Available online at www.sciencedirect.com

ScienceDirect

Ceramics International 40 (2014) 2943–2951

CERAMICS INTERNATIONAL

www.elsevier.com/locate/ceramint

Photoinduced electron transfer mechanism between green fluorescent protein molecules and metal oxide nanoparticles

Sabriye Acikgoz[a,d,*], Yakup Ulusu[b], Seckin Akin[c], Savas Sonmezoglu[d], Isa Gokce[e], Mehmet Naci Inci[a]

[a]Department of Physics, Boğaziçi University, Bebek, 34342 Istanbul, Turkey
[b]Department of Bioengineering, Faculty of Engineering, Karamanoğlu Mehmetbey University, Karaman 70100, Turkey
[c]Department of Physics, Faculty of Kamil Özdağ Science, Karamanoğlu Mehmetbey University, Karaman 70100, Turkey
[d]Department of Material Science and Engineering, Faculty of Engineering, Karamanoğlu Mehmetbey University, Karaman 70100, Turkey
[e]Department of Bioengineering, Faculty of Engineering, Gaziosmanpaşa University, Tokat 60240, Turkey

Received 4 September 2013; received in revised form 4 October 2013; accepted 4 October 2013
Available online 16 October 2013

Abstract

Green fluorescent protein (GFP) molecules are attached to titanium dioxide and cadmium oxide nanoparticles via sol–gel method and fluorescence dynamics of such a protein–metal oxide assembly is investigated with a conventional time correlated single photon counting technique. As compared to free fluorescent protein molecules, time-resolved experiments show that the fluorescence lifetime of GFP molecules bound to these metal oxide nanoparticles gets shortened dramatically. Such a decrease in the lifetime is measured to be 22 and 43 percent for cadmium oxide and titanium dioxide respectively, which is due to photoinduced electron transfer mechanism caused by the interaction of GFP molecules (donor) and metal oxide nanoparticles (acceptor). Our results yield electron transfer rates of $3.139 \times 10^8\ s^{-1}$ and $1.182 \times 10^8\ s^{-1}$ from the GFP molecules to titanium dioxide and cadmium oxide nanoparticles, respectively. The electron transfer rates show a marked decrease with increasing driving force energy. This effect represents a clear example of the Marcus inverted region electron transfer process.

Keywords: D. TiO_2; CdO; GFP; Electron transfer

1. Introduction

Green fluorescent protein (GFP) is a protein of 238 amino acids with a molecular weight of 27 kDa, which emits a bright green fluorescence with a peak wavelength at 509 nm when exposed to ultraviolet or blue light. GFP emits green fluorescence without a need in any enzyme or co-factors. The emission of the GFP of the jellyfish *Aequora Victoria* originates from the spontaneous formation of an emitting chromophore inside a rigid β-barrel structure [1]. The GFP fluorescence activity can be detected with minimal handling efforts, for example, it does not need the detection tools like use of a fluorescence microscope, a fluorometer, a fluorescence-activated cell sorting machine, an imaging micro plate reader, or a lysate preparation [2]. Many GFP mutants have been reported in the scientific literature and more than 20 crystal structures of GFP mutants and homologs are listed in the Protein Data Bank [3]. Although the GFP mutants have quite different spectroscopic characteristics, their structural features are remarkably similar [4].

GFP is an accomplished fluorescent molecule widely used in cell imaging applications, gene expression, visualizing protein–protein interactions and protein localization due to its unique characteristics [5–7]. Recently, Bogdanov et al. discovered a new feature of GFPs of diverse origins to act as the light-induced electron donors in photochemical reactions with various electron acceptors [8]. Moreover, the interaction mechanism between fluorescent proteins and nanoparticles

*Corresponding author at: Department of Material Science and Engineering, Faculty of Engineering, Karamanoğlu Mehmetbey University, Karaman 70100, Turkey. Tel.: +90 54 4218 7584; fax: +90 33 8226 2214.
E-mail addresses: sabriyeacikgoz@kmu.edu.tr, sabriyeacikgoz@gmail.com (S. Acikgoz).

0272-8842/$ - see front matter © 2013 Elsevier Ltd and Techna Group S.r.l. All rights reserved.
http://dx.doi.org/10.1016/j.ceramint.2013.10.017

could provide further control over the fabrication of nano-optic and nano-electronic devices. Quenching of green fluorescent molecules, when it is in close proximity to a metal nanoparticle, like gold or silver, has been successfully studied both theoretically and experimentally [9,10]. However, excitation mechanism of GFP-metal oxide nanoparticles (MON) has not been clarified yet, which is envisaged to be one of the most popular parts of the nano-technological applications.

Metal oxide nanoparticles are emerging as highly attractive materials for many fields of technology including catalysis, sensing, optoelectronic devices, environmental remediation and energy conversion [11–13]. The most commonly used metal oxide nanoparticles are titanium dioxide (TiO_2), zinc oxide (ZnO), iron (III) oxide (Fe_2O_3), Chromium (III) oxide (Cr_2O_3) and Cadmium oxide (CdO). Especially, TiO_2 and ZnO are the preferred nanoparticle types due to their large band gap energy and their high electron mobility [14,15]. Stable metal oxide nanoparticles cannot absorb visible light due to their relatively wide band gaps. Sensitization of these metal oxide materials with photo sensitizers – such as with organic dyes – allow absorption of the visible light. Therefore, such systems have been extensively studied in silver halide photography, electrophotography, and – more recently – in solar energy cells [16]. In the sensitization process, the excited dye molecule injects an electron into the conducting band of the metal oxide nanoparticle within a few picoseconds [17]. Then, the oxidized dye is reduced back to its ground state and the injected electron flows through the semiconductor network.

In this paper, the effects of titanium dioxide and cadmium oxide nanoparticles on the fluorescence intensity and lifetime dynamics of the green fluorescent protein molecules are examined. Recently, it has been demonstrated that the band gap energy of a metal oxide nanoparticle is strongly effective on the performance of MON based devices [18]. Therefore, two different metal oxide nanoparticles are studied in this work, one of them with a wide band gap energy (TiO_2, 3.42 eV) and the other with a relatively narrower band gap energy (CdO, 2.36 eV). It is observed that the average lifetime of the GFP molecules on the metal oxide nanoparticles is significantly shortened than that on a glass substrate. As a consequence of photoinduced electron transfer process between GFP and metal oxide nanoparticles, the fluorescence lifetime of GFP on CdO and TiO_2 nanoparticles drops from 2.419 ns down to 1.881 ns and 1.375 ns, respectively. Moreover, the electron injection yield of the GFP/TiO_2 nanoparticle system is expectedly around three times of that of the GFP/CdO nanoparticle system.

Relentless efforts are underway all over the world to obtain efficient photovoltaic energy conversion using dye sensitized metal oxide or semiconductor nanomaterials. During the last few years, a number of dyes, such as phthalocyanines, triphenyl methane, xanthenes, coumarins, porphyrins and ruthenium have been tested as sensitizer [19]. These dyes and those chemically engineered are hard to put up and are too expensive. Therefore, natural dye sensitizers should be investigated to develop low cost and environmental friendly green solar cells. In the present article, photoinduced electron transfer dynamics of GFP bound to TiO_2 and CdO nanoparticles is discussed in details. Our time resolved experimental results suggest that the green fluorescent protein molecules have a great potential to be remarkable candidates as sensitizers in photovoltaic energy conversion devices.

2. Experimental section

2.1. Expression and purification of hexa histidine tagged GFP

The plasmid vector pBAD–GFPuv carrying deoxyribonucleic acid (DNA) fragment encoding for GFP is digested using two different restriction enzymes (*Nhe* I and *Eco* RI) The GFP-encoded DNA fragment is introduced into pET28a plasmid (Merck; Novagen) using *Nhe* I and *Eco* RI restriction sites. The GFP gene is ligated into pET28a after gel purification of both vector and insert. The final plasmid is named pETGFP and DNA sequencing of this plasmid showed that the hexa histidine-tagged GFP-encoded DNA fragment is correctly inserted. Six histidine-tagged GFP is expressed in an *Escherichia coli* BL21 DE3 (pLysE) strain. The strain is transformed with pETGFP plasmid and grown on Luira Bertoni (LB) plates with kanamycin (40 mg/ml) and chloramphenicol (35 mg/ml) selection. Four milliliters of LB medium in a screw capped test tube with antibiotics is inoculated with a single *E. coli* colony and grown overnight at 37 °C. A 4 ml overnight culture is introduced into 600 mL of the LB medium in 2 L flasks containing kanamycin and chloramphenicol. Bacteria are grown up to an optical density (OD 600) of 0.8 and induced by the addition of a final concentration of 1 mM isopropyl-thiogalactopyranoside (IPTG) and then grown for additional 4 h. *E. coli* cells are harvested and resuspended in 20 mM phosphate and 300 mM sodium chloride (NaCl) (pH 8) buffer containing RNAse, DNAse, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine). The cells are lysed in a French press and the supernatant is obtained by ultracentrifugation (Beckman Coulter Optima L-80 ultracentrifuge and Ti 45 rotor) at 40,000 rpm (125,000*g*) for 1 h. The N-terminal hexa-histidine-tag facilitated purification of the GFP by means of a Ni–NTA agarose affinity resin (Qiagen). The fusion protein is washed onto the column with a 20 mM phosphate and 300 mM NaCl buffer, and then additionally washed with the same buffer containing 50 mM imidazole and eluted in 300 mM imidazole, pH 7.0. The expression of GFP protein is qualitatively analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). The concentration of protein is determined by UV absorption at 280 nm. The molecular weight of the his-tagged GFP is 28.890 kDa and its extinction coefficient is 22.015 M^{-1} cm^{-1} at 280 nm.

2.2. Synthesis of TiO_2 nanoparticle solution

In order to prepare a TiO_2 solution, firstly titanium tetraispropoxide (2.4 mL, $Ti(OC_3H_7)_4$, ex. Ti ≥ 98%, Merck) is added in ethanol (25 mL, C_2H_6O, 99.9%, Merck), and the solution is kept in a magnetic stirrer for one hour. Next, glacial acetic acid (5 mL, $C_2H_4O_2$, 99.9%, Merck), triethylamine (1.5 mL, $(C_2H_5)_3N$, 99%, Merck) and ethanol (25 mL) are added to the solution. After, it is mixed in the magnetic stirrer