Submicro photopatterning of alkanethiolate self-assembled monolayer using a negative mask and its application in the fabrication of biomolecular photodiode

Se Young Oh\textsuperscript{a,}\*, Hyung Seok Choi\textsuperscript{a}, Hang Sok Jie\textsuperscript{a}, Je Kyun Park\textsuperscript{b}

\textsuperscript{a}Department of Chemical Engineering, Sogang University, Shinsoo-dong 1, Mapo-gu, Seoul 121-742, South Korea
\textsuperscript{b}Department of BioSystems, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Deajeon 305-701, South Korea

Abstract

Alkanethiolate self-assembled monolayer (SAM) formed by the adsorption of 11-mercaptopoundecanoic acid (11-MUDA) molecules on a gold substrate. The alkanethiolate was oxidized by the irradiation of deep UV light with a 700-nm negative mask and then developed with deionized water. A uniform submicro-pattern of 11-MUDA SAM was obtained. In order to array cytochrome \( c \) molecules along the patterned substrate, the well-characterized cytochrome \( c \) was immobilized. Electrochemical properties and morphology of the cytochrome \( c \) monolayer were investigated through the measurements of cyclic voltammetry and AFM. In addition, current–voltage characteristics of biomolecular multilayers consisting of cytochrome \( c \) and green fluorescent protein (GFP) were studied with a scanning tunneling microscope (STM).

Keywords: Alkanethiolate self-assembled monolayer; Submicro-pattern; cytochrome \( c \); Electrochemical property; Current–voltage characteristics

1. Introduction

Over the past several years, self-assembled monolayers (SAMs) have attracted much attention because of interest in two-dimensional molecular assemblies and their potential applications in molecular devices, sensors, surface engineering, etc. [1–3]. Self-assembled monolayers can potentially give a robust method for fabricating protein multilayers. Moreover, the affinity of thiol moiety for gold surface makes alkanethiol suitable for the preparation of modified electrode [4]. The micro-array pattern formation of self-assembled protein is an important factor in the fabrication of molecular device composed of biomolecules. Thus, the ability to generate fine pattern of protein on a solid surface has attracted considerable interest in bioelectronic technology and fundamental study of biophysics [5,6]. Especially, studies on the characteristics for patterning and modification of solid surface at molecular level and the use of these patterns to control the immobilization of protein have been widely progressed. In this study, we have fabricated the submicro-array of biophotodiode consisting of alkanethiolate/cytochrome \( c \)/green fluorescent protein (GFP)/gold by using alkanethiolate SAMs as a very simple photosresist utilizing photochemical conversion of alkanethilates to alkanesulfonates. The physical and electrochemical properties of self-assembled protein multilayers were studied. The rectifying characteristics using a scanning tunneling microscope (STM) was also investigated.

2. Experimental

Ethyl alcohol anhydrous (99.99\%), 11-mercaptopoundecanoic acid (11-MUDA), copper(II) sulfate (CuSO\textsubscript{4}) were purchased from Aldrich Chemical (Milwaukee, USA). Cytochrome \( c \) (extracted from horse heart muscle) and GFP (rEGFP protein) were purchased from Sigma (St. Louis, USA) and Clontech (Palo Alto, USA), respectively. Au(111) substrate was purchased from INOSTEK (Korea). Other reagents were purchased from Aldrich Chemical and used without further purification.

The gold substrate was immered into 1 mM 11-MUDA ethanolic solution for 18 h. Following removal from the thiol solution, the substrate was washed with degassed ethanol, and then dried in a stream of nitrogen. Photopatterning of alkanethiolate SAM was carried out with an
exposure system of Spectral Energy, equipped with a 500-W high-pressure mercury lamp in conjunction with a narrow band pass filter for 254 nm. The self-assembled 11-mercaptoundecanoic acid monolayer on a gold substrate was oxidized by deep UV irradiation, and then developed with a deionized water. Cytochrome $c$ was sequentially immobilized onto the resulting negative patterned substrate. The carboxylic group of 11-MUDA allowed the cytochrome $c$ to form SAM. Finally, GFP molecules were adsorbed onto the self-assembled cytochrome $c$ monolayer.

Static secondary ion mass spectrometer (SSIMS) was investigated with a PHI 7200 TOF-SIMS/SALi. The topographies of self-assembled cytochrome $c$ on the photopatterned monolayer were observed by AFM (AutoProbe CP, Park Scientific Instruments, USA). Cyclic voltammetry measurements were carried out with a potentiostat measurement analyzer (IM6 system, Zahner Elektrik, Germany). The Ag/AgCl (3.0 M KCl) reference electrode, a gold substrate working electrode, and a 1-cm$^2$ Pt-gauze counter electrode were used for all experiments. Current–voltage characteristics of the protein multilayers were studied by a STM (AutoProbe CP, Park Scientific Instruments) technique. Set point for Au tip approaching was 0.5 nA and scan range for conductivity measurement was $-0.5–0.5$ V.

3. Results and discussion

Fig. 1 shows SSIMS spectra of 11-MUDA SAM on a gold substrate before and after exposure to deep UV light for 60 min. The spectrum of in situ SAM exhibited a peak corresponding to 11-MUDA at $m/z$ 217. After exposure to deep UV light, a new peak corresponding to the sulfonate...
species, \( \text{RSO}_3^- \) was observed at \( m/z \) 265. The ratio of these peak areas was used to calculate the extent of oxidation [7]. It can be found that 95% conversion of \( \text{RS}^- \) to \( \text{RSO}_3^- \) occurred by the irradiation of deep UV light.

Photopatterning of 11-MUDA SAM onto a gold substrate was carried out with a 700-nm negative mask by the irradiation of deep UV light. Alkanesulfonates in the exposed regions were developed thoroughly with a deionized water. In order to confirm the photopatterned domain of 11-MUDA SAM, the gold substrate was immersed in an aqueous solution of CuSO\(_4\) [8,9]. Fig. 2 shows the AFM image of the photopatterned 11-MUDA SAM adsorbed CuSO\(_4\). A uniform submicro-array pattern of 11-MUDA SAM was observed, indicating effective oxidation and displacement of 11-MUDA in the UV exposed regions [10,11].

Fig. 3 shows AFM images of bare gold and self-assembled cytochrome \( c \) monolayer immobilized onto the 11-MUDA molecules. It is indicated that the head moiety of cytochrome \( c \) molecule exhibited bright spot. The size and height of bright spot domain were 600 and 50 Å. The redox current of the cytochrome \( c \) SAM was observed from the measurement of cyclic voltammetry as shown in Fig. 4. It has been noted that uniform cytochrome \( c \) molecules were well immobilized onto the patterned 11-MUDA molecules.

To explore the rectifying characteristics of hetero self-assembled multilayers of cytochrome \( c \) and GFP onto the patterned substrate, the current–voltage characteristics were measured by as STM technique. The current–voltage curve was shown in Fig. 5. The electron transfer between the protein molecules and STM tip was occurred, when a forward voltage was biased in the range of \( 0.5 \)–\( 0.5 \) V. When a backward voltage was biased, no current was observed, which is attributed to the difference in redox potential between the protein molecules.
4. Conclusions

We have demonstrated the successful immobilization of protein multilayers onto carboxylate-terminated SAMs. The submicro-pattern of 11-MUDA SAM on a gold substrate was fabricated by photochemical oxidation using a 700-nm mask. Cytochrome c molecules were well immobilized onto the negative patterned 11-MUDA molecules. It has been found that the patterned biomolecular photodiode consisting of cytochrome c–GFP multilayers was successfully obtained by using the simple photopatternning of SAM.

Acknowledgements

This work was supported by Korea Research Foundation Grant (KRF-2002-005-D00002).

References