Available online at www.sciencedirect.com
SCIENCE DIRECT®
Current Applied Physics 6 (2006) 839–843
Current Applied Physics
An official journal of the KPS
www.elsevier.com/locate/cap
www.kps.or.kr

Molecular rectifier consisting of cytochrome *c*/GFP heterolayer by using metal coated optical fiber tip

Jeong-Woo Choi [a,*], Yun Suk Nam [a], Sung-Cheul Jeong [a], Won Hong Lee [a], Michael C. Petty [b]

[a] *Department of Chemical and Biomolecular Engineering, and Program of Integrated Biotechnology, Sogang University, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, Republic of Korea*
[b] *Centre for Molecular and Nanoscale Electronics, University of Durham, Durham DH1 3LE, UK*

Received 1 December 2004; accepted 9 March 2005
Available online 23 May 2005

Abstract

The molecular rectifier consisting of protein heterolayer is investigated in molecular-scale for the construction of bioelectronic device. Cytochrome *c* and green fluorescence protein were used as an electron acceptor and a sensitizer in the molecular layer by mimicking the bacterial photosynthesis. Self-assembled monolayer of thiol-modified cytochrome *c* was formed on Au coated glass, and then green fluorescence protein was adsorbed onto the cytochrome *c* surface by electrostatic attraction. The formation of cytochrome *c* layer onto the Au substrate and green fluorescence protein adsorption onto the cytochrome *c* layer were observed by the surface plasmon resonance measurement. The surface of heterolayer was observed and analyzed by the scanning tunneling microscopy. The rectifying property of proposed heterolayer was achieved by the scanning tunneling spectroscopy based current–voltage measurement. Finally, the molecular rectifying property was verified.

PACS: 85.65

Keywords: Cytochrome *c*; Green fluorescence protein; Self-assembly; Molecular rectifier; Scanning tunneling spectroscopy; Scanning tunneling microscopy

1. Introduction

The transfer of an electron from one side of a molecule to the other or between molecules is one of the most fundamental and ubiquitous processes in electronic materials and biological systems [1]. The control and exploitation of this process in organized molecular systems is a major proposition for molecular electronics and bioelectronics [2]. Progress in molecular electronic devices engineering is still rather modest due to problems associated with the elucidation and effective control of such structures and interactions at the nanometer level. Photoinduced electron transport processes in nature, such as photoelectric conversion and long-range electron transfer in photosynthetic organisms, are known to be occurred not only very efficiently but also unidirectionally guided by biomolecular functional groups [1,3,4]. The concepts for the development of new functional bioelectronic devices can be inspired from the biological systems such as the electron transfer chain or the photosynthetic reaction center. By mimicking the organization of the functional molecules in a biological electron transfer system, the biomolecular electronic devices can be realized artificially. In the initial process of photosynthesis, a biological electron transfer system, photoelectric

* Corresponding author. Tel.: +82 2 705 8480; fax: +82 2 711 0439.
E-mail address: jwchoi@ccs.sogang.ac.kr (J.-W. Choi).

1567-1739/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cap.2005.03.003

conversion occurs and then long-range electron transfer takes place very efficiently in one direction through the biomolecules [5]. The specific energy and electron transfer take place on a molecular-scale due to the redox potential difference as well as the electron transfer property of functional molecules, especially the electron acceptor, sensitizer, and electron donor [6]. Molecular heterolayers fabricated by the appropriate techniques can be used as model systems of the corresponding photosynthetic reaction center in the biological system. Substantial interest in recent years has focused upon thin film fabrication or the formation of biomaterials mono- and multi-layers on solid surfaces, by using the Langmuir–Blodgett (LB) film technique or self-assembly (SA) technique [7,8].

Based on these techniques, various artificial molecular devices have been fabricated to mimic the electron transport function of biological photosynthesis. Fujihira et al. have reported the electrochemical LB photodiode consisting of three functional organic molecules or as an aligned triad on the electrode, which worked in electrolyte solution [6,9]. Isoda et al. investigated the optical and electrical characteristics of a molecular photodiode consisting of flavin-porphyrin hetero-LB films [10]. The authors investigated the molecular diode consisting of hetero organic LB film of four functional organic molecules ferrocene, flavin, viologen, and TCNQ used as an electron donor, sensitizer, relay and acceptor, respectively [11]. Further we have investigated the fabrication of biomolecular photodiodes consisting of hetero proteins/organic molecular layers such as green fluorescent protein (GFP), viologen, TCNQ and cytochrome *c*, in which heterolayers were formed by LB film technique [12,13]. The photoinduced electron transfer of the biomolecular photodiode with metal/insulator/metal (MIM) structure was observed [14,15]. The biomolecular photodiode consisting of only hetero protein layers fabricated by SA technique without including organic molecular layers has been reported by authors [16]. And, the photoinduced electron transfer and rectifying function of hetero protein layers in molecular-scale have been previously investigated by the scanning tunneling spectroscopy (STS) based current–voltage (*I–V*) characteristics [16].

Cytochrome *c* is one of the most widely studied proteins due to its stability and solubility in water, and its general availability. The structure, physiological and physicochemical properties, and applications of cytochrome *c*, have been extensively investigated in various research fields [17,18]. The key feature of cytochrome *c*, the ability to transfer electrons, is driven by a redox state change and a conformational change of the heme group, which is covalently bound via two thioether linkages formed by two cysteine side chains and two axial ligands, histidine and methionine. Since cytochrome *c* is a constituent that acts as an electron transfer protein in the bacterial photosynthetic reaction center, cytochrome *c* could be used as an electron acceptor in molecular electronic devices, which mimic the biological photosynthetic mechanism [15,19].

The GFP from the jellyfish *Aequorea victoria* has been widely used as a reporter in the determination of gene expression and protein localization [20]. Purified GFP, a protein of 238 amino acids, absorbs blue light and emits green light (peak emission at 510 nm). This fluorescence is very stable, and no photobleaching is observed [21]. Since GFP has a very high fluorescence quantum yield, approximately 80%, it could be chosen as a sensitizer in the development of a molecular electronic device mimicking biological photosynthesis. In this study, the molecular rectifier consisting of hetero protein layer is proposed. This is the first report on the biomolecular rectifier in molecular-scale by using the Au coated optical fiber probe.

2. Materials and methods

The hetero protein layer consisted of GFP and cytochrome *c*, which functioned as a sensitizer and an electron acceptor, respectively. Cytochrome *c* (extracted from horse heart, type VI) and GFP were used as an electron acceptor (A) and a sensitizer (S), respectively. GFP (rEGFP) and cytochrome *c* were purchased from CLONTECH (Palo Alto, California, USA) and the Sigma Chemical Company (St. Louis, USA), respectively, and used without further purification. By the adsorption of GFP and cytochrome *c* onto Au substrate, hetero molecular films were fabricated. The Au coated substrate was made by thermal evaporation on cleaned cover glass substrate. To deposit the cytochrome *c* onto the Au substrate, thiol (–SH) functional group was synthesized on the surface of cytochrome *c* molecules. Modification of cytochrome *c* to have thiol group was described in author's previous reports [22,23]. Au substrate was dipped into the cytochrome *c* solution for 24 h, and then self-assembled cytochrome *c* layer was formed on Au substrate due to the thiol group on its surface. GFP layer was deposited onto cytochrome *c* layer by an electrostatic attractive force by dipping the cytochrome *c*/Au in GFP solution of pH 8. In pH 8, cytochrome *c* molecules with isoelectric point of 10.5 have a positively charged surface and GFP molecules with isoelectric point of 5.0 have a negatively charged surface. Therefore, GFP could be easily and spontaneously adsorbed onto the cytochrome *c* self-assembled layer by electrostatic attraction. The measurements of surface plasmon resonance (SPR, Multiskop, Optrel GBR, Germany) spectrum and morphology by scanning tunneling microscopy (STM, AutoProbe CP, PSI, USA) with the optical fiber were done to verify the formation of hetero protein layer. Generally, sharp metal wire was