재조합 아주린과 싸이토크롬 C의 헤테로 단백질막으로 구성된 다층 메모리 디바이스

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Multi-level Biomemory Device Composed of Recombinant Azurin/Cytochrome c Hetero-protein Layers

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Introduction

Several principles have been proposed for developing new concept-memory devices to overcome the physical and technical limitations of the conventional silicon based memory devices [1]. Our previous works including a shift register memory using biomolecular hetero Langmuir-Blodgett (LB) film to achieve simple electronic functions of the molecular diode and switching device with photocurrent generation and a rectifying property [2,3]. Starting from these simple electronic devices, various electrochemical memories have been proposed [4]. However, the fabrication of the molecular memory using biomolecule based on the electrochemical method has been developed yet. In this work, an efficient way of azurin thin film formation on Au electrodes and cytochrome c layer adsorbed onto immobilized azurin layer are introduced which will act as a memory element to achieve the basic memory function with multi-state storage capacity in the present protein-based biomemory device. Scanning tunnelingmicroscopy was carried out to investigate the morphology of the protein immobilized Au electrodes. The electrochemical properties of cytochrome c/recombinant azurin heterolayer on Au electrodes were investigated by cyclic voltammetry (CV) and the memory function wasverified using open circuit potential amperometry (OCPA).

Theory and experiments

Cytochrome c extracted from *horse heart* was purchased from Sigma-Aldrich (Sigma Chemical Company, St. Louis, USA). Cytochrome c solution of 10 mM was prepared in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer of pH 7.0. And the plasmids, to express azurin, containing genes for azurins, were transformed into *E. coli* BL21 Star (DE3). The transformants were grown to 0.6 OD at 37 °C in shake flasks containing 1L of luria-bertani medium with 50 mg/mL ampicillin. The expression was induced by adding isopropyl *β*-D-thiogalactopyranoside (IPTG) to a final concentration of 0.84 mM. The transformed cells were grown for an additional 16 hr at 37 °C. The cells were harvested by centrifugation at 5000 g for 15 min at 4 °C. The cell paste was resuspended in sucrose buffer and subjected to osmotic shock (0.5 mM $MgCl_2$). Contaminating proteins were precipitated from the periplasmic preparation by decreasing the pH to 3.8 (50 mM sodium acetate), yielding azurin-containing supernatant. Expressed azurin solution of 10 mM was prepared in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer of pH 7.0Distilled and deionized (DI) water was used for cleaning the substrates. For making heterofilms, the prepared Au substrate was immersed in recombinant azurin solution for direct immobilization of protein on Au surface. After 3hr incubation, a self-assembled recombinant azurin layer formed on the Au surface due to the thiol group being on its surface. A cytochrome c layer was deposited onto the recombinant azurin layer by electrostatic attraction by dipping the recombinant azurin/Au in cytochrome c solution of pH 7.0. In order to verify the fabricted protein biofilm, the SPR measuring were conducted using surface plasmon resonance spectroscope (Multiskop Tm, Optrel GmbH, Germany). The Kretchmann's attenuated total reflection (ATR) configuration was used for SPR experiments. The shift in the SPR angle was used to measure refractive index change at a surface. Also, the surface morphology study of the biomolecular/organic hetero-structure films (cytochrome c / 11-MUA) on gold substrates was carried out using scanning tunneling microscopy (Multimode, Vecco, USA). Atomic force microscpy (AFM) was used for the analysis of inorganic surfaces at nanometer resolution. The recombinant azurin/cytochrome c heterolayer immobilized onto Au surface, Ag/AgCl in saturated KCl (Bas, USA), and a Pt wire (Bas, USA) were used as working, reference, and counter electrodes, respectively. Cyclic voltammogram and chornoamperograms were recorded using a multi-channel potentiostat/galvanostat (CH Instruments Inc., TX, USA). The electrochemical measurements were carried out in

HEPES buffer solution (Sigma, USA) and all experiments were carried out at a constant temperature of 25 ± 2 °C.

Results and discussion

To confirm the fabricated heterofilms consisted of recombinant azurin/cytochrome c compared to recombinant azurin mixed with cytochrome c films measured by SPR. The SPR angle shift was observed for each surface and the results were compared to understand the immobilization of the recombinant azurin/cytochrome c heterolayer. It was observed that there is an angle shift from 43.000±0.016° to 43.310±0.058°, when cytochrome c absorbed directly onto self-assembled cysteine-modified azurin layer was compared with bare gold surface. Also there is a significant angle shift from 40.000±0.016°to 43.730±0.025° when cysteine-modified azurin mixed with cytochrome c layer was compared with bare gold, and it shows in fig. 2. So, finally it is concluded that recombinant azurin/cytochrome c heterolayer was efficiently immobilized on the Au surface for its biodevice application. And, the surface morphology of the fabricated biosurface was investigated measuring AFM. Fig. 3a shows the cytochrome c adsorbed onto recombinant azurin self-assembled layer is clearly visible on Au surface and the molecules have a size of 10~15 nm was formed. Fig. 3b shows the recombinant azurin mixed with cytochrome c immobilized onto Au surface. This result indicates that the protein is not well adsorbed on the Au surface. Cyclic voltammetry for the recombinant azurin/cytochrome c heterolayer immobilized on Au surface in 10 mM HEPES buffer (pH 7.0) in potential range of 0.4 to -0.1 V vs Ag/AgCl electrode at a scan rate 50 mVs^{-1} was performed. Fig. 4 shows the voltammograms obtained for the heterolayer with well defined symmetric two redox peaks with two anodic waves, at E_{pa} of 0.062 V vs. Ag/AgCl and cathodic wave at E_{pc} of 0.184 V vs. Ag/AgCl which corresponds to the redox process of recombinant azurin center $Cu^{2+/1+}$. In addition to, anodic wave at E_{pa} of 0.0131 V vs. Ag/AgCl and cathodic wave at E_{pc} of 0.294 V vs. Ag/AgCl which corresponds to the redox process of cytochrome c center $Fe^{2+/1+}$. In order to reliably store and read the charge in the recombinant azurin and cytochrome c molecules, it is necessary to eliminate the need for continuous application of potential. This is accomplished by the technique called OCPA. Aftercharge storage, the counter electrode is disconnected, and the memory element decays to an OCP. Applying oxidation voltage is another writing step whereas applying the open circuit voltage is another reading step with respect to the measurement of currents. Finally, the application of another reduction potential erases all the stored charge. Like this, input 2 pair of redox potentials are applied to the protein film for duration of 280 ms. and observed clear transient currents for 2 pair of the charge to write, read, and erase functions that are requisite for the molecular memory storage device. From these results, a multi-state protein based biomemory can be realized.

Conclusion

The multi-state biomemory device composed of cysteine-modified azurin and cytochrome c was developed. Based on the topologies by STM measurements and confirming the immobilization with SPR, the fabricated recombinant azurin/cytochrome c heterolayer on the Au surface was verified. The verified concept of protein-based memory device proposed here could be realized and produced commercially when long term storage of multi-state charge information and the new manipulation technology for ultra low signal from single molecule could be achieved

Acknowledgment

This research was supported by the Nano/Bio science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Education, Science and Technology (MEST), by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (M10644080003-06N4408-00310), and by the Ministry of Knowledge Economy (MKE) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Strategic Technology.

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Figure Cations

Figure 1. Schematic diagram of (a) recombinant azurin immobilized on Au surface, and cytochrome c adsorbed on self-assembled azurin layer. (b) recombinat azurin mixed with cytochrome c adsorbed on Au surface.

.**Figure 2.** Confirming the immobilization of heterolayer by SPR. SPR spectroscopy of a : bare gold surface, b : cysteine-modified azurin immobilized on gold surface, and c the adsorption of cytochrome c after cysteine-modified azurin immobilized onto the gold surface.

Figure 3. AFM images of (a) the adsorption of cytochrome c after cysteine-modified azurin immobilized on gold surface. (b) cysteine modified azurin mixed with cytochrome c adsorbed onto the gold surface.

Figure 4. Cyclic voltammogram of the adsorption of cytochrome c after cysteine-modified azurin immobilized on gold surface.

Figure 5. The memory function characteristics of multi-state protein based biomemory device.

Fig. 1 Lee, *et al*

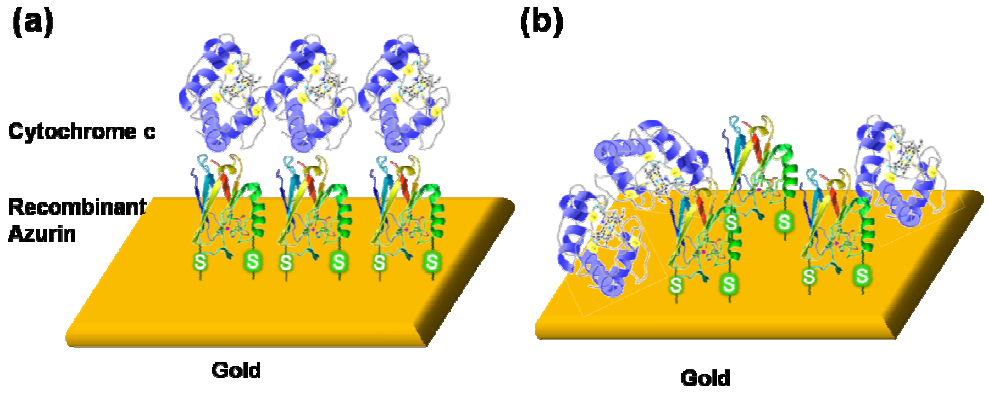

Fig. 2 Lee, *et al.*

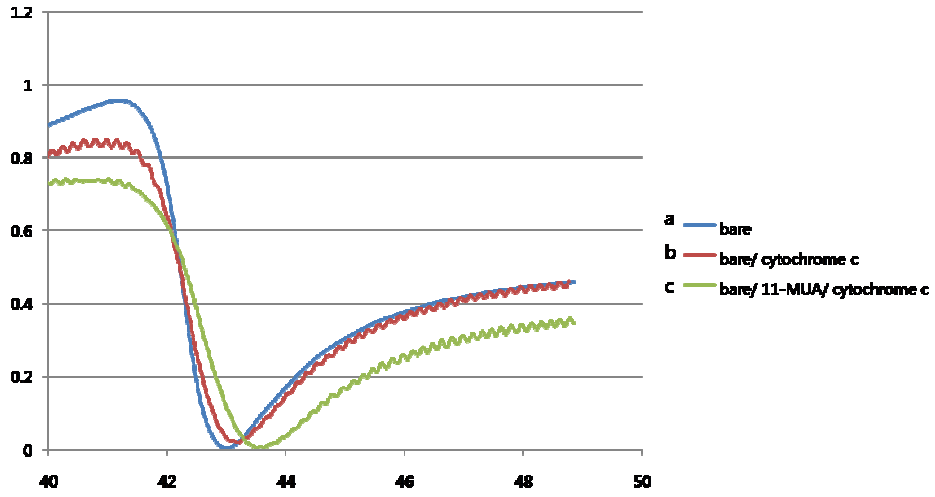

Fig. 3 Lee, *et al.*

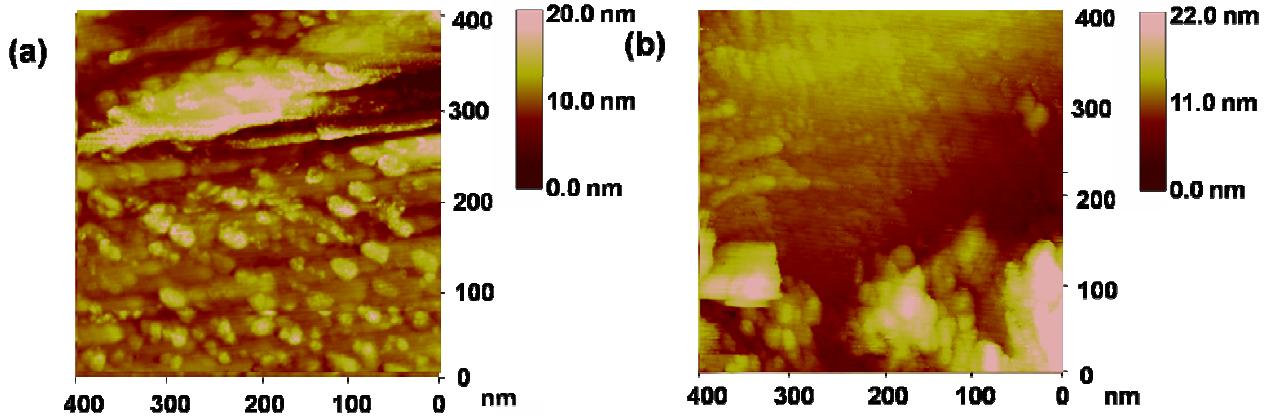

Fig. 4 Lee, *et al.*

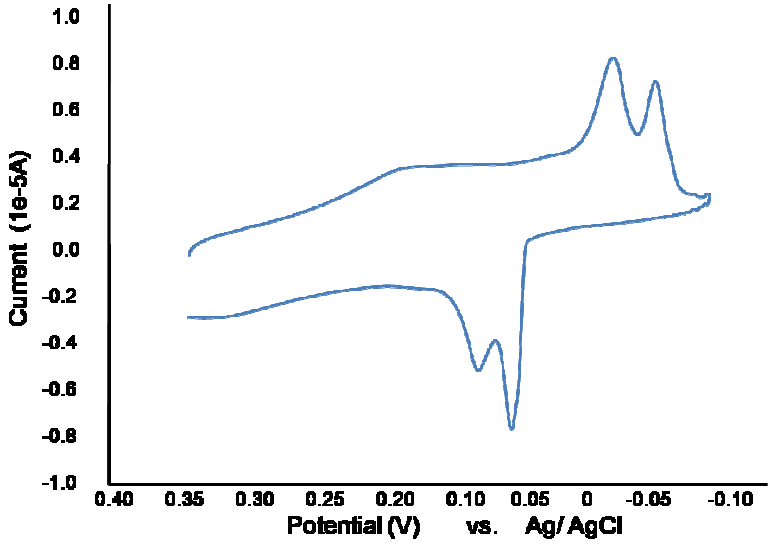

Fig. 5 Lee, *et al.*