Dual-Level Biomemory Device Composed of Cytochrome c/DNA/Myoglobin Heterolayer

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In this study, heterolayer composed of cytochrome c/DNA/myoglobin is fabricated for the demonstration of dual-level biomemory device. Metalloprotein and single stranded DNA are conjugated via chemical linkage, and then two different kinds of conjugates are connected by DNA hybridization which can provide stable and selective combination to form heterolayer on gold substrate. To verify the conjugation of metalloprotein and single stranded DNA, sodium dodecyl sulfate polyacrylamide gel electrophoresis and ultraviolet-visible spectroscopy are used. Morphological change by the heterolayer formation is investigated by atomic force microscopy. Cyclic voltammetry is used to investigate the electrochemical property of heterolayer. Dual-level memory functions are achieved by multi-potential step chronoamperometry. From results, heterolayer is well fabricated by DNA hybridization, and dual-level memory functions are well demonstrated with improved memory density. The proposed dual-level biomemory device can provide new approaching method to fabricate bioelectronic devices with high selectivity and functionality.

Keywords: Biomemory, Cytochrome c, Myoglobin, DNA Hybridization, Cyclic Voltammetry, Multi-Potential Step Chronoamperometry.

1. INTRODUCTION

Bioelectronic devices have attracted a scientific attention because of their wide range of applications such as biomedical device, diagnostic sensor, environmental sensor, biochip, and biocomputation systems.¹ Growth rate of usage and development of bioelectronic devices have led to rises of interests and intensive researches towards various biomaterials such as metalloproteins and enzymes as the new functional materials.²

Electron transfer properties of metalloproteins and enzymes play an important role in living organisms.³,⁴ The well-studied biochemical properties of metalloproteins demonstrated that these redox biomolecules were suitable tools to fabricate bioelectronic devices.⁵,⁶

Many researching groups have used several biomolecules to develop bioelectronic devices, for example, the biologic gate, bioprocessor and etc. Katz’s group suggested biologic gate using biocatalyzed enzyme reactions and Leung’s group developed biologic gate based on DNA.⁷,⁸

Among them, our group especially developed biomemory devices based on metalloproteins, which showed the basic memory functions including reading, storage and erase.⁹ To increase memory density of biomemory device, our group suggested heterolayer composed of two different metalloproteins.¹⁰ However, those researches have actually suffered from several limitations such as low electrochemical signal of biomolecules, signal merging of different biomolecules, weak selective reaction and disordered heterolayer formation.

To make selective and well-ordered heterolayer composed of metalloproteins for biomemory, we fabricated heterolayer composed of cytochrome c/DNA/myoglobin using DNA hybridization. DNA has a property of selective interaction between specific base pairs which can provide selective linkage between different metalloproteins, resulting in well-ordered heterolayer formation.¹¹
Metalloproteins used in this study were cytochrome c and myoglobin which have metal ion in its core part; it plays essential role in the electron transfer process by accepting and releasing an electron.\(^{12}\) Figures 1 (a and b) represent the scheme of cytochrome c/DNA/myoglobin heterolayer fabrication and the basic concept of dual-level biomemory function, respectively. Applying first oxidation potential induces electron transfer from cytochrome c to gold substrate resulting in writing of positive charge in cytochrome c. Conversely applying first reduction potential causes electron transfer back to cytochrome c erasing positive charge. Similarly, applying the second oxidation and reduction potentials causes the same process in myoglobin.\(^{14,15}\) Conjugation was confirmed by the ultraviolet-visible (UV-vis) spectroscopy and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Immobilization of heterolayer was investigated by atomic force microscopy (AFM). Electrochemical property of heterolayer was confirmed by cyclic voltammetry (CV). Multi-potential step chronoamperometry (MPCA) was used to evaluate dual-level memory functions.

2. EXPERIMENTAL DETAILS

2.1. Materials
Cytochrome c and myoglobin extracted from *horse heart*, and dithiothreitol (DTT) were purchased from Sigma-Aldrich (USA). Thiol-modified single stranded DNA (ssDNA) and its complementary single stranded DNA (ssCDNA) were synthesized by Bioneer Co. (South Korea). Sulfo-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) was purchased from Thermo Scientific (USA). Gold substrate was purchased from National Nanofab Center (South Korea).

2.2. Fabrication of Cytochrome c-ssDNA and Myoglobin-ssCDNA Conjugates
To make cytochrome c-ssDNA, 10 \(\mu l\) of 1.0 N DTT was added to every 100 \(\mu l\) of ssDNA solution (10 \(\mu M\)) dissolved in TE buffer. Then, the solution was incubated for 15 min at room temperature. Excess of DTT was removed using extraction by adding pure ethyl acetate. After this, 10 \(\mu l\) sulfo-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) (10 \(mM\)) was added immediately, because free sulfhydryl group becomes unstable after the removal of DTT.\(^{16,17}\) The mixture was incubated for 2 hours at 4 °C. After incubation, 10 \(\mu M\) of cytochrome c solution was added as the same volume ratio and was incubated for 6 hours at 4 °C. Finally cytochrome c-ssDNA conjugate was fabricated. Myoglobin-ssCDNA conjugate was also prepared by same process above mentioned.

2.3. Fabrication of Heterolayer Composed of Cytochrome c/DNA/Myoglobin
To immobilize the prepared cytochrome c-ssDNA conjugate, gold substrate was cleaned by piranha solution for 3 min at 70 °C and dried by \(N_2\) gas. After this, 20 \(\mu l\) 6-MHA solution was dropped on gold substrate for at least 6 hours. Then, 20 \(\mu l\) of cytochrome c-ssDNA was dropped on gold substrate and incubated for 6 hours. After this, 20 \(\mu l\) of myoglobin-ssCDNA was dropped for 6 hours at 4 °C. Finally, heterolayer was fabricated on gold substrate.

2.4. Surface Morphology Investigation of Heterolayer by AFM
To investigate surface morphology of heterolayer, AFM (Digital instruments Nanoscope (R) IV, USA) was used. A cantilever manufactured with phosphorus (n) doped silicon (RTESP, Bruker, USA) was used for AFM with tapping mode.

2.5. Electrochemical Investigation of Cytochrome c/DNA/Myoglobin Heterolayer
The electrochemical property of heterolayer was investigated using an electrochemical analyzer (CHI 660, USA). The platinum electrode was used as counter electrode, an Ag/AgCl as a reference electrode, and gold substrate as a working electrode. CV analysis was performed with the potential range of 0.5 to −0.3 V, and 50 mV/s scan rate. After CV test, MPCA was used to verify memory function.

3. RESULTS AND DISCUSSION

3.1. Confirmation of Cytochrome c-ssDNA and Myoglobin-ssCDNA Conjugation Using SDS-PAGE and UV-Vis
In Figure 2(a), lane 1, 2 displayed the protein ladder and cytochrome c. Lane 3 showed cytochrome c-ssDNA. Similarly, in Figure 2(c), lane 1, lane 2 and lane 3 exhibited protein ladder, myoglobin and myoglobin-ssCDNA, respectively. These results showed the successful fabrication of metalloprotein-DNA conjugate.

Figures 2(b and d) showed the UV-vis spectra of all components. In the case of ssDNA and ssCDNA, wavelength absorbance was at 260 nm. The UV-vis spectra of...
both metalloproteins showed a characteristic Soret peak at 410 nm. Finally, in the case of cytochrome c-ssDNA and myoglobin-ssCDNA conjugates, the UV-vis spectra showed the characteristics of both components, the Soret peak of metalloproteins at 410 nm and the absorption of ssDNA and ssCDNA at 260 nm, respectively. From results, we confirmed that cytochrome c-ssDNA and myoglobin-ssCDNA conjugates were successfully fabricated.

3.2. Surface Morphology Investigation of Cytochrome c/DNA/Myoglobin Heterolayer Using AFM

In Figure 3(a), AFM result of bare gold substrate displayed the lump size between 7 nm and 9 nm with height of 9.2 nm. Clusters of cytochrome c-ssDNA on gold substrate (Fig. 3(b)), were detected near 4 nm size. Also, these clusters were higher than bare gold substrate (height of 13 nm).

This result verified the immobilization of cytochrome c-ssDNA layer on bare gold substrate. Figure 3(c) showed the surface morphology of cytochrome c/DNA/myoglobin heterolayer immobilized on gold substrate which displayed forms with 5 nm of size and 17 nm of height. This result verified the immobilization of myoglobin-ssCDNA on cytochrome c-ssDNA via DNA hybridization. From AFM data, size and height differences of aggregation forms indicated well formation of heterolayer on gold substrate.

3.3. Investigation of Electrochemical Property of Cytochrome c/DNA/Myoglobin Heterolayer by CV

Figure 4(a) showed the comparison of cyclic voltammograms of cytochrome c and myoglobin. Figure 4(b) indicated the result obtained from cytochrome c/DNA/myoglobin heterolayer with two distinct reduction peaks and one adjoining oxidation peak. High signal intensity and selective reaction were achieved due to remarkably strong hydrogen bonds in DNA hybridization. Those specific bonds are responsible for immobilization of metalloproteins on gold substrate effectively and uniformly. So, heterolayer formation by DNA hybridization induced increased signal intensity and prevented signal merging. But, in the case of heterolayer formed by electrostatic bond, due to a weak electrostatic interaction between two metalloproteins, metalloproteins were not effectively immobilized on substrate uniformly, and signal with less intensity was obtained. Figure 4(c) showed cyclic voltammogram of cytochrome c/myoglobin layer immobilized electrostatically without DNA. From CV result, only one pair of redox peaks with 294 mV and 2 mV was detected. Thus, fabricated heterolayer showed improved redox property compared to cytochrome c/myoglobin layer.
Figure 4. Cyclic voltammogram of (a) cytochrome c and myoglobin, (b) cytochrome c/DNA/myoglobin heterolayer, (c) electrostatically linked cytochrome c/myoglobin layer, (d) conceptual biomemory function of cytochrome c/DNA/myoglobin heterolayer, (e) applied potentials for memory function, (f) Faradaic current response (1 cycle) confirming dual-level memory function for 0.8 s.

3.4. Dual-Level Memory Function Verification of Cytochrome c/DNA/Myoglobin Heterolayer Using MPCA

Figure 4(d) showed the CV of cytochrome c/DNA/myoglobin heterolayer with ‘write’ and ‘erase’ functions. The capacity of stored-charge was estimated to be $2.08 \times 10^{-5}$ C, whereas only one pair of redox signals was monitored in cytochrome c/myoglobin layer formed by electrostatic interaction. Also, heterolayer demonstrated dual-level memory functions by MPCA. The heterolayer showed $2.08 \times 10^{-5}$ C stored-charge capacity (‘write 1’) and $5.12 \times 10^{-5}$ C stored-charge capacity (‘write 2’), respectively. In conclusion, the proposed dual-level memory device can provide the new approaching method for the fabrication of biomemory device with enhanced selective reactivity using DNA hybridization. This technique can be applied to manufacture nano-scale bioelectronic device with high controllability.

Acknowledgment: This work was supported by Nanobioelectronics lab in Sogang University.

References and Notes

Received: 26 May 2015. Accepted: 11 August 2015.