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Procedia Chemistry 14 (2015) 414 – 421

Procedia Chemistry

2nd Humboldt Kolleg in conjunction with International Conference on Natural Sciences, HK-ICONS 2014

Adaptation of the Photosynthetic Unit of Purple Bacteria to Changes of Light Illumination Intensities

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Abstract

Photosynthetic purple bacteria have developed sophisticated processes to adapt their photosynthetic unit towards changes in light illumination in which the cells grow. Some purple bacteria show pronounced modification of their PSU from changing the composition and content of photosynthetic pigments, i.e. carotenoids, to replacing different composition of polypeptides that alter the Q_y absorption bands of bacteriochlorophyll. Adjusting the spectrum by shifting spectral band position or tuning absorption intensity of spectral band are keys to collect light energy at specific ecological niches they inhabit. Furthermore photoprotection system will ensure the complex from damage.

Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014

Keywords: bacteriochlorophyll; carotenoid; light adaptation; light-harvesting complex; structural diversity

Nomenclature

Bchl	bacteriochlorophyll
PSU	photosystem unit
PSI	photosystem I
PSII	photosystem II

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Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014
doi:10.1016/j.proche.2015.03.056

LH2	peripheral light-harvesting complex in purple photosynthetic bacteria
RC-LH1	core light-harvesting complex coupled with the reaction center in purple photosynthetic bacteria
N	number of conjugated double bonds (C=C)

1. Introduction

In contrast to laboratory conditions, light intensity in the natural habitat is never constant and it is an important challenge for the photosynthetic organisms to survive. The organism must reconcile the amount of incident radiation with the light-harvesting capability of the photosynthetic unit. For higher plant and cyanobacteria it is extremely important because the fragility of their photosynthetic apparatus that can lead to photo-inhibition and photo-oxidation. The sophisticated processes have evolved for this kind adaptation such as (i) adjusting the total cellular chlorophyll content and the ratio of PSII to PSI[1], (ii) thylakoid state transitions, which balance the input of light energy to PSI and PSII reaction centers for optimum electron transfer rate[2–4] and (iii) non-photochemical quenching by the Xanthophyll cycle[5,6], which epoxidase and de-epoxidase enzymes interconvert violaxanthin and zeaxanthin in response to changing light conditions. Recently, A dramatic change due to adaptation of available light has been observed in a cyanobacterium, *Leptolyngbya* sp.[7]. Under far red light condition chlorophylls *d* and *f* are synthesized, moreover there are rearrangement of core subunits of PSI, PSII and phycobilisomes proteins that enhance the ability to harvest light at 700 nm to 750 nm regions.

In purple bacterial photosynthesis the urgency of this adaptation is certainly not so immediate, nevertheless, the bacterial cell matches the light absorption capability of the PSU to the incident light intensity. Classically, the PSU is defined as a set number of interconnected Bchl molecules[8], later in general usage of the PSU has come to mean the amount of the antenna associated with the reaction center. In the purple photosynthetic bacteria the reaction center is closely associated with an antenna protein called the light-harvesting I (LH1). In addition to the RC-LH1 complex there is a different type of antenna protein called LH2 or the peripheral antenna.

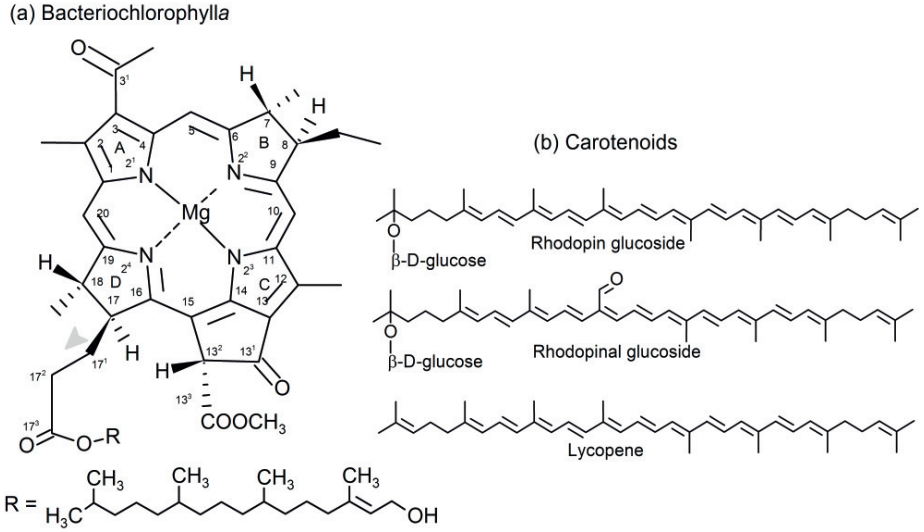

Fig. 1. Chemical structures of some photosynthetic pigments found in purple bacteria. Bacteriochlorophyll *a* ($C_{55}H_{74}N_4O_6Mg$), rhodopin glucoside ($C_{46}H_{66}O_6$), rhodopinal glucoside ($C_{46}H_{66}O_7$), and lycopene ($C_{40}H_{56}$)

The bacterial photosynthetic complexes are produced in the membranes, called intra-cytoplasmic membrane ICM)[9]. In general the antenna complex comprises of bacteriochlorophyll and carotenoid molecules (Fig.1) that are non-covalently bonded in protein. Bacteriochlorophyll molecules responsible for capturing the energy of photon in the blue or near UV region and in the red or near-infrared region. The function of carotenoid is not only for absorbing the photon energy at the visible region and transferring the energy to Bchl. This molecule is able to

quench the triplet Bchl and prevent the formation of singlet oxygen, a very reactive and destructive radical species[10]. Carotenoid is also giving the stability to the complex and in a mutant that lacks carotenoid the LH2 complexes fail to assemble[11–13]. In the *Rhodopseudomonas acidophila* strain 10050 LH2 the main carotenoid is rhodopin glucoside and in the structure this carotenoid is intertwined between the alpha- and beta-polypeptides[14]. This twisting together along with hydrophobic interaction with the Bchl phytyl chain imparts gives structural stability on the LH2 complex. The arrangement of the ring-like structure LH2 and RC-LH1 complexes in an ICM can be viewed by AFM depicted in Fig. 2. The figure shows that the complexes are arrayed like a form of batik painting where RC-LH1 complex is surrounded by LH2 complexes.

The LH1 complex consists of 15 pairs (*Rps. palustris*) or 16 pairs (*T. tepidum*) of alpha- and beta-polypeptides, which oligomerized to encircle the reaction center (RC)[15,16]. Each pair of polypeptide binds non-covalently to two Bchl and a carotenoid. This binding introduces bathochromic shift of the far-red absorption band of Bchl (Q_y band). Collectively the Bchl molecules in the LH1 complex have Q_y absorption band at around 870 nm (*Rps. palustris*) to 914 nm (*T. tepidum*) and event can use light beyond 1 000 nm with Bchl *b* based antenna (*Blc. viridis*)[17]. In LH2 complex there are nine[14] or eight[18] pairs of alpha- and beta-polypeptides that each pair binds three Bchl *a* and a carotenoid. The Bchl *a* molecules bind in two types of arrangement: two Bchl *a* molecules have their bacteriochlorin rings oriented perpendicular to the plane of the membrane, and one Bchl *a* molecule's is in parallel. When they are oligomerized to form nonameric complex those 18 Bchl *a* molecules superficially resembles the blades of a turbine, the center-to-center distance between Bchl is about 0.9 nm[19]. Collectively these 18 Bchl *a* molecules have Q_y absorption band at 850 nm, while the nine Bchl *a* molecules absorb at 800 nm.

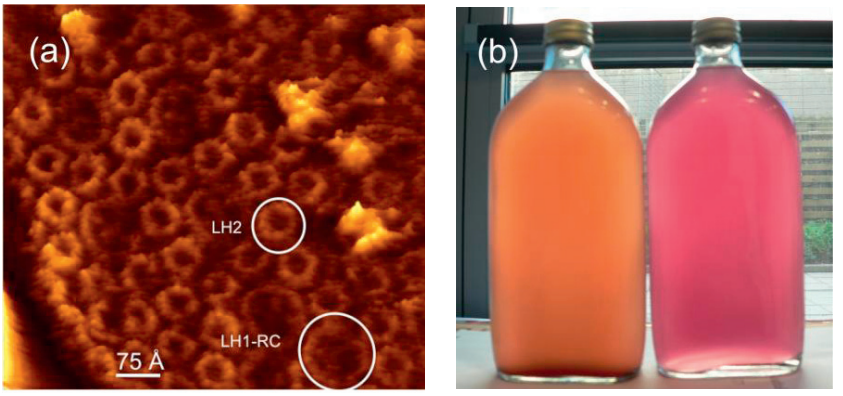

Fig. 2. (a) An image of the photosynthetic membrane from *Phs. molischianum* taken by atomic force microscopy[20] (Figure is kindly provide by Dr. Simon Scheuring). (b) A photograph of *Rps. acidophila* 7050 cells in 500 mL flat-sided bottles that has been cultivated under high light (bright orange brown) and lowlight (purple) illuminations.

2. Variation in pigment composition and content

Adaptation to light intensity can be observed by calculating the relative amount of LH2 and RC-LH1 which is expressed in the ICM. Fig. 3 shows the ratio of LH2 and RC-LH1 from the cells of *Rps. palustris* which has been cultivated under variation of illumination intensities (220 lx, 90 lx, 20 lx and 10 lx; [1 lux = 1 lm · m^{-2}]). In order to do the quantification, the solubilized membranes from cells were set to have an equal concentration before being layered onto the top of sucrose-density gradient. Then after collection of the fractions, The molar concentration of Bchl a was determined by spectroscopic measurement of the acetone/methanol (7 : 2 $^{v}/_{v}$) extracted pigment using and calculated by using the extinction coefficient at 772 nm ($\varepsilon_{772\ nm}$) of 76 $mM^{-1} \cdot cm^{-1}$ [8]. Fig. 3a shows that more LH2 complexes present in the ICM of the cells that have been grown in lower light intensities. This adaptation is consistent with other repots[21,22] and from the AFM studies on the *in situ* organization of the light harvesting complexes from *Rhodospirillum photometricum*[23].

Variation of the pigment composition has been observed in *Rps. acidophila* 7050 cells. The change from rhodopin glucoside to rhodophila glucoside as the carotenoid in the LH2 complex has the dramatic effect of the spectrum change in the visible region and turning the cell cultures from bright orange brown at high light to deep purple at low light[24] (Fig. 2b). Previously, it has been reported that the energy transfer efficiency for rhodopinal glucoside to Bchl is greater than for rhodopin glucoside to Bchl[25], so the change is advantageous to the cell that grow under low intensity of illumination. In *Rps. palustris* the effect is not as pronounced as that with *Rps. acidophila* 7050, but the situation is rather complicated. There are about five types of carotenoid molecules exist in the LH2 complex (Fig. 3). Lycopene ($C_{40}H_{56}$, $\underline{N}$ = 11), rhodopin ($C_{40}H_{58}O$, $\underline{N}$ = 11), anhydrorhodovibrin ($C_{40}H_{58}O$, $\underline{N}$ = 12), rhodovibrin ($C_{41}H_{60}O_2$, $\underline{N}$ = 12), and spirilloxanthin ($C_{42}H_{60}O_2$, $\underline{N}$ = 13) are carotenoids that have been detected by HPLC method in Fig. 4 and reported elsewhere[26] confirm spirilloxanthin pathways of carotenogenesis in *Rps. palustris*. Cultivation of *Rps. palustris* cells under high and low intensity of illumination shows that the carotenoids having 11 conjugated double bonds is major component in the LH2 complex; nevertheless the amount varies. Under high intensity of illumination rhodopin are about 74 % of the total carotenoids. In the low intensity of light illumination the concentration of rhodopin decreases to 50 % and lycopene increases to 38.4 %. The anhydrorhodovibrin content is higher in the low-light form of LH2 than in the high light form, while rhodovibrin and sprilloxanthin are lower. Assuming based on the X-ray structure shows only one carotenoid molecule in an alpha- and beta-subunit, there is still a discussion on how LH2 from *Rps. palustris* has the carotenoid molecule of $\underline{N}$ = 11 to 13 coexist in close contact with Bchl. Previously, it was reported that the interaction is associated with the process of Bchl-to-carotenoid energy transfer, as it was observed fast Bchl triplet energy transfer to the triplet state of different group of carotenoid with the number of conjugated bonds from 11 to 13[27].

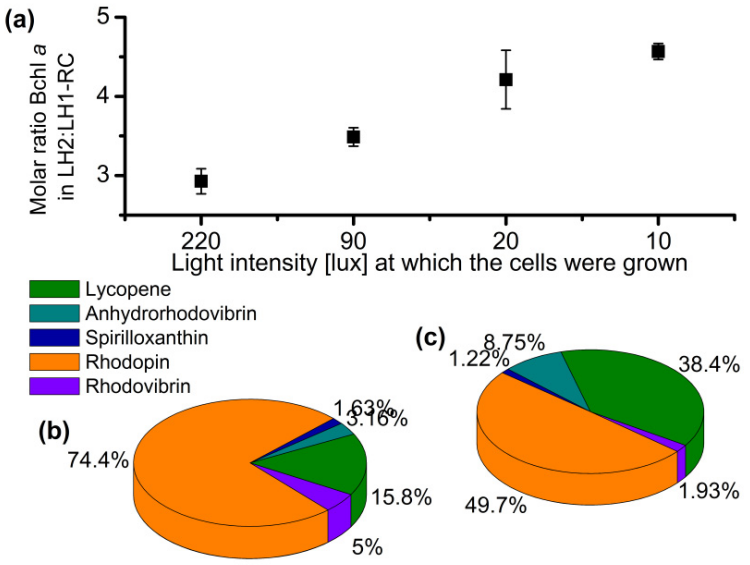

Fig. 3. The molar ratio of LH2 and core complexes depending on the light intensity under the cells were grown (a) and the comparison of the amount (%) of five identified carotenoid in the LH2 complex of *Rps. palustris* grown high light (220 lx, a) and lowlight (10 lx, b) intensities. The carotenoid composition and relative contents were determined by HPLC methods (Lichrosorb Si-60 4.6 × 300 mm column, 2.5 acetone in benzene, 0.6 mL · min^{-1} flow rate).

3. Spectral variation and structural diversity

Wild-type cells of *Rps. acidophila* strain 7750 and 7050 show a different phenotypic LH2 in response to growth at progressively decreasing light intensities[28]. At high intensity of illumination strain 7750 and 7050 produce B800-850 LH2 complex, the same complex like strain 10050 has. The complex is replaced by a B800-820 complex when the cells are grown at progressively lower light intensities. A complete removal of B800-850 LH2 can be introduced correspond to the insertion of the B800-820 complex into the ICM by low light illumination. Fig. 4a shows that the shape of the bands in the far-red region undergoes significant changes. The Q_y absorption band at 850 nm shifts to the lower wavelength at 820 nm and the Q_y band at 875 nm become more pronounced. The shape of the bands in the visible region also undergoes significant changes. This region is due to the changes of carotenoid composition that has been described above. In the case of *Rps. palustris* significant change in the absorption spectrum (Fig. 4b) is not the change of spectral band position. As it is shown the ratio of the intensity of B850 decreases correspond to the B800 band.

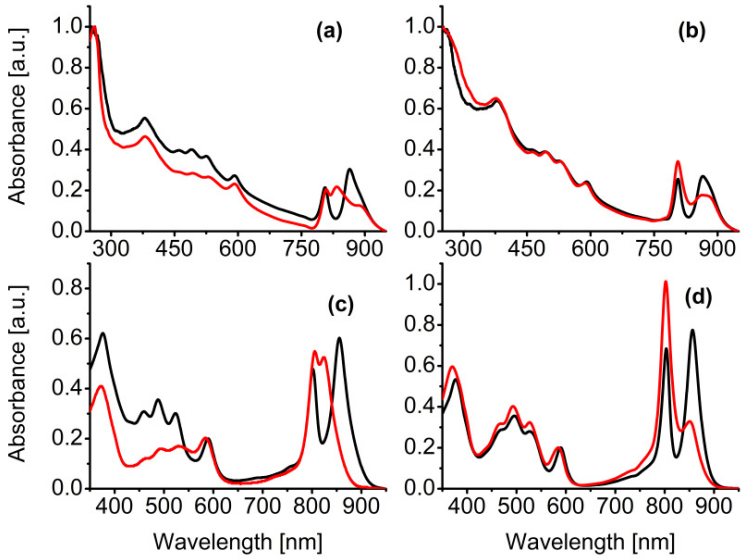

Fig. 4. UV-Vis absorption spectra of the whole cell (a and b) and the isolated LH2 complexes (c and d) of *Rps. acidophila* strain 7050 and *Rps. palustris* that have been cultivated under high and low intensity of light sources (black and red lines, respectively).
Experimental methods for cell culture and purification of stable pigment-protein complexes can be found elsewhere.

The change of the spectrum of cells becomes more clear when the photosynthetic components, RC-LH1 and LH2, are separated. Fig. 4c and 4d show the LH2 complex of *Rps. acidophila* 7050 and *Rps. palustris*, respectively, that has been cultivated under low light intensity. The spectra are compared with their high light type LH2. In *Rps. acidophila* 7050 it shown that a different LH2 complex is formed with the Q_y bands at 800 nm and 820 nm. The ability to change the type of LH2 in response to growth at different light intensity is related to the presence of multiple alpha- and beta-polypeptides, which are in the case of *Rps. acidophila* there four *pucBA* genes that have been identified so far[28,30]. When *Rps. acidophila* 7 050 is cultivated under dimmed light, the genes responsible for the production of B800-820 apoproteins are expressed rather than the B800-850 apoproteins. The origin of this spectral variation comes from the tuning of the electronic energy level of the 18 tightly coupled Bchl molecules. Specifically, it is by altering the binding site of the Bchl *a* in the protein cavity (Fig. 5). In the B800-850 complex, the carbonyl side group at position C3 of the Bchl molecule (Fig. 1a) associated with alpha polypeptide has hydrogen interaction with tryptophan at position 45 of the alpha polypeptide (Fig. 5a)[29]. The Bchl molecule associated with beta polypeptide also has its carbonyl side group in H-bonding with tyrosine at position 44 of the

alpha polypeptide. In contrast, the B800-820 complex, the respective residues in these positions are phenylalanine and leucine[29], amino acid residues which cannot provide hydrogen interaction with the carbonyl group of Bchl. Instead the carbonyl group of Bchl molecule associated with the alpha polypeptide is making hydrogen interaction with tyrosine at position 41 of the alpha polypeptide and locks the carbonyl group into an out-of-plane position with respect to the bacteriochlorin plane (Fig. 5). Similarly, the Bchl molecule associated with beta polypeptide, lacking any hydrogen bonds, has its carbonyl group into an out-of-plane position. As an effect, the extent of π-conjugation is being reduced and the result is a blue shift of the Q_y absorption band of the Bchl site energies[29,31].

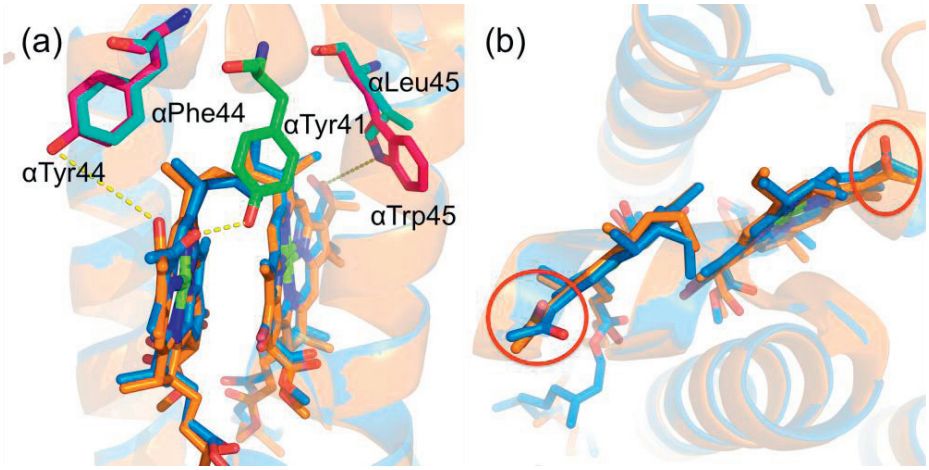

Fig. 5. Comparisons of the α- and β-bound Bchl *a* in B850 (orange) and B820 (blue) LH2. (a) A diagram showing the interaction of the C3-acetyl group of Bchl *a* with the key potential H-bonding residues. (b) Highlighting the twisting of the C3-acetyl group of Bchl *a* with respect to the bacteriochlorin 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[19] and the 3.0 Å resolution stucture of the B800-820 LH2 from *Rps. acidophila* 7050 (PDB: 1IJD)[29]

Rps. palustris also has five different *pucBA* genes which their expression is regulated by light intensity[32]. The regulation of LH2 complex from *Rps. palustris* is rather complication as there are six bacteriophytochrome-like genes have been identified and they are located near to genes coding for photosynthetic light-harvesting apoprotein[33]. Attempts to resolve X-ray crystal structure of the lowlight type of LH2 with low intensity of 850 band from *Rps. palustris* has been done, but still at 5.6 Å resolution[34], however peptide identification by nLC-ESI-MS/MS has provided an important insight. The lowlight type LH2 from *Rps. palustris* comprises of at least two types of polypeptide composition, $PucAB_a$, $PucAB_d$ and $PucB_b$[34]. The $PucAB_d$ polypeptide contains phenylalanine and methionine at the position that it supposes to have hydrogen interaction with the carbonyl group of Bchl. If $PucAB_d$ co-presents with $PucAB_a$ to form an LH2 complex, it can be expected that there is inhomogeneous interaction in one subunit to the other subunit of the complex. Further investigation suggest that inhomogeneous distribution of Bchl site energies within the LH2 ring creates high exciton state in the 18 tightly coupled Bchl and this is responsible to the decrease of 850 band in the LH2 from *Rps. palustris*[35].

Currently more species of purple bacteria become characterized it is apparent that there is consideration in the PSU of these species. Some species such as *Blastochloris viridis*[36] and *Allochromatium vinosum*[37,38] has an extremely complicated PSU and show sophisticated mechanism to adapt at specific ecological niche that they inhabit. This in turn would shed light on understanding the intricacy of efficient light energy utilization in the photosynthesis process.

Acknowledgements

The authors acknowledge the financial support from TWAS The Academy of Science RGA No: 13-059 RG/BIO/AS_I – UNESCO FR: 3240277699 and from DIKTI for National Strategic (STRANAS) research grant No. 040/SP2H/P/K7/KM/2014.

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