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Charge Distribution of Cytochrome *c* Monolayer Using Electrostatic Force Microscope

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The charge trapping of the cytochrome *c* was investigated by electrostatic force microscopy (EFM). The cytochrome *c* was modified by SPDP and deposited onto the gold substrate by self-assembly technique. The charge was transported to heme groups of cytochrome *c* by scanning tunneling microscopy (STM) tip, and it was verified that the charge was trapped onto the cytochrome *c* film surface by EFM. The charge distribution on the cytochrome *c* surface was observed by EFM measurement.

Keywords : Cytochrome *c*; Electrostatic force microscopy; Self-assembly technique; Scanning tunneling microscopy

INTRODUCTION

Recently molecular engineering and its application to molecular electronic device have been emerged as one of the challenging fields with the advancement of biomimetic engineering and nanotechnologies. EFM is advantageous to be able to measure topography and other physical properties simultaneously at the same sample positions. In addition to detecting surface topographical changes, EFM is capable of measuring variations in surface potential and capacitance gradient on the sample surface[1,2]. So the efforts have revealed a much broader potential for the measurement of local mechanical properties and electrical properties such as surface potential, surface charge by EFM[3] Cytochrome *c* is one of the most widely studied proteins due to its

stability and solubility in water as well as an electron transport property. The key feature of cytochrome *c*, the capability of electron transfer, is driven from the redox state change and conformational change of heme groups[4].

In this study, it was investigated that the charge distribution of cytochrome *c* film surface. To deposit the cytochrome *c* onto the gold substrate, the functional group of cytochrome *c* was modified by SPDP. Also UV absorption spectra were measured to verify the charge transfer in cytochrome *c*. To investigate the charge distribution of cytochrome *c* surface, EFM measurement was carried out.

EXPERIMENTAL DETAILS

STM and EFM measurements were carried out using Autoprobe CP(PSIA, KOREA). In STM measurement, electrochemically etched-tungsten wire(ϕ 0.5mm diameter) was used as STM tip. Tunneling images were obtained in constant-current mode. Typical tunneling tip bias, set point, and servo gain were 0.84mV, 0.2-0.3nA and 2, respectively.

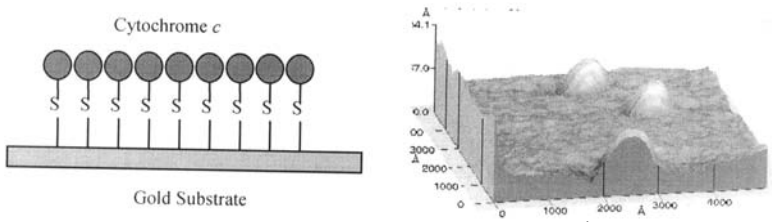
The conventional non-contact EFM is used to maintain constant tip-sample distance, thus producing topographic images. In addition, V_{ac} and V_{dc} are applied between the conducting tip and sample. The frequency *f*, time constant, sensitivity, amplification, and harmony are 17kHz, 10ms, 20mV, 5V, and 1, respectively.

Cytochrome *c* (extracted from horse heart, type VI) was purchased from Sigma Chemical Company (St. Louis, USA) and CLONTECH CO. (Palo Alto, California, USA), respectively. N-succinimidyl-3-[2-p-yridyldithio]propionate (SPDP) and dithiothreitol (DTT) purchased from Sigma Chemical Company were used as a cross-linking and a cleavage reagent, respectively. The gold-coated substrate was made with thermal evaporation on cleaned glass substrate.

RESULTS AND DISCUSSION

Fig. 1 shows the schematic diagram of pattern fabrication of cytochrome *c* onto the gold substrate(a) and 3-D image of AFM(b). To deposit of cytochrome *c* particle onto the gold substrate, the functional

group of cytochrome *c* was translated to thiol group using SPDP and DTT. To fabricate the pattern formation, cellulose membrane was used as a mask. The cellulose membrane has a lot of pore with 0.1μm and thus the modified cytochrome *c* could be deposited onto gold substrate with 0.1μm. As shown in Fig. 1(b), the size of cytochrome *c* is observed as about 0.1μm.



(a) (b)

FIGURE 1. The schematic diagram of pattern fabrication of cytochrome *c* onto the gold substrate(a) and 3D image(b)

Fig. 2 shows the UV absorption spectra of cytochrome *c* for investigation of charge transfer. As shown in Fig.2(a), the peak at 410nm means the existence of cytochrome *c* and peaks between 500 and 600nm indicates the reduction state and the oxidation state of cytochrome *c*, respectively. The peak at 280nm indicates the existence of the aromatic amino acids. In Fig.2(b), the peak at 695nm results from an interaction between the heme iron and its ligands. Therefore this indicates the charge-transfer(CT) in cytochrome *c*.

Fig. 3 shows the topography of NC-AFM and non-contact mode EFM. The modified cytochrome *c* aggregates were deposited onto the gold substrate as shown in Fig. 3(a). Bright spots are cytochrome *c* aggregates, and the height and size of the cytochrome *c* aggregates are 200Å and 0.1μm, respectively. In Fig. 3(b), the image is obtained by non-contact mode EFM for charge distribution on cytochrome *c* surface. Bright spots are charge trapped cytochrome *c* regions. It means that electron is transferred from STM tip to cytochrome *c*, and then the electron is trapped into cytochrome *c*.

From these results, it can be suggested that the charge trap of biomolecules such as proteins can be applied to construct the biomolecular memory.

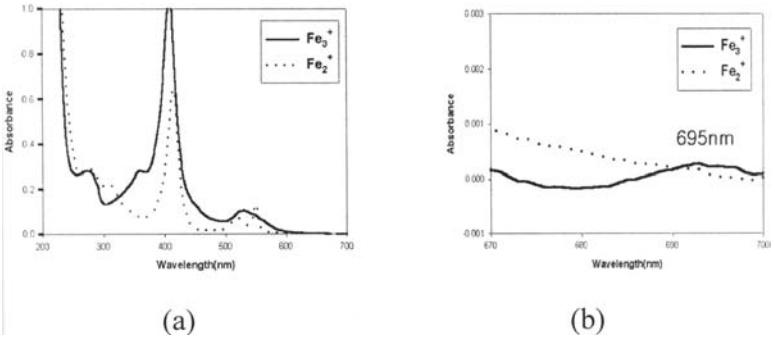


FIGURE 2. UV absorption spectra of cytochrome *c* at pH 7.0, (a), absorption spectra from 200nm to 700nm, (b), absorption spectra from 670nm to 700nm

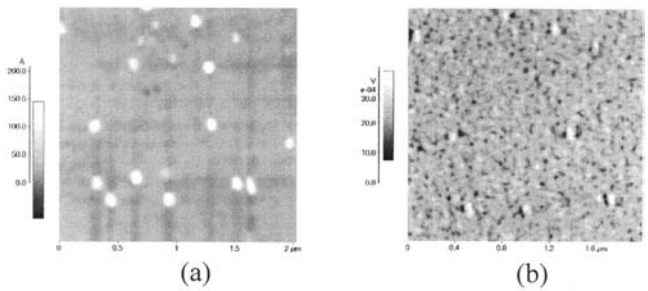


FIGURE 3. (a), Topography of NC-AFM, (b), non-contact mode EFM for charge distribution on the cytochrome *c* surface

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