BIOCHIP JOURNAL, Vol. 3, No. 2, 157-163, June 2009

Charge Storage in Redox-active Azurin Monolayer on 11-MUA Modified Gold Surface

Jeong-Woo Choi[1,2], **Jin Seok Kim**[1], **Sang-Uk Kim**[2] **& Junhong Min**[3]

[1]Department of Chemical & Biomolecular Engineering, Sogang University, #1 Shinsu-dong, Mapo-gu, Seoul 121-742, Korea
[2]Interdisciplinary Program of Integrated Biotechnology, Sogang University, #1 Shinsu-dong, Mapo-gu, Seoul 121-742, Korea
[3]College of Bionanotechnology, Kyungwon University, Bokjeong-dong, Sujeong-gu, Seongnam 461-701, Korea
Correspondence and requests for materials should be addressed to J.-W. Choi (jwchoi@sogang.ac.kr)

Accepted 17 April 2009

Abstract

The charge storage in the protein-based biomemory consisting of *P. aeruginosa* azurin was investigated. Azurin was used as an electron donor or acceptor. To immobilize the azurin on the Au surface, 11-mercaptoundecanoic acid was used as a linker material. The azurin-immobilized biofilm on Au surface was optimized by surface plasmon resonance spectroscopy and the surface morphology of protein-based biofilm on Au surface was investigated by atomic force microscope. The memory device characteristics, including the "write", "read" fuctions of the self-assembled azurin biofilm, were well demonstrated with two distinct electrical states of protein-based biofilms by cyclic voltammetry. From the results, it could be concluded that the azurin biofilm could be used for the construction of nanobiochip with memory function.

Keywords: Charge storage, Cyclic voltammetry, Surface plasmon resonance, Atomic force microscope, Open circuit potential amperometry, Nanobiochip

Introduction

Over the past four decades, various principles of molecular electronics have been proposed as promising information storage concepts to overcome the physical and technical limits of conventional silicon-based memory concepts such as the regional charge trap and transfer[1-10]. Beratan *et al*. proposed molecular electronic device with shift register memory function based on electron transfer reaction. The proposed device used two main energy sources, the photon and the electron, in order to obtain memory characteristics based on switching system[3]. Hersam *et al*. showed that the electrical properties of single molecule can be investigated by high vacuum STM (scanning tunneling microscopy) in order to prove that a single molecule can be realized to silicon-based molecular electronic devices[4]. Roth and co-workers investigated the electron-transfer rates of charge-storage molecular monolayers on Si (100) to demonstrate the hybrid molecular/semiconductor information storage device[5-7].

The biomolecular information storage system can be directly incorporated to organic/biomolecular mimicking systems, as well as to building blocks such as neurosystems, if memory element composed of metalloprotein. Capstick *et al*. recently proposed an information storage logic gate that was composed of biomolecule such as DNA, enzymes[8]. The possibility of encoding information in the base sequences of DNA was shown by the manipulation of DNA with enzymes or DNAzymes[8]. We also developed the shift register memory effects using the hetero biomolecular langmuir-blodgett layer to achieve simple electronic functions of the biomolecular diode and switching device with photocurrent generation and a rectifying property[11-13]. However, the characteristics of memory device, including the "Write", "Read" functions, have not yet been demonstrated using well-defined azurin layer on self-assembled 11-MUA (11-mercaptoundecanoic acid) layer to sustain simple and stable information signals.

P. aeruginosa azurin (about 14 kDa) is electron transfer protein; azurin is a sub-class of the type 1 blue copper protein family. Although the physiological role of azurin has not been established, it most likely functions as a soluble electron carrier, transferring charges between redox partners in membrane or soluble conditions[14]. From the previous research, it has also been shown that azurin can function as an electron donor or acceptor for nitrite reductase from *P. aeruginosa*[15-17].

Here we realize the concept of protein-based biomemory devices, in which a well-organized single protein can act as an individual memory element[18]. One of the most important technologies to introduce protein to molecular electronics is the immobilization of protein possessing capabilities of its orientation and

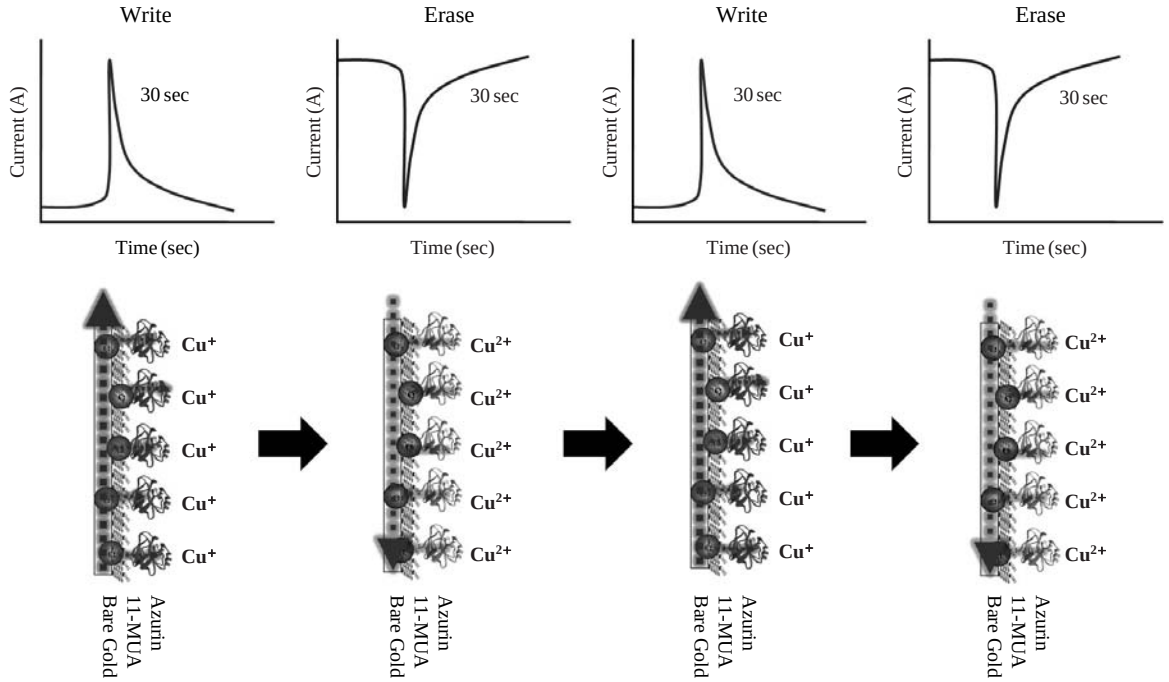

Figure 1. Schmatic diagram of charge storage function of the 11-MUA/azurin modified gold electrode.

stability. The non-specific adsorption of protein on the gold surface prevents the orientation of protein.

Our approach to demonstrating the practical protein-based biomemory device utilizing protein as memory element is the validation of information manipulation principle and the robustness of biodevice through the stable redox properties of well-oriented immobilization layer by electrostatic interaction. We fabricated the alkanethiol modified gold surface using 11-MUA, which has -SH (thiol group) and -COOH (carboxyl group) at the both end sides. The azurin-immobilized layer on Au surface is optimized by SPR (surface plasmon resonance) spectroscopy and AFM (atomic force microscope). Redox property of azurin molecules on 11-MUA modified gold surface is validated by using CV (cyclic voltammetry) technique. When an oxidation potential is applied to the fabricated protein modified electrode, the azurin in the monolayer loses an electron to the Cu metal, and thereby entrapment of positive charge occurs in the biofilm, as shown in Figure 1. This trap process of positive charge inside the protein layer corresponds to the function of storage (write) of information. The trapped charge (written information) in the biofilm is measured (read) when an OCP (open-circuit potential) is applied to the fabricated biomolecule based electrode. When a reduction potential is applied to the fabricated electrode after the initial step of oxidation the inorganic base gives back the electron to the azurin monolayer. Thus, the initial charge trapped in the protein layer during the time of oxidation, is neutralized (erase).

Results and Discussion

SPR Spectroscopy Analysis

In Figure 2, we confirmed the increased immobilization capability of azurin when gold surface was modified by 11-MUA linker material. The SPR angles mean the thickness of film. In random immobilization system, SPR angle was 0.30 degree (Figure 2a). But in well-oriented immobilization system, SPR resonance angle shift was 0.49 degree (Figure 2b). From this result (Figure 2c), we could obtain the well-oriented film by electrostatic force when 11-MUA was used to modify the gold electrode.

Topography Analysis Using AFM

Figure 3 represented the surface morphology of the proposed biofilm by the AFM. The bare gold surface has about 40-50 nm width and 1-2 nm height (Figure 3a). When azurin was fabricated on the 11-MUA modified gold substrate, the height is changed to 15-20 nm (Figure 3b). We can assume the formation of 3-4 azurin layers on the 11-MUA modified gold substrate. When we detect the redox property of the target molecules, redox property of the unknown molecules could be added in the signal of target molecules. So in inves-

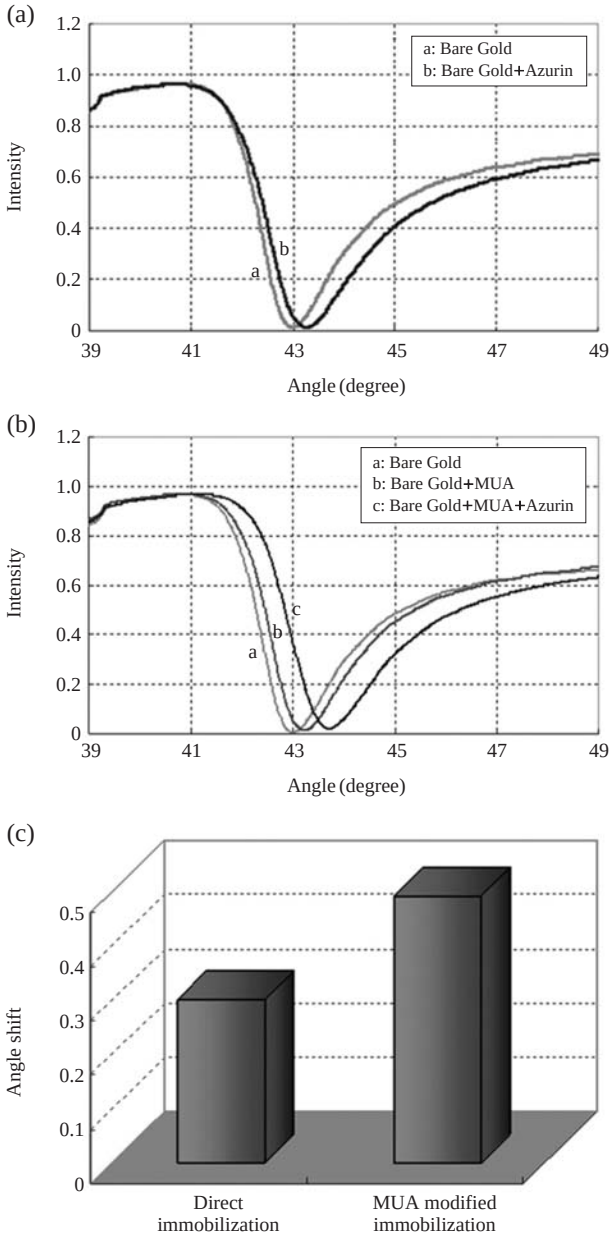

Figure 2. Confirming the immobilization of azurin layer by surface plasmon resonanace. (a) Characterizing the immobilization of the azurin on gold substrate. a: bare Au surface, b: direct azurin adsorption. (b) Characterizing the immobilization of the azurin on gold substrate. a: bare Au surface, b: 11-MUA-modified electrode, c: electrostatic adsorption on the 11-MUA-modified electrode. (c) Compare angle shift of direct azurin adsorption and electrostatic azurin adsorption on the 11-MUA-modified electrode.

tigation of redox property, we removed the possibility of other redox property. It is important to maintain the original structure of the redox molecule for obtaining the redox property. But when the fabrication of redox molecule layer on the metal electrode was fabricated, 3-D structure of protein was denatured. Since the supporting layer between the protein and metal substrate not only increase the capability of immobilization but also assist to maintain the protein sturcture on the metal substrate. In this study, the 11-MUA was used as the supporting layer to immobilize azurin on the gold electrode.

Characterization of Redox-active Azurin by Using CV

The CV measurement of the immobilized azurin layer was investigated from 0.8 V to 0.3 V. Figure 4 shows cumulative charge data of azurin by cyclic voltammetry in 500 cycles. The scan direction was from the positive voltage to the negative voltage and the scan range was from 0.8 V to −0.3 V with 50 mV/s scan rate. The reduction peak was observed at 0.35 V, and oxidation peak was observed at 0.20 V.

This result means that the assembled azurin on electrode still had redox property. Generally, the structure originality of the biomolecule was very important to maintain the function of the biomaterial. In the 11-MUA modified immobilization method, the fabricated azurin had their original redox property. It implies the proposed biofilm has original structure. Therefore, the well-oriented immobilization system was very efficient method to maintain the activity of the biomaterial.

The stability of the biodevice consisting of the immobilized azurin layer using 11-MUA was investigated. As shown in Figure 4, oxidation and reduction peaks of the biofilm were observed continuously until 500 times of CV. This result means the biodevice consisting of azurin immobilized on Au electrode using 11-MUA was stable until 500 times continuous cycling nevertheless a linear signal of the redox signal for the multicycle is very difficult due to the instability of biomolecules. In the 500 cycles, the oxidation potential and the reduction potential were moved to reduce the interval of the oxidation and reduction potential. It means the resistance of electron transfer was deceased and the more reversible electrochemical reaction system was settled.

Novel Biomemory Device

Figure 5a and b shows the oxidizable and reducible currents by OCP. In Figure 6, we obtained different current profiles (two stages) by the applied redox potential. The obtained current profiles of azurin immobilized can be controlled by the change of applied potential. It means azurin immobilized on Au surface can store the charge. The two stages controlled by applied potential can be utilized as “read” and “write”

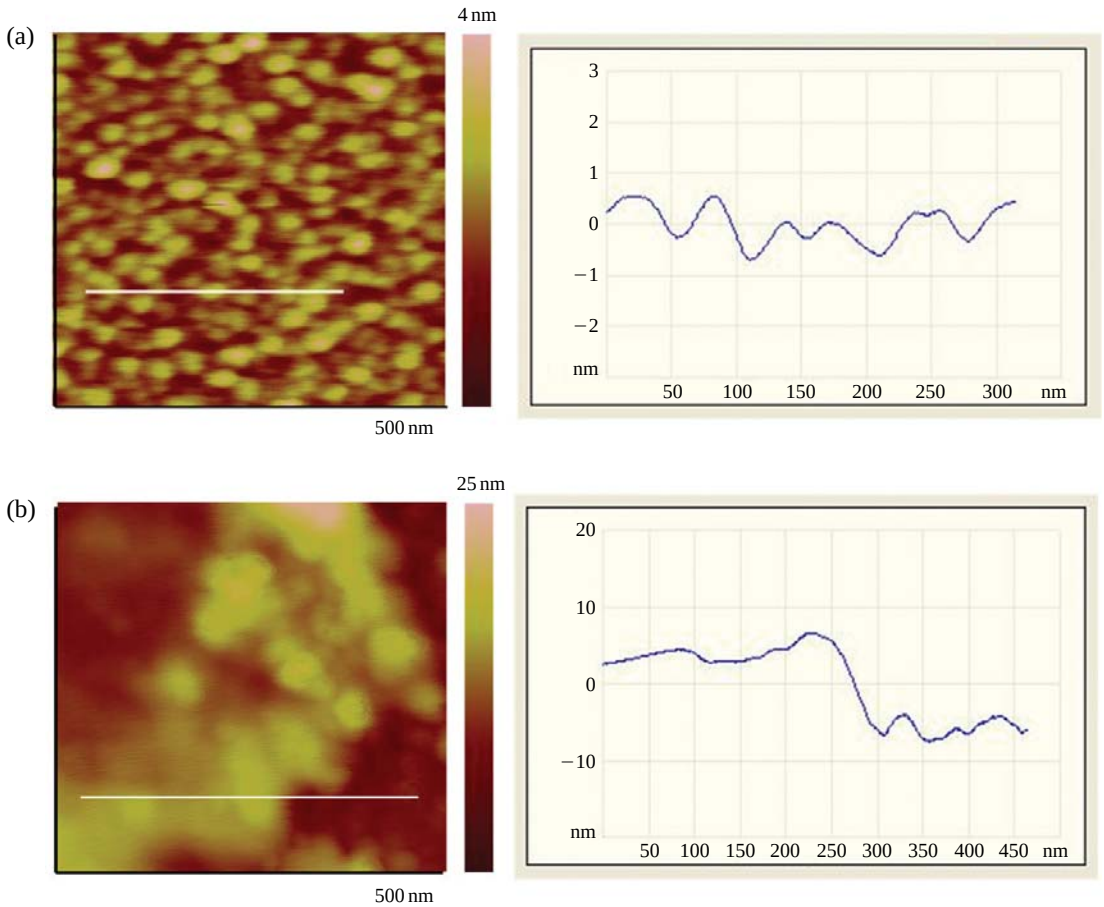

Figure 3. Surface analysis of the bare gold and (b) the azurin modified electrode.

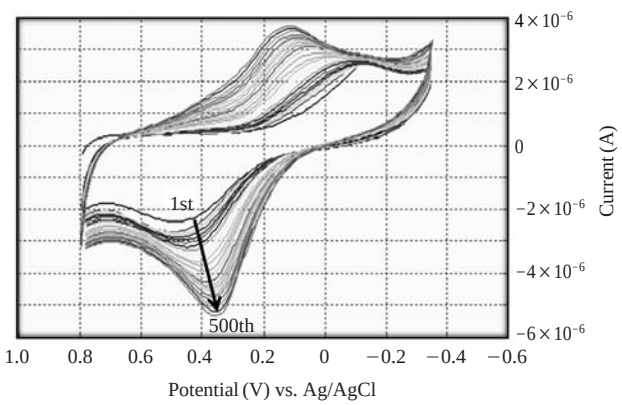

Figure 4. Cumulative charge data of azurin by cyclic voltammetry in 500 cycles.

function or "0" and "1" stages in the current memory. The charge occupied in the biofilm when applying oxidation or reduction potentials can be measured by OCPA. But, in current stage, we could not obtain the uniform current signal. If we improve the assembly and detection system, we can obtain the more improved current switchable signal.

Conclusions

From these results, the charge storage in the proposed biomemory device composed of azurin was investigated. To fabricate the azurin on the Au surface, 11-mercaptoundecanoic acid was used as a linker material. The immobilization of the biofilm on Au surface was optimized by SPR spectroscopy and the surface morphology of protein-based biofilm on Au surface was investigated by AFM. The memory device characteristics, including the "write", "read" fuctions of the self-assembled azurin biofilm, were well demonstrated with two distinct electrical states of protein-based biofilms by cyclic voltammetry. From the results, it could be concluded that the proposed molecular device could be used for the construction of

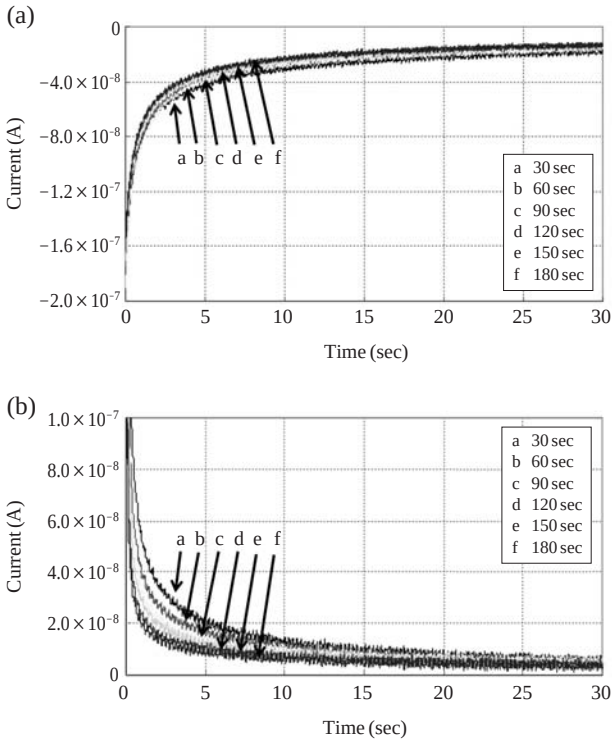

Figure 5. (a) The oxidizable currents measured from the reduced azurin layers after selected disconnect times followed by reconnection at the OCP and (b) The reducible currents measured from the oxidized azurin layers after selected disconnect times followed by reconnection at the OCP.

bioelectronic device with memory function.

Materials and Methods

Materials

Gold substrate used as working electrode was purchased by Inostek (Korea). Pt Counter electrode and Ag/AgCl Reference electrode were purchased by BAS (USA). *P. aeruginosa* azurin was purchased by Sigma Aldrich Co. (USA) 11-MUA (11-mercaptoundecanoic acid), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) used as solvent to prepare azurin sample and washing buffer solution[19,20]. Distilled and deionized Milipore [(Milli-Q) water (DDW; $>18\ M\Omega$)] was used in this experiment. Benzyl benzoate (Merck, Germany) was purchased and used as index matching fluid for SPR measurement.

Immobilization Methods

For the fabrication of gold (Au) thin film, cover glass (BK7, 18 mm × 18 mm, Superior, Germany) was used chromium (Cr) was sputtered onto the glass substrate initially as an adhesion promoter material to a thickness of 20 nm. Au sputtering to a thickness of 430 nm was followed on the sputtered Cr layer[21]. The sputtered Au substrate was cleaned using piranha solution composed of 30 vol% H_2O_2 (Sigma Aldrich Co., USA) and 70 vol% H_2SO_4 (Duksan Chemical Co., Korea) at 70˚C for 5 min, and then the cleaned substrate was immersed into pure ethanol solution for 1 hr. The prepared substrate was passed three steps, the first immersed into the solution, the second was washed by DI water, and the last step was immersed into pure ethanol solution for several hours. Next step is gold substrate modification process using by 11-MUA. 11-MUA is a kind of alkanethiol that have eleven carbon chains, the end of both sides was consisted of carboxyl group (-COOH) and thiol group (-SH) at the both sides. It processes linker material of protein interaction. Thiol group connected to gold substrate and carboxyl group electrostatic interacted with amine group of protein. 11-MUA solution was dissolved in ethanol by the 20 mM. And the substrate was immersed on the 11-MUA solution for 6 hr. The substrate washing was processed by ethanol and DI water for eliminate excess alkanethiols.

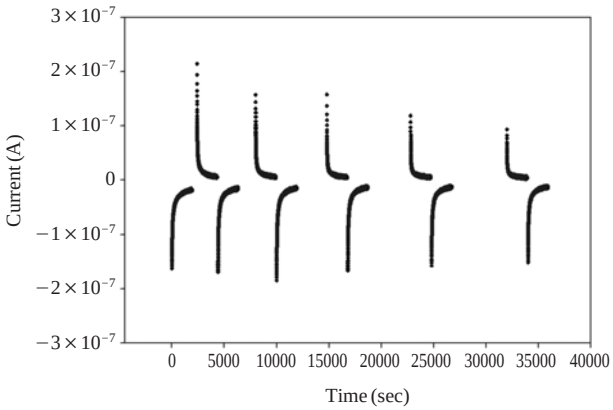

Figure 6. The memory function of the protein-based biomemory device.

Last step was the electrostatic protein adsorption step. Azurin dissolved in the HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution. Optimal concentration of this sample to adsorb the 11-MUA modified gold was 0.5 mg/mL by the SPR spectroscopy measurement. We dropped azurin solution on the modified gold substrate for 6 hr and washed by the phosphate buffered saline (PBS) buffer solution.

Fabrication of Thin Biofilm

For the confirming of immobilization of azurin, sur-

face plasmon resonance spectroscopy (Multiskop™, Optrel GbR, Germany) was used. In accordance of the thickness of metal substrate, the resonance angle was changed by the proportion to the increased film thickness. Therefore, we optimize the film formation process by the change of resonance angle of the SPR spectroscopy[22-24].

For the investigation of the fabricated thin biofilm, Atomic force microscope (AFM) study was carried out[25]. AFM analysis may be used to subsidiary method of SPR. The benefit of combining SPR and scanning probe microscope (SPM) imaging allows the interrelationships between surface morphology and biological interaction with biomaterials to be efficiently analyzed.

Investigation of the Redox Property by the Electrochemical Methods

For the confirming of electrochemical property of azurin immobilized substrate, the cyclic voltammetry technique was used. Electrochemical cell consisted of gold working electrode, Ag/AgCl reference electrode and Pt counter electrode. The working electrode fabricated by 150 nm gold deposition. It was modified with 11-MUA. And azurin was immobilized on the 11-MUA modified gold substrate. Working electrode size was 5 mm × 20 mm. HEPES buffer (pH=5.1) was used by electrolyte.

OCP (open circuit potential) means the equilibrium potential of the electrochemical system[26,27]. At the OCP, azurin become the equilibrium state. From the cyclic voltammetry investigations, we can obtain the redox potential of the adsorbed azurin layer. So From the open circuit potential measurement, we can assume the OCP was 20 mV of this electrochemical system. If some voltage was applied, the system can moved other redox state. But when the OCP was applied to the electrochemical system, the system was moved again to the equilibrium-state. Therefore, From this potential change process, we can trap the charges of the redox molecule layers on the target electrode.

OCPA (open circuit potential amperometry) was used for investigation of surface charge switchable characteristic. When the reduction voltage was applied to the system, adsorbed azurin on the modified gold electrode was oxidized. Therefore, negative current was measured. With a contrary concept, When the OCP was applied to the system, oxidized azurin return to the neural-state by the reduction process. Therefore, positive current was measured by the working electrode. These results showed that the charge storagable system based on the assembled redox biomolecules can be regulated by the oxidation and reduction control. And it shows the possibility of the improvement to fabricate the redox biomolecule organized molecular electronic device.

Acknowledgements

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 Research Foundation and The Korean Federation of Science and Technology Societies Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund), 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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