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ScienceDirect

Energy Procedia 47 (2014) 189 – 195

Energy Procedia

Conference and Exhibition Indonesia Renewable Energy & Energy Conservation [Indonesia EBTKE CONEX 2013]

Photostability Assay on Light-Harvesting Complex as a Material of Biophotovoltaic

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Abstract

Purple photosynthetic light-harvesting (LH2) is attractive complex module for hybrid nanostructures. Bacteriochlorophyll *a* in LH2 absorb at 320-400 nm, 585 nm and at near-infrared region (B800, B850 bands), while carotenoids absorb at 400-550 nm. Prior for application, LH2 has to be extracted from its native membrane and placed in suitable matrix. This study reports photostability assay of LH2 from *Rhodopseudomonas palustris* under continuous illumination in its native membrane and in solubilized condition in different detergent buffers. Continual degradation has been observed by the decrease of intensity at λ_{max}, yet can be slowed-down and restrained by addition of glycerol or polyvinyl-alcohol.

Selection and peer-review under responsibility of the Scientific Committee of Indonesia EBTKE Conex 2013

Keywords: light-harvesting complex; photostability; detergent buffer; glycerol; PVA

Nomenclature

LH2	light-harvesting 2
nm	nanometer
B800	absorption at 800 nm
B850	absorption at 850 nm
λ_{max}	wavelength at which maximum absorption is observed
PVA	polyvinyl alcohol
TW	terraWatt
DSSC	dye-sensitized solar cell

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Selection and peer-review under responsibility of the Scientific Committee of Indonesia EBTKE Conex 2013
doi:10.1016/j.egypro.2014.01.213

BChl	bacteriochlorophylls
UV	ultra-violet
NIR	near infra-red
ps	picosecond
Au	Aurum
Si	silicon
$\mu A \bullet cm^{-2}$	microAmpere per centimetre squared (units for photocurrent density)
HPLC	high performace liquid chromatography
HCl	hydrochloric acid
LDAO	lauryldimethyl-N-oxide
DDM	*n*-dodecyl-β-D-maltophyradoside
MWCO	molecular weught cut-off
AU	absorbance units
mW/cm^2	milliWatt per centimetre squared (units for surface power density)
min	minutes
t	time
cars	carotenoids

1. Introduction

Energy from sunlight covers a wide spectrum from ultraviolet to infrared regions (300 – 2000 nm), which is important for running cellular activities in living organisms on Earth, including photosynthesis. The total energy that arrives on Earth surface is about 120,000 TW (1 TW = 10^{12} W) [1, 2]. With assistance of chlorophylls and carotenoids, photon derived from sunlight energy is harvested by photosynthetic units and then converted into electrons. Afterwards, the electron transfer process triggers the generation of fuel. This process has been adapted to create photovoltaic devices, including organic solar cells [3-6] and dye-sensitized solar cells [7-10]. However, due to narrow absorption spectrum of early-developed solar cells, it seems that the efficiency of solar cells has become problem, which is only 11% [11], while the efficiency in commercial silicon solar cell is about 18±2% [12]. Thus, creating higher efficiency of solar cells is needed for longer application.

Nature has provided light-harvesting system in photosynthetic organisms, such as purple photosynthetic bacteria, which is able to harvest photon derived from sunlight. In comparison to the recent developed DSSC, photosynthesis mostly uses different types of pigment embedded in a protein cavity. As an example, purple photosynthetic bacteria have evolved their photosynthetic unit to incorporate carotenoid and bacteriochlorophyll (BChl) molecules embedded with proteins as a complex molecule, i.e. the light-harvesting complexes and the reaction centres. These well-structure molecules function as devices that are able to absorb photon in the UV-visible regions as well as in the NIR regions by carotenoid and bacteriochlorophyll molecules. The key feature in farming the light energy is the energy transfer that is faster enough to compete with unfruitful decays. Upon the absorption, the energy is stored in the excited states and then transferred to the reaction centres at near-infrared region (NIR, at 875 nm) within a few hundred ps [13, 14].

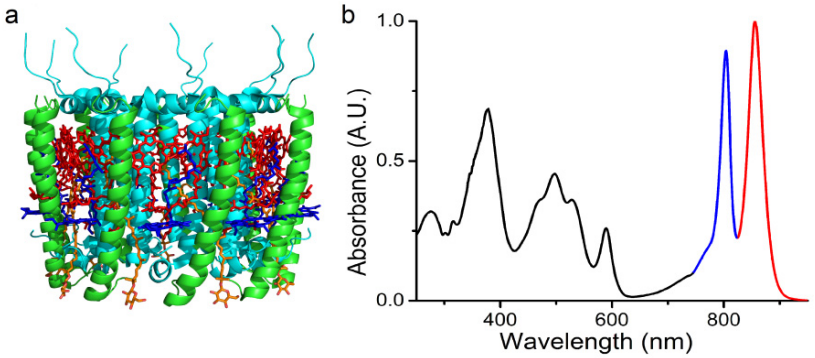

Fig. 1.(a) An X-ray crystal structure of light-harvesting LH2 complex that shows polypeptides, B800 BChl (blue) and B850 (red) [15] (b). Absorption spectrum of LH2 that spans from ultraviolet to NIR.

Light harvesting (LH2) complex from purple photosynthetic bacteria contains 9 carotenoids and 27 Bchl *a* molecules, which are arranged and oriented in two different ways that lead to the absorption at 800 (B800 band) and 850 nm (B850 band) (Figure 1a) [16-18]. In the case purple bacteria *Rps. palustris*, there are different polypeptides that responsible for binding the pigments [19]. The Bchl *a* molecules in LH2 also absorb at UV regions (300-400 nm) and at 585 nm (Q_y band), while the carotenoid molecules absorb at regions between 400 to 500 nm (Figure 1b). The unique feature of having broad absorption spectrum and the capability of energy transfer is making LH2 highly attractive for assembling hybrid nanostructure for photovoltaic.

Recently, several successful experiments have been conducted to corporate the biological light-harvesting systems into photovoltaic. His-tagged light-harvesting/reaction center complexes have been self-assembled on Au electrode [20]. It was shown that electron current was measured upon the illumination of light. An attempt also has been carried out to couple photosystem I with p-doped Si and it was then deposited on the semiconductor. It was shown that the average photocurrent density is high up to 875$\mu A \bullet cm^{-2}$ [21]. In practical, photovoltaic application requires long-term stability. In this paper, we show a photostability study of light-harvesting complex as a biomolecule for photovoltaic applications. In addition, we also try to give additives as an attempt to increase the robustness of the biomolecule.

2. Materials and method

Light-harvesting LH2 complex was isolated from *Rhodopseudomonas palustris* and prepared according to Brotosudarmo *et al.* [19] with a modification on the final step of purification. The final purification step was performed using high performance liquid chromatography (HPLC) with a size-exclusion column (BioSep SEC s-3000, Phenomenex). The mobile phase was 20 mM Tris-HCl buffer containing 0.1% LDAO (flow rate 0.5 ml/min). Detection was at 280 and 370 nm. Prior to assay the stability, the detergent was exchanged into 0.02% *n*-dodecyl-β-D-maltophyradoside (DDM) and 0.1% Triton X-100 according to the protocol published by Howard *et al.* [22]. The sample was centrifuged with a vivaspin (50.000 MWCO) to remove LDAO. The sample was then re-suspended again in 20 mM Tris HCl buffer (pH 8.0) containing new detergent. The process of detergent exchange was repeated 3-4 times to ensure complete removal of the previous detergent.

In order to perform stability assay, the LH2 sample was adjusted to give absorption of 0.5 AU at B850 band. Intralux 4100 (Volpi AG) was used as a source of light. In order to eliminate exposure of heat that is generated by the light, cold water column was set onto the cell holder. The sample was illuminated with a 11.71 mW/cm^2 for 360 min with interval of 30 min. Absorption spectrum was determined with UV-Vis Spectrophotometer (Shimadzu UV Vis-1700 MultiSpec) in the region of 250-950 nm.

3. Result and discussion

Figure 2 compares the evolution of the absorption spectra of the LH2 in its native membrane and in three different detergents (i.e. DDM, LDAO and Triton X-100) after illumination of light. It is shown that the absorption maxima (λ_{max}) of the selected bands decrease consistently and also shift to the blue region of the wavelength. In the native membrane, the absorption maxima (λ_{max}) of the B850 and B800 bands is decreasing to about 54.96% and 54.87%, respectively, after exposure of light for 360 min. The decrease of the absorption maxima at the carotenoids band (490 nm) is also apparent.

Similar pattern can also be observed in the LH2 complexes, which were solubilized in DDM, LDAO and Triton X-100. In DDM and LDAO, the λ_{max} of B800 band decrease fast during the first 120 min and then consistent until the remaining 240 min. In triton X-100, the λ_{max} of B800 band also shows fast degradation at the first 120 nm, but it also shows stabilization stage at t = 150 and 180 min, before another fast degradation until the remaining 150 min. In comparison, the evolution of the λ_{max} of B850 band show interesting feature. In DDM, the λ_{max} of B850 band increases at the first 30 min, and then it shows consistent decrease. In LDAO, the λ_{max} of B850 band remains the same at the first 30 min, and then it is followed by consistent decrease. In case of triton X-100, the λ_{max} of B850 band decreases directly. In the absorption region of the Q_x band and the carotenoids, small variations were observed in the DDM, LDAO and triton X-100. The absorption intensity of a small band at 693 nm has become stronger in parallel to the decrease of the intensity of B800 and B850 bands. This band is corresponding to the chlorin formation in the mixture as a derivative of bacteriochlorophylls degradation [23]. At UV region where polypeptide absorbs, changes can also be clearly observed as the protein unfolded. In this case, the bacteriochlorophyll is slowly detached from the protein to become free molecule. The bacteriochlorophyll molecules are still undergoing degradation to become chlorin products.

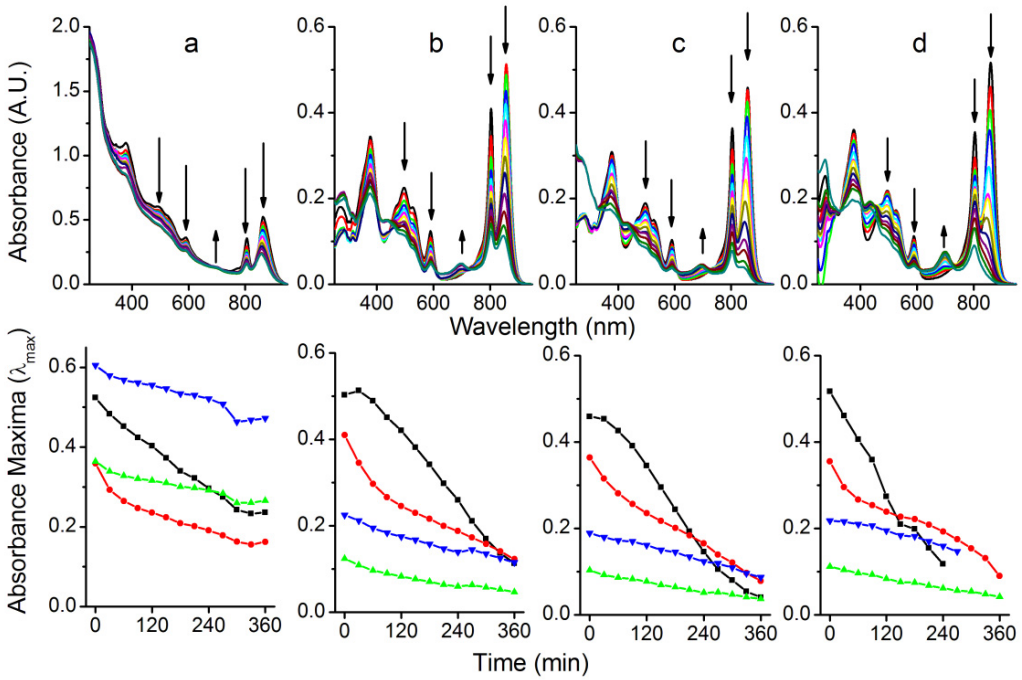

Fig. 2. Photostability of LH2 from *Rps. palustris* in (a) native membrane, (b) DDM, (c) LDAO and (d) Triton X-100. Top: Absorption spectra that evolve upon illumination for 360 min. Bottom: Absorption maxima (λ_{max}) of B850 (black), B800 (red), Qx (green) and carotenoid bands (blue).

Effort to stabilize LH2 complexes was carried out by introducing LH2 complexes with 66% of glycerol and 2% of PVA. Figure 3 compares changes the absorption maxima of selected band during 360 min observation. A common feature shown on the spectra is that the addition of glycerol or pva can slow down the degradation process. This is clear that at t = 240 min, the λ_{max} of B850 band of LH2 in the triton X-100 solution containing glycerol or pva is still higher than in the control. Similar feature can also be observed for the LH2 in the DDM or LDAO. Quite interesting is the combination between DDM and 66% glycerol. In this condition, the degradation of LH2 can be observed in the first 60 minutes, then the absorption maxima of the selected bands of LH2 remain constant until 360 continual exposure of light. Additionally, the spectra of LH2 complexes solubilized in the detergent containing glycerol or pva show minor increase of the band at 693 nm. Table 1 summarizes the percent degradation of LH2 in the detergent solution as well as shows the contribution of glycerol and pva as additives for stabilizing the LH2 complexes. The addition of glycerol in the LH2 solubilized in DDM solution also contributes significantly to buffer the degradation of carotenoid. It shows that the percent decrease after 360 min of carotenoid band is only 1%, in comparison to other conditions. The ability to stabilize the LH2 complex from degradation could relate to the number of hydrogen bonds provided by combination of glycerol and DDM. Glycerol was reported to have a contribution in stabilization effect on protein due to its ability to increase the protein free energy of denaturation. It also increases the intensity of hydrophobic interaction between non-polar groups; thus, makes protein structure becomes more rigid and prevents from unfolding. Glycerol also has ability to absorb water at the surface of protein, hence, protects protein from degradation [24, 25].

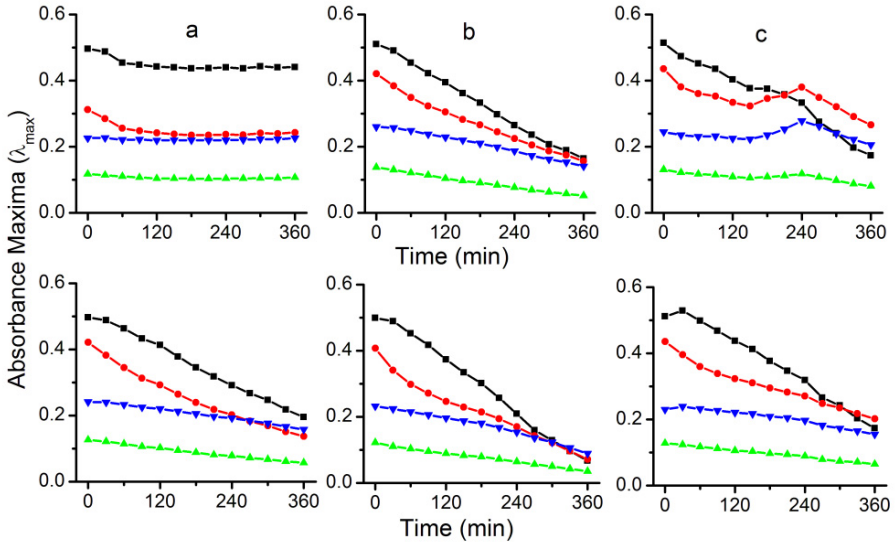

Fig. 3. Photodegradation of LH2 in (a) DDM, (b) LDAO and (c) Triton X-100 with addition of glycerol (66%, Top) and PVA (2%, Bottom). The blank-dotted, red-dotted, green-dotted and blue-dotted lines indicate the absorption maxima (λ_{max}) of B850, B800, Q_x, and carotenoid bands.

Table 1. Percent decrease of the absorption maxima at selected bands in LH2 complexes diluted in DDM, LDAO and Triton X-100 and with additional of glycerol or PVA after 360 min exposure of light.

Condition in solution	Percent decrease (%)			
	Q_y		Q_x	Cars
	B850	B800		
DDM	77.53	70.00	62.09	48.89
DDM + 66% glycerol	11.09	22.12	8.55	1.33
DDM + 2% PVA	60.77	67.46	54.76	34.44
LDAO	91.29	78.57	64.08	53.97
LDAO + 66% glycerol	67.84	62.71	62.32	45.77
LDAO + 2% PVA	86.57	82.56	71.31	61.64
Triton	not detected	74.65	62.5	not detected
Triton + 66% glycerol	66.15	38.99	38.17	15.92
Triton + 2% PVA	66.02	53.67	49.22	32.61

4. Conclusion

The light-harvesting complexes show instability against the exposure of light. It is shown that the protein is unfolded and the pigments are detached from the complex. The use of additive such as glycerol can restrain the continual degradation of light-harvesting complexes under the exposure of light.

Acknowledgements

We acknowledge financial support from Indonesian Ministry of Research and Technology through National Innovation System research grant (RT-2013-166, No: 187/M/Kp/XI/2012) and International Collaboration Research Grant from Directorate General for Higher Education (DIKTI No. 088/SP2H/PL/DIT.LITABMAS/V/2013).

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