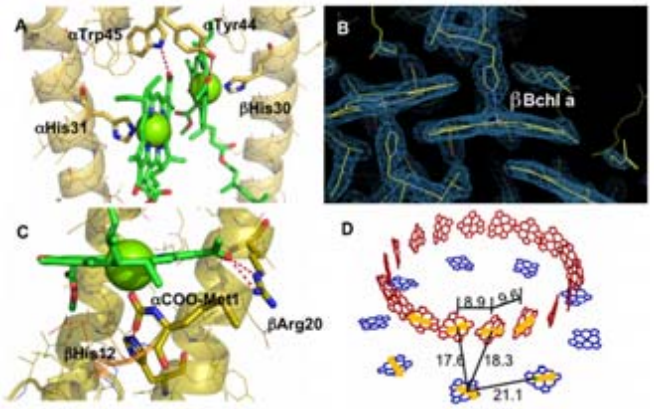

Fig 6. The binding pocket of the B850 Bchl *a*s (A and B) and the B800 Bchl *a* (C). The coordinates for this figure were taken from the high resolution (2.0 Å) structure of the LH2 complex from *Rps. acidophila* 10050 (PDB: 1NKZ) [16]. (B) The electron density of a B850 Bchl *a* molecule shows that the bacteriochlorin ring is rather bent and the histidine residue, which is the fifth coordinated to the central Mg^{2+}atom, is clearly seen. (D) The spatial organisation and distances between the Bchl *a* pigments in LH2. The numbers indicate the size of LH2 and centre-to-centre distances between the macrocyles of the Bchl *a* molecules in Å. The arrows indicate the direction of the Q_y transition moments. The phytol chain is cropped off for clarity.

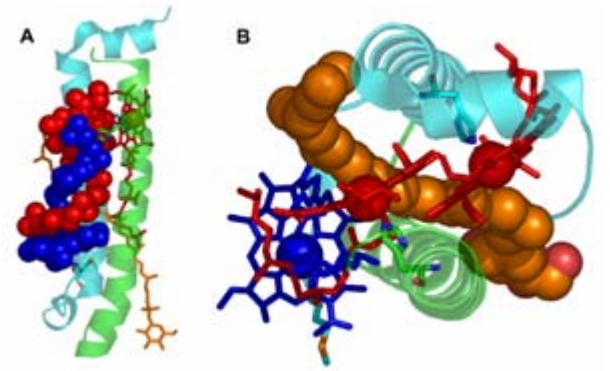

Fig 7. Diagram showing (A) the interaction of the phytol chain of the B800 Bchl *a* (dark spheres) and β-bound Bchl *a* (grey spheres) and (B) the position of all-*trans* carotenoid rhodopin-glucoside in the heterodimeric pair of αβ-polypeptides. The coordinate is taken from high resolution (2.0 Å) structure of LH2 complex from *Rps. acidophila* 10050 [16].

each other (Fig. 6D). A further eighteen Bchl *a* molecules have their bacteriochlorin rings oriented perpendicular to the membrane plane. They are responsible for the absorption band at about 850 nm. The B850 Bchl *a*s (red in Fig. 5 and 6D) sit very close to each other and, when viewed from above, superficially resembles the blades of a turbine. The central Mg^{2+} atoms of these B850 Bchl *a*s are separated 0.95 nm within one αβ pair, and by 0.88 nm from one αβ pair to the next (Fig. 6D). The 2.0 Å resolution structure suggests that there is a single well-defined carotenoid rhodophin–glucoside per αβ pair. In case of *Phaeospirillum (Phs. previously Rhodospirillum) molischianum* it has been found that the LH2 complexes are $\alpha_8\beta_8$ octamers [17]. Until now, it is not clear what structural features cause one to be nonamer and the other octamer, or indeed whether this difference has any significant functional consequences.

Looking at the binding site, the B850 Bchl *a*s have their central Mg^{2+} liganded to the imidazole ring sidechain of histidine residues, α-His31 and β-His30 (Fig. 6A and B) [15-16]. A hydrogen bond is formed between the acetyl group in ring A and the phenol sidechain of α-tyrosine44 for α-B850 Bchl *a*s and the indole sidechain of α-tryptophan45 for β-B850 Bchl *a* molecules (Fig. 6A) [16]. The B800 Bchl *a*s are stabilised by coordination between the Mg^{+2} of the Bchl *a* and COO-α-Methionine1, and a H-bond between the acetyl group of Bchl *a* and the guanidinium group of β-Arginine20 (Fig. 6C) [16]. On a closer inspection, there is an interesting interaction between Bchl *a* molecules of B850 and B800, within an αβ-apoprotein pair, conducted by their hydrophobic phytyl chains. The B800 phytyl chain from B800 folds around the phytyl chain of β-B850 Bchl, crossing it and passes across the macrocycle ring of the β-B850 (Fig. 7A). The B800 phytyl chain interacts *via* van der Waals contact with rings A and D of the β-B850 macrocycle.

The Car found in the *Rps. acidophila* strain *10050* is rhodopin-glucoside. The X-ray structure of LH2 from *Rps. acidophila* also reveals interactions between the Car and the polypeptide as well as between Car with the Bchl *a* molecules (Fig. 7B). In the LH2 complex from *Rps. acidophila* 10050, the rhodopin-glucoside passes in close contact (3.4 Å) to the edge of the bacteriochlorin ring of the B800 Bchls. The polyene chain then runs perpendicular to the macrocycle of the α-B850 Bchl *a* (Fig. 7B). The glucosyl group of the rhodopin-glucoside molecule is located in a hydrophilic binding pocket on the cytosolic side of the transmembrane-spanning protein. This Car has an extremely important structural role in LH2. It holds the αβ-polypeptide pairs together (Fig. 7B). In a mutant that lacks Car the LH2 complexes fail to assemble [18].

The structural arrangement of the pigments in the LH2 brings a significant consequent in the energy transfer and funnelling down of that energy. The energy transfer processes (B800→B800, B800→B850 and B850→B850) in the LH2 complexes have been also well reviewed [19-20]. If the carotenoids in the LH2 from

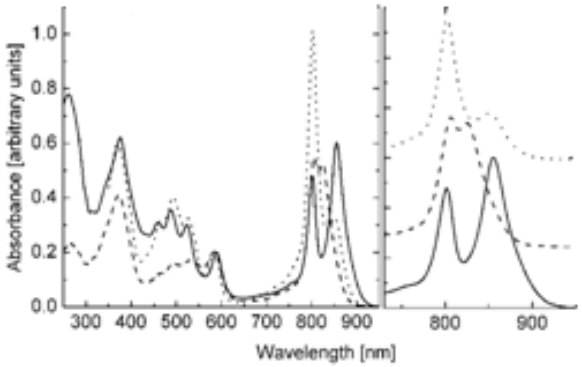

Fig 8. Room temperature (RT) steady state absorption spectra of the B800-850 (black line), the B800-820 (dashed line) LH2 complexes from *Rps. acidophila* 7050 and B800-low-850 (dotted line) LH2 complexes from *Rps. palustris* 2.1.6.

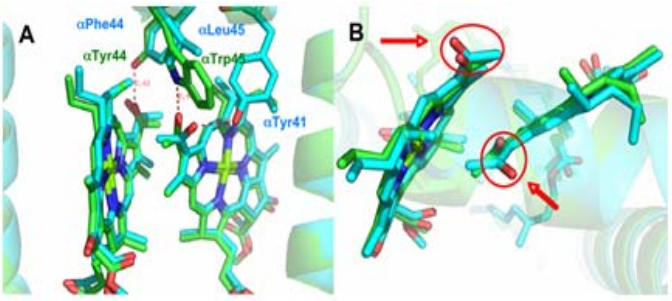

Fig 9. Comparisons of the α- and β-bound Bchl *a* in B850 (green) and B820 (cyan) LH2. A. Diagram showing the interaction of the C_3-acetyl group of Bchl *a* with the key potential H-bonding residues. B. Highlighting the twisting of the C_3-acetyl group of Bchl *a* with respect to the bacteriochlorin plane. The coordinates used to produce this figure were taken from the high resolution (2.0 Å) structure of B800-850 LH2 complex from *Rps. acidophila* 10050 [16] and the 3.0 Å resolution structure of the B800-820 LH2 from *Rps. acidophila* 7050 (PDB: 1IJD) [14].

Rps. acidophila are excited, the energy is transferred to the Bchl *a*s within 61 fs [21]. About two-thirds of this energy is transferred to the B850 molecules and one-third to the B800 molecules [22]. Upon the excitation of B800 Bchl *a*s, the hopping of excitation between B800 monomers occurs in 1.5 ps in the case of *Rps. acidophila* 10050 [23] and 1-3 ps for *Phs. molischianum* [24]. The B800→B850 energy transfer in *Rps. acidophila* takes place with a time constant of 0.7-0.9 ps at room temperature [23] and only slows down to 1.8-2.4 ps at 1.4K [25]. Exciton relaxation in the B850 ring of the LH2 from *Rba. sphaeroides* has been measured to take place on the 100-200 fs timescale [26]. In *Rps. acidophila* 10050 the exciton relaxation in B850 ring has been recorded to be 160 fs [27]. The LH2→LH1 energy transfer has been measured to be 3 ps at 296 K in *Rba. sphaeroides* [28] and LH1→RC transfer has been recorded to be about 20-50 ps in *Rba. sphaeroides* [29].

VARIANTS OF LH2 COMPLEXES

In some species of purple bacteria, such as *Rps. acidophila* 7050/7750 or *Rps. palustris* 216, the Q_y absorption band of the Bchl in the LH2 complexes can vary depending on the growth conditions. When *Rps. acidophila* strain 7050 and 7750 are grown under low-light conditions, the B800-850 LH2 is replaced by a different LH2 complex with the Q_y absorption bands at 800 and 820 nm (Fig. 8) [7,14,30]. The ability to change the type of LH2 in response to growth at different light intensities is related to the presence of multiple αβ-polypeptides, which are (in the case of *Rps. acidophila*) encoded by at least four different αβ-apoprotein gene pairs [30-31]. In order to distinguish these two types of peripheral LH2 complexes they are often referred to as B800-850 and B800-820 complexes, or LH2 and LH3, respectively.

The origin of this spectral variation comes from the fine-tuning of the electronic energy levels of the "B850" molecules, by altering the binding site of the Bchl *a* in the protein matrix. In the B800-850 complex, the C_3-acetyl group of the α-bound B850 Bchl *a* molecule is H-bonded to the αTrp45 residue and the C_3-acetyl group of the β-bound B850 Bchl *a* molecule is H-bonded to the αTyr44 residue (Fig. 9A) [14]. In contrast, in the B800-820 complex, the respective residues in these positions, i.e. αPhe44 and αLeu45, are unable to form hydrogen bonds (Fig. 9A). Instead the C_3-acetyl group of the αB820 Bchl *a* molecules is H-bonded to the αTyr41 residue and this locks the acetyl group into an out-of-plane position with respect to the bacteriochlorin plane (Fig. 9B). Similarly, the β-bound B820 Bchl *a* molecule, lacking any hydrogen bonds, has its acetyl in an out-of-plane position with respect to the bacteriochlorin plane. Rotation of the acetyl group of the Bchl *a* molecule into an out-of-plane position reduces the extent of π-conjugation and results in a blue shift of the Bchl *a* site-energies [14,32]. This then is reflected in the shift of the absorption band from 850 nm to 820 nm. Conclusions from the structural studies comparing B800-850 with B800-820 are strongly supportive of the previous conclusion derived from site-directed mutagenesis experiments carried out on LH2 from *Rba. sphaeroides* [33-34]. In this study Fowler used two double mutants of *Rba. sphaeroides*, that replace the αTyr44 and αTyr45 to Phe-Tyr and Phe-Leu, respectively, and thus produced a B800-839 and B800-826 LH2s, respectively [35]. The resonance

Raman studies of these mutants identified the breakage of one or two H-bonds, respectively, between the protein residues and the respective C_2-acetyl carbonyl group of the B850 Bchl *a* molecules [33]. The removal of a H-bond to the acetyl carbonyl group was signalled by a shift of the Raman peak expected for interaction-free acetyl carbonyl, i.e. 1635 cm^{-1} in the wild-type LH2 to 1659 cm^{-1}. Similarly it is also observed in the *Phs. molischianum* mutant, when αTyr43 in the B800-850 LH2 is replaced a Phe in the B800-820 LH2 [36]. The B800-820 LH2 mutant from *Rs. molischianum* shows loss of H-bound C_2-acetyl RR-stretching mode of B850 Bchl *a* molecule at 1642 cm^{-1} and a dramatic increased of free-from-interaction acetyl carbonyls RR-stretching mode at 1663 cm^{-1}. The importance of the H-bonding residues has also been shown in controlling the site-energy of Bchl *a* molecules [6,37].

This phenomenon of chromatic adaptation is also observed in *Rps. palustris*. When *Rps. palustris* 216 is grown under low light intensity, it replaces the B800-850 LH2 complexes with the B800-low-850 LH2 complexes (Fig. 8 dotted line). The complete genome of *Rps. palustris* has been sequenced [38]. This ability to adapt is related to the five different *pucBA* genes present in the *Rps. palustris* genome (*pucBA-a, b, c, d,* and *e*), which their expression is regulated by light intensity [39-40]. The regulation of the LH2 complex from *Rps. palustris* is even more complicated as 6 bacteriophytochrome (Bph)-like genes have also now been identified in the genome [38]. Four of these genes are located near to genes coding for photosynthetic LH-apoproteins or pigment biosynthetic genes. This suggests that these Bphs are also involved in the regulation of the PSU. The regulation of the LH2 complex in *Rps. palustris* is, therefore, influenced by the light intensity as well as the light quality.

In *Rps. palustris* 261, the structural explanation of the spectral changes going from B800-850 to B800-low-850 LH2 is still a matter of debate [41-44]. The ability to shift the type of LH2 allows these bacteria to be able to grow at ten times lower light intensity than most species that cannot do this. It is not currently understood how changing the types of LH2 allows the bacteria to grow photosynthetically at these lower light intensities.

It was reported in 2002 that Hartigan et al. managed to isolate the B800-only LH2 from the low-light B800-low-850 LH2 by employing a strong anion exchanger as additional purification protocol [45]. The B800-LH2 has been crystallized and a structural model based on the low-resolution crystals (7.5 Å) has been proposed [45]. This model suggests that this complex is an octamer and that each of its $\alpha\beta$-subunit binds an extra B800 Bchl *a* relative to LH2 from *Phs. molischianum*. An AFM study of LL membranes from *Rps. palustris* also supports that the low light grown LH2 is predominantly octameric [46]. The unusual B800-LH2 (LH4) complex, which has Q_y absorption band at 800 nm only, is encoded by *$pucB_d$ and $pucA_d$*, producing LH4 $PucB_dA_d$ peptides, and is regulated by two Bph genes, Rpa3015 (Bph4) and Rpa3016 (Bph5) [47]. The $PucA_d$ apoprotein does not contain Tyr and Trp, but rather Phe and Met at the position 44 and 45, thus unable to H-bonding with the C_3-acetyl group of both Bchl *a* pairs.

A trial expressing two LH2 gene pairs, which encode the high-light ($pucBA_a$) and the low-light ($pucBA_d$) proteins, from *Rps. palustris* in *Rba. sphaeroides* has been carried out and the synthesis of the high-light B800-850 LH2 complex ($pucBA_a$) has been a great success [43]. However expressing *$pucB_d$ and $pucA_d$* geen pairs in *Rba. sphaeroides* resulted unstable low-light B800-830 LH2 complexes.

Studies on the native B800-low-850 LH2 complexes (Fig. 8 dotted line) have been carried out [42,48]. The polypeptide composition of the B800-low-850 LH2 complexes has been characterised by mass spectroscopy. It was found that B800-low-850 LH2 complexes contain mixtures of $pucBA_a$, $pucBA_d$ and $pucB_e$ polypeptides [49]. Previously, it was hypothesised that the LH2 fro *Rps. palustris* may have a heterogeneous polypeptides composition [42]. This hypothesis suggests that the LL LH2 from *Rps. palustris* is composed of a rings that consists of a heterogeneous mixture of different α-polypeptides, where some have Tyr/Trp residues able to provide a H-bond with the acetyl group of B850 Bchl *a*s, and the other have the residues, providing no H-bond. However it was not clear whether the LH2 complexes consisted of rings where each ring has a mixture of apoprotein types or whether the preparation contains a mixture where each individual ring in the mixture has a homogeneous apoprotein composition, but where different rings have different compositions. Quite recently by the use of single molecule spectroscopy it was shown that a single B800-low-850 LH2 complex is composed of heterogeneous apoprotein composition. This heterogeneous apoprotein composition modulates the site energies of Bchl *a* molecules, producing exciton bands both at 820 and 850 nm, within a single 18 tightly coupled Bchl ring [50]. The result from single molecule study was also supported by the femtosecond transient absorption spectroscopy. Although transient spectroscopy unable to probe individual molecules, all the energy transfer data could be explained with a simple kinetic model, that is, the presence of a high-energy exciton state in the region of 820 nm [51]. As a consequent of it, the rate constant for energy transfer from B800 Bchl *a*s to B850 Bchl *a*s is only slightly reduced (by about 10%) in the B800-low-850 LH2 in comparison to high-light ones. Attempts to obtain its

crystal structure are still rather at low-resolution diffraction (6.7 Å) [49]. From these low-resolution data, the best molecular replacement solutions suggest that these LL LH2 complexes are nonameric. However, definite structural conclusion must await better quality crystals.

SUMMARY

In this review it is shown that the polypeptides in LH2 do not just provide inert scaffolding for the pigments – their role is far subtler. They are able to control the electronic properties of the pigments, for example, the absorption of Bchl, which can be altered by changing the number of hydrogen bonds between Bchl and the protein backbone. Understanding the mechanism of this optical tuning is particularly important, as in the purple bacteria the funnelling of the excitation energy (LH2, B800→B850, LH2→LH1, B850→B875) towards the reaction centre is strongly controlled by the gradient energy of the electronic transition of the Bchl Q_y transition band of the different light harvesting complexes.

ACKNOWLEDGEMENT

RJC acknowledges Biotechnology & Biological Science Research (BBSRC), UK. THPB is grateful for the fellowship from the European Commission through the Human Potential Program (Marie-Curie RTN BIMORE, Grant No. MRTN-CT-2006-035859) and from the United States Department of Energy, the Photosynthetic Antenna Research (DE-SC0001035).

REFERENCES

1. Blankenship, R.E. 2002, *Molecular mechanisms of photosynthesis*, Blackwell Science, Oxford.
2. Cogdell, R.J., Gardiner, A.T., Hashimoto, H., Brotosudarmo, T.H., 2008, *Photochem. Photobiol. Sci.,* 7, 1150–1158.
3. Scheuring, S., and Sturgis, J.N., 2009, *Photosynth. Res.,* 102, 197–211.
4. Bahatyrova, S., Frese, R.N., Siebert, C.A., Olsen, J.D., Van Der Werf, K.O., Van Grondelle, R., Niederman, R.A., Bullough, P.A., Otto, C., and Hunter, C.N., 2004, *Nature,* 430, 1058–1062.
5. Hu, X., Ritz, T., Damjanovic, A., Autenrieth, F., and Schulten, K., 2002, *Q. Rev. Biophys.,* 35, 1, 1–62.
6. Cogdell, R.J., Howard, T.D., Isaacs, N.W., McLuskey, K., and Gardiner, A.T., 2002, *Photosynth. Res.,* 74, 135–141.
7. Cogdell, R.J., and Scheer, H., 1985, *Photochem. Photobiol.,* 42, 6, 669–678.
8. Michel, H., and Oesterhelt, D., 1980, *Proc. Natl. Acad. Sci. U.S.A.,* 77, 3, 1283–1285.
9. Michel, H., 1982, *EMBO J.,* 1, 10, 1267–1271.
10. Garavito, R.M., and Rosenbusch, J.P., 1980, *J. Cell. Biol.,* 86, 327–329.
11. Michel, H., 1982, *J. Mol. Biol.,* 158, 567–572.
12. Papiz, M.Z., Hawthornthwaite, A.M., Cogdell, R.J., Woolley, K.J., Wightman, P.A., Ferguson, L.A., and Lindsay, J.G., 1989, *J. Mol. Biol.,* 209, 833–835.
13. Cogdell, R.J., and Hawthornthwaite, A.M., In *The photosynthetic reaction center*; Eds. Deisenhofer, J., Norris, J. R., Academic Press, INC., 1993, Vol. 1.
14. McLuskey, K., Prince, S.M., Cogdell, R.J., and Isaacs, N.W., 2001, *Biochemistry,* 40, 30, 8783–8789.
15. McDermott, G., Prince, S.M., Freer, A.A., Hawthornthwaitelawless, A.M., Papiz, M.Z., Cogdell, R.J., and Isaacs, N.W., 1995, *Nature,* 374, 517–521.
16. Papiz, M.Z., Prince, S.M., Howard, T., Cogdell, R.J., and Isaacs, N.W., 2003, *J. Mol. Biol.,* 326, 1523–1538.
17. Koepke, J., Hu, X., Muenke, C., Schulten, K., and Michel, H., 1996, *Structure,* 4, 581–597.
18. Hunter, C.N., Hundle, B.S., Hearst, J.E., Lang, H.P., Gardiner, A.T., Takaichi, S., and Cogdell, R.J., 1994, *J. Bacteriol.,* 176, 12, 3692–3697.
19. Sundström, V., and van Grondelle, R., In *Anoxygenic Photosynthesis Bacteria, vol 2. Advances in Photosynthesis*; Ed. Blankenship, R. E., Madigan, M.T., and Bauer, C.E., Kluwer Academic Publisher, Dordrecht, 1995.
20. Sundström, V., Pullerits, T., and van Grondelle, R., 1999, *J. Phys. Chem. B,* 103, 2327–2346.
21. Polli, D., Cerullo, G., Lanzani, G., De Silvestri, S., Hashimoto, H., and Cogdell, R.J., 2006, *Biophys. J.,* 90, 7, 2486–2497.
22. MacPherson, A.N., Arellano, J.B., Fraser, N.J., Cogdell, R.J., and Gillbro, T., 2001, *Biophys. J.,* 80, 2, 923–930.
23. Kennis, J.T.M., Streltsov, A.M., Vulto, S.I.E., Aartsma, T.J., Nozawa, T., and Amesz, J., 1997, *J. Phys. Chem. B,* 101, 7827–7834.
24. Novoderezhkin, V., Wendling, M., and van Grondelle, R., 2003, *J. Phys. Chem. B,* 107, 11534–11548.
25. Kennis, J.T.M., Streltsov, A.M., Permentier, H., Aartsma, T.J., and Amesz, J., 1997, *J. Phys. Chem. B,* 101, 8369–8374.
26. Agarwal, R., Rizvi, A.H., Prall, B.S., Olsen, J.D., Hunter, C.N., and Fleming, G.R. 2002, *J. Phys. Chem. A,* 106, 7573–7578.
27. Mercer, I.P., El-Taha, Y.C., Kajumba, N., Marangos, J.P., Tisch, J.W., Gabrielsen, M.,

Cogdell, R.J., Springate, E., and Turcu, E., 2009, *Phys. Rev. Lett.,* 102, 5, 057402.
28. Hess, S., Chachisvilis, M., Timpmann, K., Jones, M.R., Fowler, G.J.S., Hunter, C.N., and Sundstrom, V., 1995, *Proc. Natl. Acad. Sci. U.S.A.,* 92, 12333–12337.
29. Visscher, K.J., Bergstrom, H., Sundström, V., Hunter, C.N., and Vangrondelle, R., 1989, *Photosynth. Res.,* 22, 211–217.
30. Gardiner, A.T., Takaichi, S., and Cogdell, R.J., 1993, *Biochem. Soc. Trans.,* 21, 6S.
31. Bissig, I., Brunisholz, R.A., Suter, F., Cogdell, R.J., and Zuber, H., 1988, *Z. Naturforsch., C: Biosci.,* 43, 77–83.
32. Sturgis, J.N., Jirsakova, V., Reiss-Husson, F., Cogdell, R.J., and Robert, B., 1995, *Biochemistry,* 34, 33, 517–523.
33. Fowler, G.J., Sockalingum, G.D., Robert, B., and Hunter, C.N., 1994, *Biochem. J.,* 299, 3, 695–700.
34. Olsen, J.D., Sturgis, J.N., Westerhuis, W.H., Fowler, G.J., Hunter, C.N., and Robert, B., 1997, *Biochemistry,* 36, 12625–12632.
35. Fowler, G.J.S., Visschers, R.W., Grief, G.G., Vangrondelle, R., and Hunter, C.N., 1992, *Nature,* 355, 848–850.
36. Sauer, P.R.R., Lottspeich, F., Unger, E., Mentele, R., and Michel, H., 1996, *Biochemistry,* 35, 6500–6507.
37. Cogdell, R.J., Gall, A., and Köhler, J., 2006, *Q. Rev. Biophys.,* 39, 227–324.
38. Larimer, F.W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., Land, M.L., Pelletier, D.A., Beatty, J.T., Lang, A.S., Tabita, F.R., Gibson, J.L., Hanson, T.E., Bobst, C., Torres, J.L., Peres, C., Harrison, F.H., Gibson, J., and Harwood, C.S., 2004, *Nat. Biotechnol.,* 22, 55–61.
39. Tadros, M.H., and Waterkamp, K., 1989, *EMBO J.,* 8, 1303–1308.
40. Tadros, M.H., Katsiou, E., Hoon, M.A., Yurkova, N., and Ramji, D.P., 1993, *Eur. J. Biochem.,* 217, 3, 867–875.
41. Evans, M.B., Hawthornthwaite, A.M., and Cogdell, R.J. 1990, *Biochim. Biophys. Acta,* 1016, 1, 71–76.
42. Gall, A., and Robert, B. 1999, *Biochemistry,* 38, 16, 5185–5190.
43. Fowler, G.J.S., and Hunter, C.N., 1996, *J. Biol. Chem.,* 271, 23, 13356–13361.
44. Nishimura, Y., Shimada, K., Yamazaki, I., and Mimuro, M., 1993, *FEBS Lett.,* 329, 3, 319–323.
45. Hartigan, N., Tharia, H.A., Sweeney, F., Lawless, A.M., and Papiz, M.Z., 2002, *Biophys. J.,* 82, 2, 963–977.
46. Scheuring, S., Goncalves, R.P., Prima, V., and Sturgis, J.N., 2006, *J. Mol. Biol.,* 358, 83–96.
47. Evans, K., Fordham-Skelton, A.P., Mistry, H., Reynolds, C.D., Lawless, A.M., and Papiz, M.Z., 2005, *Photosynth. Res.,* 85, 169–180.
48. van Mourik, F., Hawthornthwaite, A.M., Vonk, C., Evans, M.B., Cogdell, R.J., Sundström, V., and Vangrondelle, R., 1992, *Biochim. Biophys. Acta,* 1140, 85–93.
49. Brotosudarmo, T.H.P., 2009, PhD Thesis, University of Glasgow.
50. Brotosudarmo, T.H., Kunz, R., Bohm, P., Gardiner, A.T., Moulisova, V., Cogdell, R.J., and Kohler, J., 2009, *Biophys. J.,* 97, 1491–1500.
51. Moulisova, V., Luer, L., Hoseinkhani, S., Brotosudarmo, T.H., Collins, A.M., Lanzani, G., Blankenship, R.E., and Cogdell, R.J., 2009, *Biophys. J.,* 97, 3019–3028.
52. Goncalves, R.P., Bernadac, A., Sturgis, J.N., and Scheuring, S., 2005, *J. Struct. Biol.,* 152, 221–228.