BIOCHIP JOURNAL, Vol. 1, No. 1, 65-69, March 2007

Self-assembled Monolayer Fabrication of Cysteine-modified Ferredoxin

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Accepted 13 February 2007

Abstract

Rhodobacter sphaeroides **ferredoxin is a metalloprotein with ferric ion in its active site. Ferredoxin has redox property and it can transfer the electron. These molecules can be applied to the bioelectronics by fabricating them as a self-assembled bio-film. The significant key of film fabrication is the immobilization method of bio-molecule. In our previous works, it has been reported that metalloprotein film can be fabricated by using chemical linker material that have thiol-group to assemble it on gold substrate. However, the chemical linker can interfere with electron transfer because it is acted as an insulator of the system. So, we used recombinant protein with cysteine functional residue at the end of the protein which can be directly immobilized on the gold (Au) surface. It could be confirmed the immobilization of the protein and surface morphology of thin film by surface plasmon resonance (SPR) and scanning tunneling microscope (STM). These results show that cysteine-modified ferredoxin can be used for making high quality protein film, and applied to the fabrication of nano-scale bioelectronics.**

Keywords: *R. sphaeroide* ferredoxin, Recombinat protein, Self assembly, Metalloprotein, Surface plasmon resonance, Scanning probe microscope, Bioelectronics

Introduction

In a biological electron transfer system, photoelectric conversion occurs and then long-range electron transfer takes place very efficiently in one direction through the biomolecules[1-3]. If we could control the arrangements of these molecules, we can make a nano-scale electronic biodevice[2-6]. According to in vitro studies, ferredoxin has redox property as a metalloprotein. It has been shown that ferredoxin can function as an electron donor or acceptor. However biomolecules are unstable and fragile. Therefore an efficient film fabrication technology should be needed to overcome these disadvantages.

In recent decade, self-assembly technique has been studied which offers an useful method to make a thin layer onto the metal substrate for various applications[7-9]. The most general system of self-assembly is to use alkanethiols as chemical linker. In the long hydrocarbon chains structure, one side is reacted with solid substrate and another side is reacted with target protein. It has been found that sulfur compounds coordinate very strongly to various metal surfaces, such as gold (Au), silver (Ag), copper (Cu) and platinum (Pt). In most work to date, Au surface has been used for the self assembly monolayer (SAM) formation of alkanethiols, because gold can not be oxidized easily. Therefore it can be handled in the ambient conditions. However, self-assembly method using alkanethiols is no good method for bioelectronics because it could be functioned as an insulator[12-14].

The general way for efficient immobilization is recombinant protein that modified with cysteine residue[15]. It can be immobilized on Au surface directly. In this study, well-ordered cysteine-modified ferredoxin layer is fabricated on Au surface, and the formation of film on Au surface was confirmed by surface plasmon resonance (SPR) and scanning tunneling microscopy (STM) topography analysis was carried out for the fabricated ferredoxin layer. The schematic description of ferredoxin immobilization is shown Figure 1 It has not been reported that ferredoxin with cysteine residue by recombinant technique is immobilized onto Au surface.

Results and Discussion

The formation of ferredoxin film was investigated using SPR. Figure 2 shows the change of SPR angle with respect to the immobilized concentration of cysteine-modified ferredoxin. In principle, a surface plasmon is a bound electromagnetic wave propagating at

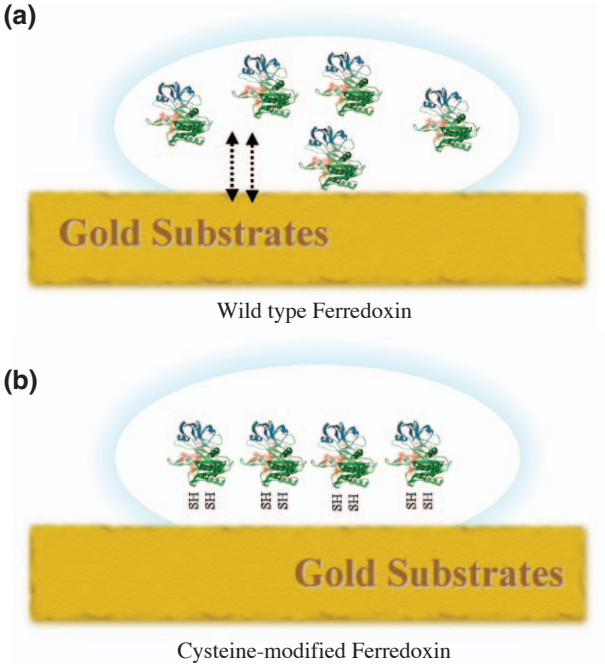

Figure 1. Schematic description of ferredoxin immobilization (a) Directly immobilization of wild type ferredoxin. (b) Immobilization of cysteine-modified ferredoxin.

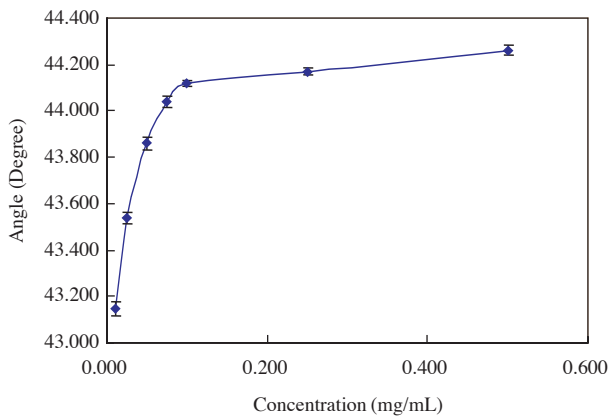

Figure 2. Change of SPR angle shift with respect to the ferredoxin concentration.

the metal-dielectric interface. The external laser field drives the free electron gas of metal in a distinct mode. The spatial charge distribution creates an electric field which is localized at the metal-dielectric interface. So, the plasmon resonance is extremely sensitive to the interfacial architecture. An adsorption process leads to a shift in the plasmon resonance and allows to monitor the mass coverage at the surface with a high accuracy. Therefore, the shift in the SPR

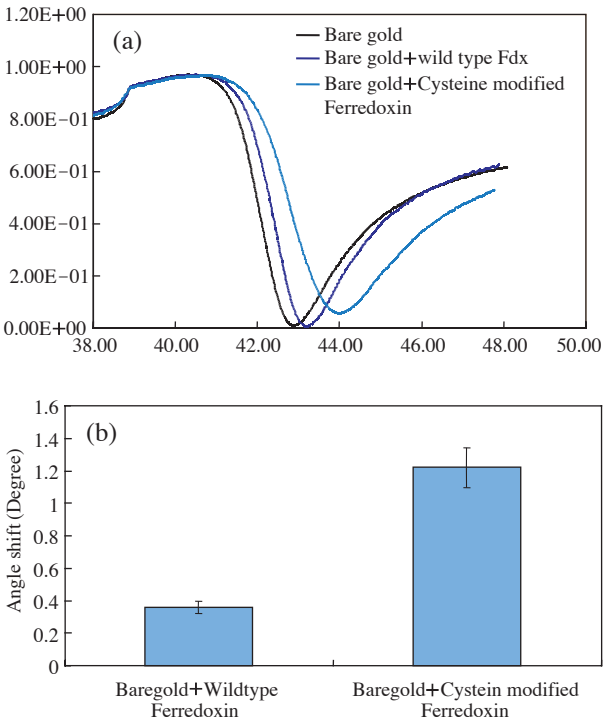

Figure 3. (a) SPR spectroscopy of bare gold surface, cysteine-modified ferredoxin immobilized surface, and wild type ferredoxin immobilized surface. (b) Comparison of SPR angle shift of cysteine-modified ferredoxin and directly immobilized wild type ferredoxin.

angle verified that thin layer of cysteine-modified ferredoxin on Au surface was formed. Also, as the concentration of cysteine-modified ferredoxin was increased, the amount of SPR angle shift was also increased and finally saturated. The results are presented in Figure 2. Moreover we determined the optimized cysteine-modified ferredoxin concentration from the saturated SPR angle curve. After saturation concentration was determined, all experiment was carried out using optimized concentration. Optimal protein concentration was 0.100 mg/mL.

Figure 3-(a) shows the SPR angle shift according to the deposition on Au surface. To confirm effective immobilization of cysteine-modified ferredoxin, the angle shift is composed with wild type ferredoxin. For confirming effectiveness of modified cysteine group, it was compared the angle shift of cysteine-modified ferredoxin with wild type ferredoxin. Therefore it can be naturally immobilized on Au substrate without any chemical modification. When 0.100 mg/mL of cysteine-modified ferredoxin was introduced on the cleaned Au surface, the SPR minimum angle shift was 1.02 degree were observed. But in the case of wild type ferredoxin, the SPR minimum angle shift

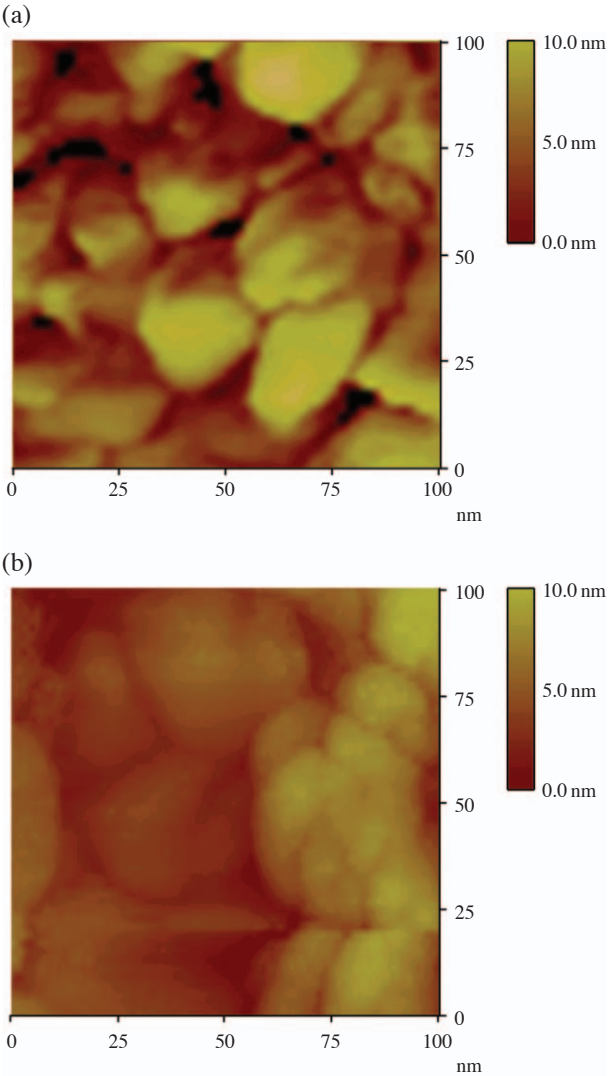

Figure 4. STM topography analysis : (a) Au substrate image (100 nm) (b) Cysteine-modified ferredoxin immobilized layer.

was only 0.27 degree at the same condition by non-specific binding. The results were compared in Figure 3-(b). It means that cysteine-modified ferredoxin was well immobilized than wild type one. That is, cysteine-modified ferredoxin could be successfully immobilized without any linker materials.

SPR data can explain only about optical thickness of the fabricated protein layer. Therefore surface morphology analysis is needed for accuracy. STM analysis may be used to compliment method of SPR. The benefit of combining SPR and Scanning Probe Microscope (SPM) imaging allows the inter-relationships between surface topologies and biological interaction with biomaterials to be efficiently analyzed[16]. Figure 4 shows the topography of the clean bare gold surface and ferredoxin immobilized gold substrate. Figure 4-(a) shows the bare gold image. And Figure 4-(b) show cysteine-modified ferredoxin immobilized surface. In 100 nm scale, immobilized cysteine-modified ferredoxin assumes the form of small lumps. Whereas adsorbed wild type ferredoxin covered whole surface by forming the aggregates of 15-20 nm in height in our previous work. Furthermore, it can be expected that cysteine modified proteins are immobilized with good orientation probably due to the effective linking of thiol-group onto Au surface.

Conclusions

The cysteine-modified ferredoxin layer was self-assembled on the Au surface. Optimal concentration for ferredoxin is determined as 0.100 mg/mL using SPR saturation curve. And it shows the effect that can be immobilized very well without linker material. STM topography showed that less aggregated layer was fabricated on the Au substrate. It is suggested that the formation of the functional proteins is well fabricated on Au substrate. It is a novel method for fabricating ferredoxin thin film without any linker material. From this work, we suggested about bio-film fabrication. The results can develop the nano-bio device using biomolecules.

Materials and Methods

Materials

R. sphaeroide Ferredoxin was recombined from Korea Research Institute of Bioscience and Biotechnology (KRIBB). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma-Aldrich, USA) and Phosphate buffer saline tween 20 (PBS-T Sigma-Aldrich, USA) were used as solvent to prepare and wash ferredoxin sample and washing buffer solution. The water used for all experiments was distilled and deionized by Milipore [(Milli-Q) water (DDW; >18 MΩ)].

Thin Film Fabrication

For the fabrication of Au substrate, cover glass composed of BK7 (18 mm × 18 mm, Superior, Germany) was used as a solid support. Chromium (Cr) was sputtered onto the glass substrate initially, as an adhesion material, to a thickness of 20Å and followed by an Au sputtering to a thickness of 430Å. The sputtered Au substrate was cleaned using pirana solution composed of 30 vol% H_2O_2 (Sigma-Aldrich MO USA) and 70 vol% H_2SO_4 (Duksan Chemical Co. Ltd,

Korea) at 70°C for 5 min, and then the cleaned Au substrate was immersed into pure ethanol solution for 1 hr. The Au substrate was rinsed with acetone and deionized water.

For the preparation of ferredoxin sample, ferredoxin of 0.1 mg/mL is dissolved in 10 mM HEPES (PH 5.14). Drop 0.10 mg/mL cysteine-modified ferredoxin solution on the substrate for 2 h. After 2 hrs, washing the substrates with DI water slightly. It is important thing to wash the substrate sufficiently. And then the residual solution on Au surface was removed by N_2 gun. It is very simple step compared with reported previously method using alkanethiol.

SPR Spectroscopy

SPR depends on a bound electromagnetic wave propagating at a metal-dielectric interface. The attenuated total reflection (ATR) configuration by Kretchmann, which is well known as the design for SPR instrumental method, relies on the phenomenon of total internal reflection. The external laser field drives the free electron of metal in a distinct mode. The spatial change distribution creates an electric field, which is localized at the metal-dielectric interface.

Bi-molecular interaction was monitored by surface plasmon resonance spectroscopy (Multiskop^{TM}, Optrel GmbH, Germany) using He-Ne laser light source with a wavelength of 632.8 nm. The p-polarized light beam by the polarizer was used as a reference and the intensity of the reflected beam was measured by photo multiplier tube (PMT) sensor. A glass prism (BK 7, n=1.5168) with 90° angle was used as a Kreschmann ATP coupler. The plane face of the 90° glass prism was coupled to cover glass via index matching oil. The resolution of the angle reading of the goniometer was 0.01 degree. All samples were monitored at a constant temperature of 20°C. The incidence angle was verified from 38 degree to 50 degree.

Topography Analysis using STM

The surface topography of the prepared metalloprotein film was obtained by commercially available scanning probe microscopy (XE-100, PSIA, Korea), image acquisition was carried out under the condition of I_{set}=0.5 nA , When the applied voltage was 0.1 V-1.0 V. STM image can support with SPR data for confirming immobilization.

Acknowledgements

This research was supported by the Nano/Bio Science & Technology Program (2006-00955) of the Ministry of Science and Technology, and by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-005-J02301), and by the Korea Science and Engineering Foundation (KOSEF) through the Advanced Environmental Monitoring Research Center at Kwangju Institute of Science and Technology.

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