Original article

Photocurrent generation by recombinant allophycocyanin trimer multilayer on TiO₂ electrode

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ABSTRACT

A recombinant allophycocyanin trimer was successfully immobilized on a mesoporous TiO₂ electrode. The formation of the immobilized surface was confirmed by multilayer adsorption of protein complexes. The key biophotovoltaic parameters were obtained, which showed that the recombinant allophycocyanin trimer could be a candidate for photosensitizer materials. The values of short-circuit current, open-circuit voltage, fill factor, and conversion efficiency were up to 0.73 mA/cm², 0.52 V, 0.69, and 0.26%, respectively.

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1. Introduction

Nature has been developing and optimizing photosynthesis for billions of years. Recently, the development of Grätzel cells was inspired by the possibility of using biological macromolecules with pigments as sensitizers for dye-sensitized solar-cells [11]. So far, studies have focused on bacteriorhodopsin (BR), photosystem I (PSI), photosystem II (PSII), and light-harvesting complexes (LHCl) to create direct-conversion photovoltaic devices [2–5].

The allophycocyanin (APC) trimer is a primary composition of phycobilisomes cores, containing only phycocyanobilin, for efficient lower-energy photon (maximum 650 nm) funneling pathways. Relatively high-resolution X-ray crystallography and electron microscopy images have revealed the details of four native APC trimer structures from cyanobacteria [6,7]. A recent study has shown that, similar to the LHCl, photon energy transfer from the core to the chlorophylls reaction centers in the APC trimer follows the Förster resonance mechanism on a time-scale of 430 – 440 femtoseconds with a very high efficiency [8]. In 2009, Liu et al. assembled a highly soluble self-assembled bioengineered APC trimer with N-terminal polyhistidines (14 unstructured residues including 6 × His tags) from Synechocystis sp. PCC 6803, which were expressed in Escherichia coli (Supplementary Fig. S1) [9]. The native structure, stability, and fluorescent characteristics of the recombinant APC trimer were determined using fluorescence and absorption spectrometry, and the results supported a previous report that the longer His tag near the N-terminus did not significantly interfere with the trimer assembly [9,10].

Large-scale purification of the recombinant APC trimer has also been achieved. In a previous attempt to develop its potential biophotocatalytic capacities, we fabricated an APC-trimer-sensitized solar-cell. In this study, we report the generation of light-induced electrical currents and voltages by self-assembled biophotovoltaics in which the recombinant APC trimer was sensitized on a nanostructured mesoporous TiO₂ electrode. In addition, the electrical and optical properties were tested under the standard air-mass 1.5 sun light (100 mW/cm²) and a lower light intensity (75 mW/cm²). To confirm the characteristics of APC trimer adsorption, we used confocal laser scanning microscopy (CLSM), field-emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM), and fluorescence spectrophotometer to detect the sensitized surface of the TiO₂ electrode.

2. Experimental

In the preparation of the bioengineered APC trimer, the strains and purification methods reported previously were used [9]. Briefly, the recombinant APC trimer was purified through nickel affinity chromatography. Nano-scale spherical particles of TiO₂ (approximately 20 nm in diameter) were deposited on the surface.
of the prepared FTO glass to fabricate semiconductor films as the anode, using a screen-printing method. CLSM, AFM, and FE-SEM were used to examine the immobilization of the sensitized photoanode at the nanoscale. More details of this experiment were given in the supplementary materials.

3. Results and discussion

Fig. 1 shows a set of images (CLSM, AFM, and FE-SEM), demonstrating the deposition of an APC trimer complex multilayer on the sensitized surface of the photoanode. CLSM is a sensitive and convenient method for detecting signals from the recombinant APC trimer because of its maximum fluorescence emission at 661 nm under 600 nm excitation and fluorescence quantum yield at 0.72 [9,11]. The bright fluorescence image revealed the presence of adsorbed APC trimer complexes with non-uniform patterns, compared with the faint visual field of the blank control (unsensitized photoanode) under the same conditions (Fig. 1a and b). FE-SEM is a powerful method that enables the visualization of the nanoscale geometry of the sensitized surface at high resolution.
resolution (ranging from 1 to 2 nm) [12]. Fig. 1c shows that the blank control had an uneven mesoporous structure, with pore sizes ranging from 8 nm to larger than 100 nm, and TiO2 nanoparticles (approximately 20 nm in diameter), as described above. The two-dimensional SEM image in Fig 1d showed that there were rigid spherical particles larger than 50 nm on the sensitized surface, highlighting the distinct differences from the blank control image. These observations suggested that the surface might have formed by multilayer protein adsorption. As a verification to the SEM data, AFM designed for complex biosurface investigations provided the three-dimensional topography with almost identical atomic resolution and super-sensitivity [13,14]. As shown by the AFM three-dimensional height images, the features of the sensitized surface were quite different from those of the blank control on an area of 1 µm × 1 µm (Fig. 1e and f). There were many sharp tall peaks in the height image of the nanoscale surface of the blank control (Fig. 1e), and the size axes of the peaks were approximately 20 nm in the peak force error image (Supplementary Fig. S3), as observed in the SEM image in Fig. 1c. Compared to the blank control image, the surface morphology of the sensitized surface showed fewer and lower hills, with rougher wrinkles, like "valleys and peaks", coated with non-rigid particles; this was consistent with the smooth peak force error image (Supplementary Fig. S3) and the SEM image (Fig. 1d). In addition, the AFM scan clearly demonstrated a more than 20 nm decrease in height, larger than the values of the maximum diameter of the trimer molecule (approximately 3 nm short axis, 12 nm long axis) obtained by electron microscopy; this was probably caused by cross-linking of deposited APC trimer complexes with each other (Fig. 1f) [7]. The SEM image of cross section of a sensitized photoanode (Supplementary Fig. S4) also showed a more than one-layer adsorbed protein thickness, supporting the formation of irregular multilayer protein agglomerates.

The results of quantitative morphological investigations shown in Fig. 1 confirmed our hypothesis that the immobilized self-assembled APC trimers on a sensitized photoanode formed a multilayer with a non-specific orientation, similar to the findings from earlier reports [15–17]. Moreover, the very similar fluorescence emission spectra from the APC trimer complexes in solution and on TiO2 both exhibited a fluorescence emission maximum at 660 nm with a shoulder at around 640 nm (Fig. 2a); this demonstrated that the APC trimer complexes were not disassembled following the formation of the multilayer structure [9]. It is possible that electrostatic interactions, especially hydrogen bonds, which are strong noncovalent forces in biological systems through functional groups such as carbonyl, amide, or imidazole, especially in macromolecules, are responsible for the multilayer adsorption, as noted previously [5,15,18]. Previous reports suggested that photocurrent generation based on photoelectrode complex structures (BR, PSI, and PSII) is a result of electron transfer and continuous re-reductions in solution, after charge separation originating from the initial photoexcitation [4,17,19]. However, as in antenna complexes, the photocurrent in this case was ascribed to the photoexcited trimer and subsequent electron transfer, as demonstrated in previous reports (Supplementary Fig. S5) [5,20].

The results in Fig. 2b show the key parameters that were measured for a typical performance of the APC-trimer-sensitized solar-cells. The maximum values of the short-circuit current ($I_{oc}$) and open-circuit voltage ($V_{oc}$) are 0.73 mA/cm$^2$ and 0.52 V, respectively, under 100 mW/cm$^2$; the maximum value of the fill factor (FF) is 0.69 for measurements of the diode behavior of the cell; the maximum efficiency ($\eta$) is 0.26% for measurements of the electrical power of the cell under 100 mW/cm$^2$; the values of $I_{sc}$, $V_{oc}$, FF, and $\eta$ under 75 mW/cm$^2$ are 0.57 mA/cm$^2$, 0.54 V, 0.73 and 0.30%, respectively. The $I$–$V$ curve (Fig. 2b) for this system on an exposure to 100 mW/cm$^2$ is similar to those recently reported in similar sensitized solar-cell systems (TiO2 photoanodes and similar electrolyte solutions), in which the performance of a LHCI-sensitized solar-cell was $I_{sc} = 0.49$ mA/cm$^2$ and $V_{oc} = 0.47$ V, and the photocurrent response of a PSI-sensitized solar-cell was $I_{sc} = 0.362$ mA/cm$^2$ and $V_{oc} = 0.5$ V [2,5]. The $I_{sc}$ achieved with the recombinant APC trimer is over 50% higher than those with LHCI and PSI under a $V_{oc}$ of roughly 0.5 V, which indicates an enhanced photocurrent.

4. Conclusion

In the present study, a recombinant APC trimer was successfully sensitized on a nanostructured mesoporous TiO2 electrode. The immobilized self-assembled APC trimer was confirmed to be a multilayer with a non-specific orientation, using CLSM, AFM, and FE-SEM. The performance of a biohybrid artificial solar-cell suggests that the APC trimer shows great potential as a photosensitizer material for visible light photon utilizations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2012.12.011.

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