Potential of light-harvesting of bacteriorhodopsin co-sensitized with green fluorescence protein: A new insight into bioenergy application

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20 **Abstract**

21 Herein we report for the first time on **efficient** and environmentally friendly bioenergy
22 production from bacteriorhodopsin (bR) and green fluorescent protein (GFP) as co-sensitizers. bR
23 as a transmembrane protein, acts like a light-driven proton pump in *Halobacterium salinarum*,
24 converting light energy into a proton gradient. Employing GFP beside bR can enhance the photo-
25 bioenergy production efficiency in two aspects: GFP can increase short circuit current by
26 improvement in light absorption either by extending the sensitization spectrum or making
27 fluorescence in absorption region of bR. It can also enhance open circuit voltage more than 150
28 mV by improvement in photoelectrode converging and extending electron lifetime in
29 photoelectrode. Maximum photovoltage of 680 mV and photocurrent of 1.2 mA cm^{-2} have been
30 achieved upon co-sensitization with bR/GFP. With the power conversion efficiency of 0.45%,
31 the highest efficiency of photovoltaic cell based on bR has been reported in this research.

32 **Keywords:** Bio-sensitized solar cell; Green fluorescent protein; Bacteriorhodopsin; Nanostructured
33 photoelectrode

34

35 **Introduction**

36 Bio-sensitized solar cells (BSSCs) are a promising new kind of thin film solar cells that combine
37 natural and technological components to make some kind of artificial leaves; they convert
38 sunlight into electric energy based on the sensitization of wide band gap semiconductors by
39 specific photo-active bio-molecules such as bacteriorhodopsin (bR) protein [1] and chlorophyll
40 (Chl) [2].

BSSCs and dye-sensitized solar cells (DSSCs), third generation of photovoltaic devices, differ in the type of employed light-harvesting molecules. The advantages of bio-sensitizers over synthetic dyes include their abundant availability, renewability, low cost, and easy extraction methods using cheap solvents which leads to production of environmentally friendly and fully biodegradable solar cells. In addition, like various types of natural leaves, they can operate at very low intensities of illumination and the power conversion efficiencies reaches saturation values at quite low light intensities[1-4].

However research in BSSCs is still in its infancy, but the progress in this field is really exciting.

Since the first report in 2009, several studies have been performed to improve the performance of bR based BSSCs. Applying various strategies, including using ZnO as a semiconductor[5], bR LB film formation [5], co-sensitization[1], control of dye-loading time[6], TiCl₄ treatment[6], and coating of a scattering layer[6] have led to improvement of the BSSCs. Photovoltaic characteristics of bR-based BSSCs reported in the literature are summarized in Table 1.

Table 1

Photovoltaic features of bR-based BSSCs.

Sensitizer	Nanostructured semiconductor	scattering layer	TiCl ₄ treatment	J _{sc} (mA cm ⁻²)	V _{oc} (mV)	Efficiency (%)	Reference
3Glu mutant	TiO ₂ NPs	no	no	0.09	350	-	[4]
wild-type bR	ZnO NPs	no	no	0.39	500	0.1	[5]
bR+bacterioruberin	TiO ₂ NPs	no	yes	0.45	570	0.16	[1]
wild-type bR	TiO ₂ NPs	yes	yes	1	533	0.35	[6]

In our previous study, we reported the efficient bR based BSSC with optimization of the materials at the nano-bio interface and also morphology design [6]. This paper is a continuation of our study on BSSCs; we propose the use of green fluorescent protein (GFP) beside bR to extend light absorption spectra and enhance the efficiency of BSSC. As it is shown in Fig. 1a, employing GFP beside bR can enhance cell performance in two aspects, either by extending in light absorption spectrum or making fluorescence in the range of bR light absorption spectrum. Additionally as it has been shown in Fig. 1b, the lowest unoccupied molecular orbital (LUMO) of bR and GFP located at -3.8 eV and -3.43 eV respectively, which shows the favorable electron injection to the conduction band of TiO₂ located at -4.2 eV; on the other hand the highest occupied molecular orbital (HOMO) of bR and GFP located at -5.4 eV and -6.8 eV correspondingly which is suitable respect to redox potential of iodine based electrolyte at -5 eV [7,4]. On the other hand the fluorescence spectrum of GFP can absorb by bR which can help the light absorption efficiency.

Green fluorescent protein isolated from jellyfish *Aequorea victoria* has attracted a lot of attention since Shimomura discovered the first GFP in 1962 [8], and more so after it was cloned in 1992[9]. This 27-kDa protein, contains a highly fluorescent fluorophore that is formed autocatalytically by internal cyclization and oxidation of the Ser-Tyr-Gly sequence at positions 65–67 within the 238 amino acid[10,11]. Because of its unique properties, GFP has been considered in recent years as a unique and promising material for biosensors and bioelectronics[12-15]. Here we present potential application of this protein in solar energy conversion.

Fig. 1 (a) Schematics of co-sensitization of photoelectrode with bR and GFP. (b) Energy level diagram of BSSC respect to vacuum energy based on TiO_2 photoelectrode co-sensitized with bR and GFP

Experimental

Gene cloning, protein expression and purification

Synthetic sequence was designed from the wild type sequence of GFP mRNA, with the insertion of NdeI (5' end) and XhoI (3' end) sites, subsequently. This sequence was cloned into EcoRV site of pBluescriptIIISK(-) (Bio S&T company, Toronto, Canada). In this synthetic sequence, nucleotide "t" in position 231 was replaced with nucleotide "c" to remove the restriction site of NdeI for its cloning between NdeI and XhoI site in pET21(a). Also six histidine codon, (cac) 6, was designed after start codon (atg) and restriction site of NdeI for one-step purification by immobilized metal-ion chromatography.

GFP was produced in E. coli cells, strain BL21 (DE3) using pET expression system, and purified with chelating Sepharose fast flow column (Roche Company, Germany) that was charged with 0.2 M NiSO₄. The purity of GFP was confirmed by SDS-PAGE. The protein concentration was measured by Bradford method. The purified recombinant GFP was mixed in phosphate buffer saline (PBS, 20 mM Na₂HPO₄, 0.15 M NaCl, pH 7.4) with or without 30 mM sorbitol as stabilizer and stored at -80 °C.

Purple membrane (PM) of *Halobacterium salinarum* containing bacteriorhodopsin was isolated by the well-known method of Oesterhelt and Stoebenius [16].

2.2. Fabrication of BSSCs

A nanostructured photoanode was prepared as described previously [6] utilizing 10 nm transparent TiO₂ layer (20 nm) as the active medium (Fig. 2a) for sensitization and micron-length nanofibers synthesized through electro-spinning method employed as scattering layer (Fig. 2b). The parameters obtained from our previous study on bR based BSSC were applied to improve the interface of protein and nanostructures [4]. Prepared photo-anodes were immersed in 2 cm³ of 1 kg m⁻³ bR and 324 g m⁻³ GFP for 1 h at 70°C separately. Also, both bR and GFP were adsorbed

on a TiO_2 electrode to harvest the broader range of wavelengths over the solar spectrum. Therefore, the electrode was first immersed in 1 kg m^{-3} bR for 1 h at 80°C , and then immersed in 324 g m^{-3} GFP for 1 hour at 22°C . The Platinum coated FTO sheets were used as counter electrode. The working and counter electrodes, were assembled into sealed sandwich solar cell with hot-melt Surlyn film ($30 \mu\text{m}$ thickness) as spacer between the electrodes. Redox electrolyte was introduced through small holes drilled in the counter electrode that were sealed afterward. The composition of the electrolyte was as follows: 0.1 M I_2 , 0.1 M LiI , 0.6 M tetrabutylammonium iodide, 0.5 M 4-tertbutylpyridine in acetonitrile.

Characterization and measurement

LEO 1550 field – emission scanning electron microscope was employed for morphological characterization of photoelectrodes. The absorption spectrum of samples was determined using Perkin Elmere UV-Vis-NIR Model Lambda 950 double beam spectrophotometer in the wavelength range $300\text{--}700 \text{ nm}$. For measuring emission spectrum, Cary Eclipse fluorescence spectrophotometer has been employed. The current–voltage (I–V) curves of BSSCs were measured with an Ivium stat potentiostat under simulated AM 1.5 illumination (100 mWcm^{-2}) provided by a Luzchem solar simulator. Electron lifetime in nanostructured photoelectrodes were obtained through photovoltage–decay measurements using Autolab potentiostat.

Results and discussion

The absorption spectrum for bR and GFP, and the emission spectra of GFP are shown in Fig. 2c. Wild-type GFP (GFP wt) exhibits two absorption maxima, at 395 nm and 475 nm , with the emission around 508 nm [10]. bR shows a characteristic absorption band at about 570 nm due to the retinal chromophore [17]. The related absorption/emission peaks are shown accordingly; as it

could be expected the place of peaks shifts to smaller wavelength upon immobilization of biomolecules on TiO_2 substrates. Adding GFP to bR can enhance the light absorption in two aspects; the relative absorption spectrum of bR and GFP are complementary to each other in UV and visible range of spectrum so it can extend the absorption range. Additionally, the fluorescence of GFP occurs in absorption range of bR, so it can enhance light absorption by bR.

Fig. 2 Scanning electron microscopy of (a) transparent layer consists of 20 nm TiO₂ nanoparticles, (b) scattering layer consist TiO₂ of microfibers (c) The absorption spectrum of bR and absorption and emission spectra of GFP.

Fig. 3a illustrates current-voltage characterization of BSSCs sensitized with bR, GFP and co-sensitized with bR and GFP and the extracted data has been shown in Table 2. As it is obvious, bR-sensitization resulted in relatively low open circuit voltage, around 533 mV and quite high current density around 1 mA cm^{-2} , with power conversion efficiency of 0.35%. On the other hand, sensitization with GFP caused relatively higher open circuit voltage around 640 mV and quite lowers current density around 0.7 mA cm^{-2} with power conversion efficiency of 0.23%. The relatively lower short circuit current can be related to lower absorption in visible range of spectrum. On the other hand, the higher open circuit voltage can be as a result of some interface aspects or higher coverage ratios. From the current-voltage curves of the BSSCs in Fig. 3a, the highest photovoltaic performance of the bR-GFP hybrid cell can be seen compared to the other

150 BSSCs. bR-GFP based BSSC exhibits open circuit voltage of 680 mV and short circuit current of
 151 1.2 mA cm^{-2} , with overall efficiency of 0.4% that is much higher than that of the individual cells.
 152 In addition, the fairly high V_{oc} of 680 mV achieved for the bR/GFP sensitized solar cell is well
 153 comparable with those of even DSSCs and is highest among previously reported BSSCs [18].

Fig. 3 Photovoltaic performance (a) and electron life time of photoanodes (b) sensitized by bR, GFP and co-sensitized by bR/GFP

Table 2.Photovoltaic performance of solar cells sensitized by bR, GFP and co-sensitized by bR/GFP.

Type of protein sensitizer	dye loading duration	Voc (mV)	Jsc(mA cm ⁻²)	Efficiency (%)	FF
bR	10 hours	533	1	0.35	0.66
GFP	1h	640	0.709	0.23	0.50
bR/GFP	10 hours/ 1 hour	680	1.2	0.4	0.49

Results show visible sensitization of nanocrystalline TiO₂ by GFP and bR together, with complementary absorption spectra is an effective approach to enhance the photovoltaic performance of the BSSCs. This strategy can efficiently enlarge the absorption region that leads to converting more photons into electrons at longer wavelengths. Additionally, the fluorescence spectra of GFP can enhance light absorption by bR molecules which resulted in improved photo-current.

On the other hand, the significant higher values of open circuit voltage utilizing GFP can be a sign of more efficient sensitizer coverage. It can be observed adding GFP to bR resulted in more than 150 mV enhancement in open circuit voltage. To investigate the interface features, the electron lifetime has been measured accordingly and the results have shown in Fig. 2b; it demonstrates the values of electron lifetime in these three types of photoelectrodes in various quasi-Fermi level positions where E_{Fn} and E_{f0} are the quasi Fermi level under illumination and in

dark respectively. As it was expected, much longer electron lifetime has been achieved in photoelectrode sensitized with bR and GFP together. The result indicates that GFP protein can fill in the spaces on the surface of TiO_2 nanoparticles that are not covered by bR, which results in longer electron lifetime and larger open circuit voltage in bR/GFP photoelectrodes.

The results of this report beside the unique properties of GFP including great thermal stability at temperatures up to 70 °C, stability against chemicals, such as ethylene oxide, extreme photostability, no requirement for cofactors (intrinsic or extrinsic) or staining for fluorescence, being easily expressed in bacteria, being exploited in a variety of experimental systems, and easily delivered to cells, imply the need for future studies in this field. We hope that this report will encourage further studies on BSSCs.

Conclusion:

In this paper we discuss our approach to the utilization of such a biologically inspired molecules, bR and GFP as photosensitizer to harvest the solar energy and generate photon triggered electron ejection. Employing GFP beside bR as a sensitizer on the mesoporous photoelectrode of DSSCs, the enhancement in photovoltage as well as photocurrent has been achieved. The reported result in this work is highest among the previously reported BSSCs based on bR. This result shows that GFP has the potential to consider as a bio-sensitizer and it can efficiently increase the open circuit voltage of BSSC.

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