Jurnal Teknologi

Full Paper

# PHOTOSTABILITY OF PURPLE BACTERIAL LIGHT–HARVESTING COMPLEXES TOWARDS EXPOSURE OF LIGHT ILLUMINATION TRACED BY PIGMENT RATIO

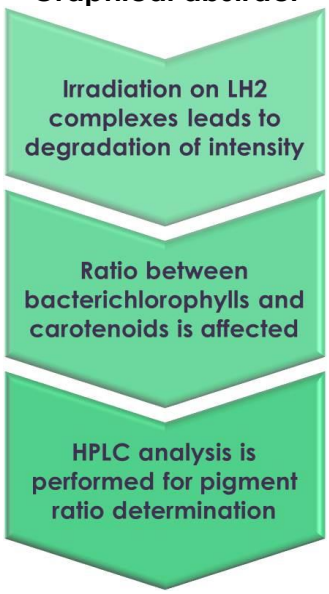
**Article history**
*Received*
*30 November 2015*
*Received in revised form*
*30 January 2015*
*Accepted*
*6 Februari 2016*

Monika Nur Utami Prihastyanti[a], Heriyanto[a,b] Tatas Hardo Panintingjati Brotosudarmo[a*]

[a]Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Villa Puncak Tidar N-01, Malang 65151, East Java, Indonesia
[b]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Krakow, Polland

*Corresponding author
tatas.brotosudarmo@machung.ac.id

**Graphical abstract**




## Abstract

Purple photosynthetic light–harvesting (LH2) is an attractive complex module for assembling hybrid nanostructures that feature energy transfer. LH2 has a broad absorption spectrum range from ultraviolet (UV) to near-infra red (NIR) region. Bacteriochlorophyll *a* molecules absorb at 320 nm to 400 nm (Soret band), 585 nm (Qx) and at NIR region (B800 and B850 bands), while carotenoid absorption bands span from 400 nm to 550 nm. LH2 has to be extracted from its native lipid bilayer membrane and placed in suitable matrix that less mobile and better adherent than the native lipid environment to determine its function. Previous results on pigment ratio determination in different strains of purple photosynthetic bacteria suggested a variation during initial log phase and late log phase. In this experiment, the goal is to reveal the behavior of pigment ratio in LH2 of *Rhodopseudomonas palustris* during irradiation of certain intensity of light. Photostability assay of LH2 from *Rhodopseudomonas palustris* in *n*-dodecyl-$\beta$-D-maltoside or DDM was determined under continuous illumination (3 000 $\mu$ mol·m$^{-2}$·s$^{-1}$) for 300 min at room temperature by recording the absorption spectra. Degradation was observed in B850 and B800 at about 67 % and 64 %, respectively, as well as blue shift in B850. Initial pigments isolated from LH2 suggested a mixture of carotenoids and bacteriochlorophylls which was determined further using a high-performance liquid chromatography (HPLC).

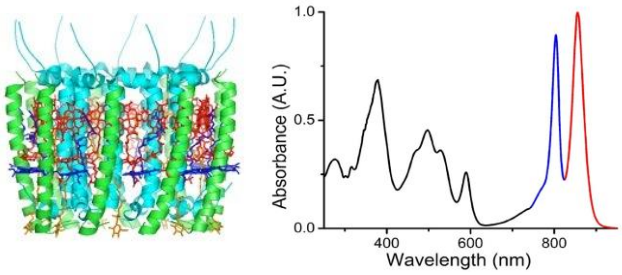
*Keywords*: Light-harvesting complexes, photostability, pigment ratio, purple bacteria



## 1.0 INTRODUCTION

Sunlight provides source of energy with a wide spectrum that ranges from ultraviolet (UV) to infrared regions (300 nm to 2 000 nm). Purple photosynthetic bacteria have evolved their photosynthetic unit to incorporate carotenoid and bacteriochlorophyll (BChl) pigments embedded with protein as complex molecules, i.e. the light-harvesting complexes and the reaction centers. These well-structure molecules function as devices that are able to absorb photon in the UV-visible regions by carotenoid and chlorophyll molecules and transfer that absorbed energy. Upon the

absorption, the energy is stored in the excited states and then transferred to the reaction centers at near-infrared (NIR) region (at 875 nm) within a few hundred picosecond [1].




(a) (b)

**Figure 1** (a) Side view of crystal structure of light-harvesting (LH2) complex that shows polypeptides, B800 Bchls (blue) and B850 Bchls (red) [6] and (b) Absorption spectrum of LH2 that spans from UV to NIR region

The peripheral light-harvesting (LH2) complexes used in this experiment were isolated from purple photosynthetic bacterium *Rhodopseudomonas (Rps.) palustris*. A single LH2 complex contains nine carotenoids and 27 Bchl *a* molecules, which are arranged in two rings: B800 and B850 (Figure 1a blue and red colored molecules, respectively) [1–3]. The Bchl *a* molecules in LH2 also absorb at UV region (300 nm to 400 nm) and at 585 nm, while the carotenoid molecule absorption bands span from 400 nm to 500 nm (Figure 1b). This broad absorption spectrum due to large number of pigments comprising the LH2 has made this biomolecule highly attractive for assembling hybrid nanostructure for solar cell. However, isolating this LH2 from its native membrane bilayers may minimize interaction with the surrounding lipids, which are important for maintaining the stability [5, 6].

LH2 integral membrane antenna complex in purple photosynthetic bacteria is similar but clearly distinct to LH1. Bchl pigments in LH2 exhibit spectrally different compared to those from LH1. There are two well-separated absorption bands which centered at around 800 nm and 850 nm, referring to B800 and B850 pigments. For B800 pigments, the Bchls exist in monomeric form and forming a ring that parallel to the plane of the membrane that the complex embedded in; while for the B850 pigments, each subunit consists of two Bchls, in the form of arrangement of coupled dimer.

The light-harvesting complexes showed instability against exposure of light. Protein incorporated in the structure was unfolded and the pigments were detached from the complexes. Moreover, spectral analysis revealed a blue-shift on the absorption maxima during photostability assay [8]. This phenomenon suggested a change in Bchl and carotenoid ratio in the complexes. This paper would show behavior of pigment ratio in LH2 complexes under illumination of light exposure by means of spectroscopy and chromatography analysis.

## 2.0 MATERIAL AND METHOD

### 2.1 Isolation and Purification

Cells of *Rps. palustris* were grown with succinate media [8] under certain illumination. The cells were collected by separation using centrifugation at 8 000 g for 15 min and resuspended in 20 mM Mes buffer (pH 6.8) containing 100 mM KCl. Native membranes of *Rps. palustris* were isolated from the cells with a French Press (9 500 psi) [1 pascal = 0.000145037738007 psi] in a presence of DNAse and $MgCl_2$ and then pelleted by centrifugation at 44 400 rpm for 2 h (1 rpm = 1/60 Hz). The membrane pellet was resuspended and homogenized in 20 mM Tris-HCl buffer (pH 8.0). The concentration was adjusted to give an absorption of 60 $cm^{-1}$ at 850 nm. After addition of 1% of lauryldimethyl-N-oxide (LDAO), the membrane was solubilized for 1 h in the dark. Unsolubilized material was removed by centrifugation at 10 000 rpm for 10 min. Supernatant was collected and layered onto a sucrose step gradient. The gradient consisted of 0.8 M, 0.6 M, 0.4 M, and 0.2 M sucrose in 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 % LDAO. The gradients were centrifuged at 43 900 for 16 h at 4 °C [9].

The band containing LH2 complexes (upper pigmented band) was collected and purified on DE–52 cellulose (Whatman, Maidstone, England) column. The column was prewashed with 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 % LDAO. Using 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM NaCl in 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 % LDAO, collected band of LH2 complexes was washed through the column. A desalting step was performed using a PD-10 column afterwards. Final step of purification was performed using a high-performance liquid chromatography (HPLC) with BioSep SEC s-3000 as the purifying column and 20 mM Tris-HCl buffer (pH 8.0) containing 0.1% LDAO as the mobile phase (flow rate, 0.5 ml $min^{-1}$). Detection was at 280 nm and 370 nm.

### 2.2 Photostability Assay

LDAO was removed from LH2 complexes by performing centrifugation with a vivaspin. Detergent was changed into 0.02 % of *n*-dodecyl- $\beta$ -D-maltoside (DDM). LH2 complexes in DDM were suspended again in 20 mM Tris HCl buffer (pH 8.0) containing 0.02 % DDM and adjusted to give absorption of 1 AU at B850 band. The sample was illuminated with 3 000 $\mu$mol $m^{-2}$ $\cdot s^{-1}$ intensity from an artificial daylight with a broad spectra at visible region. Absorption spectrum was determined with

UV-Vis Spectrophotometer (UV-Vis-1700 MultiSpec) from UV to NIR region (250 nm to 1 000 nm).

### 2.3 Determination of Pigment Ratio

Pigment extraction from LH2 complexes was carried out based on developed method [10]. The light-harvesting complex was loaded onto DE-52 ion exchange column. To remove remaining detergent, column was washed with water, subsequently $N_2$ flow was applied to remove residual water until the column was dry. Pigments were extracted with a mixture of acetone and methanol (7: 2, v/v). The pigments were dried using nitrogen flow and redissolved in acetone to measure the absorption spectra and pigment composition in HPLC.

Pigment composition was determined using reversed-phase HPLC (Shimadzu, Kyoto, Japan) with photodiode array detector (PDA) [11]. The column was Shimpack VP-ODS C18 with a mixture of acetonitrile, methanol and dicloromethane (70:15:15, v/v/v) as mobile phase at a flow rate of 1 mL $min^{-1}$. Detection was at 470 nm and 770 nm.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Degradation Analysis and Pigment Composition

Pigment-protein complexes isolated from *Rps. palustris* were irradiated to determine degradation of intensity. Three peaks representing pigments in the complexes were monitored. i.e., peak at 858 nm for B850 band, 804 nm for B800 and 497 nm for carotenoids. After 300 min of irradiation, each peak underwent degradation of intensity at different rates.

Figure 2 shows degradation rates of B850, B800 and carotenoid band. Degradation of intensity of 67 % was observed at B850, while B800 underwent 64 %. This rate of intensity led to faster degradation of B850 as can be seen in Figure 2b. In the beginning of experiment, higher intensity can be observed at B850 peak compared to B800, however, after 300 min of irradiation, B850 intensity was lower than B800 due to its faster degradation rate. At the same time, small peak slightly appeared at 700 nm region indicating formation of degradation products from Bchl, which most likely suggested chlorin formation [12, 13]. Faster degradation rate at Bchl, especially at B850, was more likely due to energy transfer process. B850 as the final receiver receives excess amount of energy as a result of energy transfer process, after which, the energy is dissipated as heat, thus, damaging the pigment. Carotenoid band at 497 nm underwent slower degradation of intensity than Bchl band, i.e. 40 % of degradation after 300 min and it also changed in spectral shape.

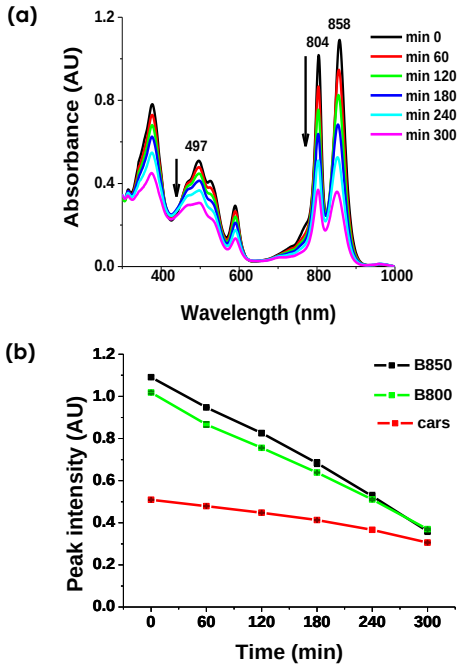



**Figure 2** (a) Absorption spectra of LH2 complexes irradiated for 0 min, 60 min, 120 min, 180 min, 140 min and 300 min; (b) Peak intensity of B850, B800 and carotenoid band upon irradiation for 0 min, 60 min, 120 min, 180 min, 140 min and 300 min.

**Table 1** Tentative identification of pigments in LH2 complexes from *Rps. palustris* based on maximum absorption and retention time of HPLC peaks

| No. | Retention time (min) | $\lambda_{max}$ (nm) | Tentative pigment identification |
|---|---|---|---|
| 1 | 5.17 | 458; 486; 519 | rhodovibrin |
| 2 | 5.44 | 473; 505, - | rhodopin |
| 3 | 6.48 | 499; 530; - | spiriloxanthin |
| 4 | 8.34 | 458; 486; 519 | anhydrorhodovibrin |
| 5 | 8.87 | 449; 473; 505 | lycopene |
| 6 | 9.6 | 363; 601; 739 | Bchl *a* |

Further analysis using HPLC revealed composition of pigments extracted from the complexes. Table 1 shows tentative identification of pigments in LH complex from *Rps. palustris* based on maximum absorption and retention time of the separated peaks [11]. Rhodovibrin, rhodopin and spiriloxanthin were observed in a low amount at 5.17 min, 5.44 min and 6.48 min, respectively, while anhydrorhodovibrin and lycopene appeared in a higher intensity at 8.34 min and 8.87 min, respectively. Bchl *a* was also observed at 9.6 min. Each peak number in Table 1 is corresponding to peak number in the chromatograms (Figure 4).

### 3.2 Determination of Pigment Ratio

Spectral analysis on pigments from irradiated complexes (before and after irradiation for 300 min) was performed to determine changes in pigment ratio. From Figure 3, absorption of Bchl (770 nm) and carotenoids (480 nm) clearly underwent degradation of intensity, however, only in carotenoid bands, changes in spectral pattern were observed. This suggested changes in carotenoid composition in the complexes.

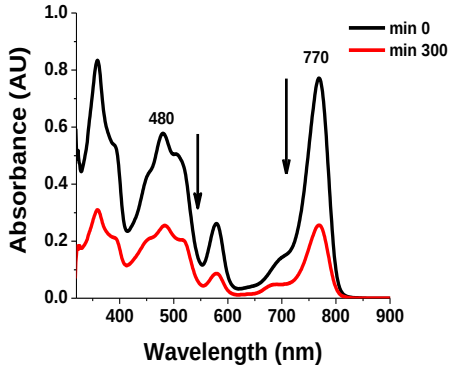



**Figure 3** Absorption spectra of extracted pigments from irradiated complexes after 0 min (black) and 300 min (red) of irradiation

Moreover, HPLC analysis showed more detailed results in pigment ratio due to the availability of peak area data from designated peak. Figure 4 shows the comparison between HPLC area of Bchl group and carotenoid group. Before irradiation was applied, Bchls showed higher amount than carotenoids of which the ratio was 1:0.96. After 300 min of irradiation, HPLC area of both groups decreased, however, the ratio of Bchls to carotenoids changed (1:3.51) which suggested that carotenoids could still maintain their stability. Based on calculated concentration using Beer-Lambert law and extinction coefficient from each identified pigment, a massive degradation occurred in Bchls (93 %). While carotenoids underwent 21 % of degradation. This calculation confirm the behaviour of pigment degradation which was previously analyzed by spectrophotometer and HPLC area.

Further analysis on HPLC area (Figure 5) on dominated carotenoids (anhydrorhodovibrin and lycopene) shows that lycopene underwent a faster degradation than anhydrorhodovibrin. HPLC area of lycopene (detection at 473 nm and $t_R$ 8.87 min) decreased at value of 83.16% while in anhydrorhodovibrin (detection at 486 nm and $t_R$ 8.34 min), the HPLC area decreased for 71.03 % after 300 min of irradiation. Anhydrorhodovibrin, having 12 conjugated double bonds, was more stable than lycopene which has 11 conjugated double bonds. This result confirmed that carotenoids which have longer conjugated double bonds are more stable towards light exposure [14].

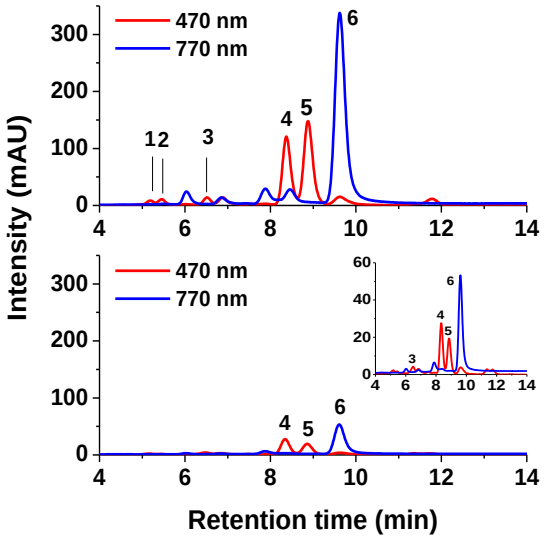



**Figure 4** HPLC chromatograms of pigments from irradiated samples upon 0 min (top) and 300 min (down) of degradation. Inset: magnification of HPLC chromatogram of pigments upon 300 min of irradiation

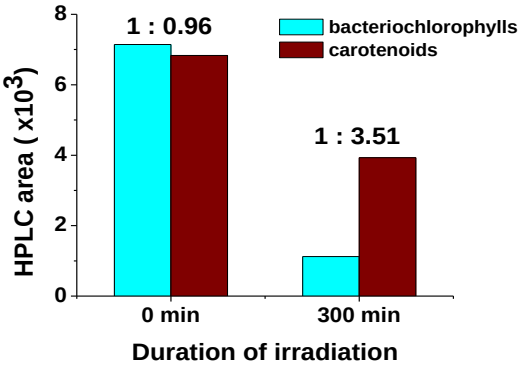



**Figure 5** Bchl to carotenoid ratio on irradiated LH2 complexes

Several factors contribute to the structural stability of carotenoids, such as hydrophobic interactions of carotenoids with apolar amino acid side chains, intermolecular π-π stacking interaction between carotenoids and aromatic amino acid side chains, and packing of carotenoids with their protruding methyl groups between rigid conjugated double bond chains and LH1 αβ-Bchls structural units. Moreover, carotenoids which have a longer conjugated double bond chain are good in giving protection to Bchls towards photooxidative damage [6, 14, 15]. Structure of carotenoids is relevant to a process named photoprotection towards Bchls. Due to the low-lying triplet states, carotenoids are able to quench the Bchl triplet states. Singlet oxygen which is

formed from excitation on Bchl is highly reactive and damaging for biological molecules. Therefore, the existence of carotenoids in photosynthetic system is able to prevent the formation of singlet oxygen from excited states of Bchls [16].

## 4.0 CONCLUSION

Light-harvesting complexes underwent degradation of intensity as well as peak shift which suggested change in Bchl-to-carotenoid ratio after irradiation. Carotenoids in light-harvesting complexes were able to maintain the stability during irradiation due to the stable structure and the ability for photoprotection.

## Acknowledgements

This work is supported by The World Academy of Science (TWAS) research grant (13-059 RG/BIO/AS_I; UNESCO FR: 3240277699) awarded to Tatas H.P. Brotosudarmo.

## References

[1] Blankenship, R. E. 2002. *Molecular Mechanisms of Photosynthesis*. Oxford: Blackwell Science.
[2] Cogdell, R. J., P. K. Fyfe, S. J. Barret, S. M. Prince, A. A. Freer, N. W. Isaacs, P. McGlynn, P., and C. N. Hunter. 1996. The Purple Bacterial Photosynthetic Unit. *Photosynthetic Research*. 48: 55–63.
[3] Roszak, A.W., T. D. Howard, J. Southall, A. T. Gardiner, C. J. Law, N.W. Isaac, and R. J. Cogdell. 2003. Crystal Structure of the RC-LH1 Core Complex from *Rhodopseudomonas palustris. Science*. 302: 1969–1972.
[4] Hartigan, N., H.A. Tharia, F. Sweeney, A.M. Lawless, and M.Z. Papiz, 2002. The 7.5 Å Electron Density and Spectroscopic Properties of a Novel Low-Light B800 from *Rhodopseudomonas palustris*. *Biophysical Journal*. 82: 963–977.
[5] White, S. H. and W. C. Wimley. 1999. Membrane Protein Folding and Stability: Physical Principles. *Annual Review of Biophysics and Biomolecular Structure*. 28: 319–365.
[6] Papiz, M. Z., S. M. Prince, T. Howard, R. J. Cogdell, and N. W. Isaacs. 2003. The Structure and Thermal Motion of the B800–B850 LH2 Complex from *Rps. acidophilla* at 2.0 Å Resolution and 100 K: New Structural Features and Functionally Relevant Motions. *Journal of Molecular Biology*. 326: 1523–1538.
[7] Prihastyanti, M.N.U. Indriatmoko, and T. H. P. Brotosudarmo. 2014. Photostability Assay on Light-Harvesting Complex as a Material of Biophotovoltaic. In Praptiningsih G. A., N. Anggi, S. Y. Agus, and S. Andi (eds.). *Energy Procedia*. 47: 189–195.
[8] Bose, S. K. 1963. *Bacterial Photosynthesis*. Ohio: Antioch Press.
[9] Brotosudarmo, T. H. P., A. M. Collins, A. Gall, A. W. Roszak, and A. T. Gardiner, R. E. Blankenship, and R. J. Cogdell. 2011. The Light Intensity under which Cells are Grown Controls the Type of Peripheral Light-Harvesting Complexes That Are Assembled in a Purple Photosynthetic Bacterium. *Biochemical Journal*. 440: 51–61.
[10] Nakagawa, K., S. Suzuki, R. Fujii, A. T. Gardiner, R. J. Cogdell, M. Nango, and H. Hashimoto. 2008. Probing the Effect of the Binding Site on the Electrostatic Behavior of a Series of Carotenoids Reconstituted into the Light-harvesting 1 Complex from Purple Photosynthetic Bacterium *Rhododpirillum rubrum* Detected by Stark Spectroscopy. *The Journal of Physical Chemistry B*. 112: 9467–9475.
[11] Nelis, H. J. and A. P. de Leenheer. 1989. Profiling and Quantitation of Bacterial Carotenoids by Liquid Chromatography and Photodiode Array Detection. *Applied and Environmental Microbiology*. 55(12): 3065–3071.
[12] Limantara, L., Koehler, P., Wilhelm, B., Porra, R. J. and Scheer, H. 2006. Photostability of Bacteriochlorophyll a and Derivatives: Potential Sensitizers for Photodynamic Tumor Theraphy. *Photochemistry and Photobiology*. 82: 770–780.
[13] Fiedor, J., L. Fiedor, N. Kammhuber, A. Scherz, and H. Scheer. 2002. Photodynamics of the Bacteriochlorophyll–Carotenoid System. 2. Influence of Central Metal, Solvent and β-carotene on Photobleaching of Bacteriochlorophyll Derivatives. *Photochemistry and Photobiology*. 76(2): 145–152.
[14] Fiedor, L., J. Akahane, and Y. Koyama. 2004. Carotenoid-Induced Cooperative Formation of Bacterial Photosynthetic LH1 Complex. *Biochemistry*. 43: 16487–16496.
[15] Wang, Y. and X. Hu. 2002. A Quantum Chemistry Study of Binding Carotenoids in the Bacterial Light-Harvesting Complexes. *Journal of The American Chemical Society*. 124: 8445–8451.
[16] Koyama, Y., M. Kuki, P.O. Andersson, and T. Gillbro. 1996. Singlet Excited States and the Light-Harvesting Function of Carotenoids in Bacterial Photosynthesis. *Photochemistry and Photobiology*. 63(3): 243–256.