Fabrication and Electrical Characteristics of Ferredoxin Self-Assembled Layer for Biomolecular Electronic Device Application

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Abstract A ferredoxin adsorbed hetero self-assembled layer was fabricated on chemically modified Au substrate. 4-Aminothiophenol (4-ATP) was deposited onto Au substrate and then N-succinimidyl-3-[2-pyridyldithio]propionate (SPDP) was adsorbed on the 4-ATP layer, since SPDP was used as a bridging molecule for ferredoxin adsorption. Ferredoxin/SPDP/4-ATP structured hetero layer was constructed because of strong chemical binding of ferredoxin, SPDP, and 4-ATP. The surface of the ferredoxin-adsorbed SPDP/4-ATP layer was observed by scanning tunneling microscopy. The hetero film formation was verified by surface plasmon resonance measurement. The current flow and rectifying property based on the scanning tunneling spectroscopy I-V characteristics was achieved in the proposed hetero layer. Thus, the hetero layer structure of ferredoxin functioned as a molecular diode with rectifying property. The proposed molecular diode can be usefully applied for the development of molecular scale electronic devices.

Key words: Ferredoxin, self-assembly layer, STS, SPR

In the recent decade, self-assembled monolayers (SAMs) have been studied, because SAM offers an easy method to modify a metal substrate for various applications. Substrate modification by chemical adsorption can control the protein immobilization onto solid substrate. The most extensively studied system of SAM is alkanethiols, long hydrocarbon chains with a sulfur atom at one terminus, on gold surface. In 1983, Nuzzo and Allara [1, 2] reported first in this area, showing that dialkyldisulfides form oriented monolayers on gold surfaces. Since then, many studies on SAM formation of alkanethiols on gold have been carried out, dealing with structure, kinetics, effect of chain length, and solvent effect [3–7]. It has been found that sulfur compounds coordinate very strongly to various metal surfaces, such as gold, silver, copper, and platinum. In most works to date, however, gold surface has been used for the SAM formation of alkanethiols, because gold cannot form a stable oxide, and can therefore readily be handled in ambient conditions. Generally, modification of gold substrate by alkanethiol forms a well-oriented and ordered monolayer. Thus, it can be used to fabricate a well-ordered biomolecular heterolayer because of its high coverage and strong sulfur-gold bond.

In particular, fabrication techniques using biomolecules could be applied to fabricate biochips, bioelectronic devices, and biosensors. In our previous research, fabrication techniques of biomolecular hetero self-assembly (SA) layers by electrostatic attraction, based on the Langmuir-Blodgett (LB) technique, and by modifying functional groups of proteins have been studied for biomolecular electronic device application [8–12]. However, the application of a biomolecular layer for an electronic device is more difficult because of their low molecular orientaion, cluster formation in solution, and low coverage on a metal substrate.

The purpose of this research was to fabricate a ferredoxin-adsorbed hetero SA layer for molecular electronic devices application. In this study, the ferredoxin-adsorbed hetero SA layers were investigated, and their electrical properties were examined. A 4-aminothiophenol (4-ATP) SA layer was fabricated onto a gold substrate. N-succinimidyl-3-[2-pyridyldithio]propionate (SPDP) was used to bind the ferredoxin onto the 4-ATP layer. SPDP is widely used to modify protein surface for its adsorption onto metal substrate [13]. Furthermore, SPDP can bind easily to the CH_3 terminal group of alkanethiol SAM. Thus, if SPDP is the interfacial layer, proteins will immediately immobilize

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on the alkanethiol SAM. SPDP-based protein modification has been reported in our previous research [8]. SPDP was used as the interfacial layer to immobilize ferredoxin onto a 4-ATP layer. Surface morphologies of hetero SA layers were observed to verify the SA layer formation by scanning tunneling microscopy (STM). To verify the SA layer deposition, surface plasmon resonance (SPR) measurement of 4-ATP, SPDP, and ferredoxin was performed. The electrical property of ferredoxin-adsorbed hetero SA layer was investigated by scanning tunneling spectroscopy (STS), based current-voltage (I-V) measurement, for molecular electronic device performance.

MATERIALS AND METHODS

Ferredoxin extracted from *Spinach* was purchased from the Sigma Chemical Company (St. Louis, USA), and was dissolved in tris-HCl buffer solution at pH 7.0 and degassed with nitrogen gas. 4-ATP and SPDP used to immobilize the ferredoxin onto the substrate were purchased from Pierce Biotechnology (IL, U.S.A.), and they were dissolved in ethanol at nitrogen atmosphere. The substrate used for the adsorption of ferredoxin was gold-coated glass of size 1×1 cm.

The prepared Au substrate was immersed into the 4-ATP solution for 24 h, and then rinsed with absolute ethanol and deionized water before drying under a nitrogen atmosphere. The 4-ATP layer on an Au surface can be used as a template for the adsorption of ferredoxin because of the NH_2 group of 4-ATP. Since the ferredoxin surface is composed primarily of negatively charged amino acids, like aspartate and glutamate, it can be adsorbed on 4-ATP layers by electrostatic attraction. However, ferredoxin adsorption by electrostatic force is a weak physical interaction, and therefore, the water-soluble ferredoxin is easily dissolved in electrolyte solution at electrochemical property measurement, and electrostatic force interaction cannot control the adsorption orientation of ferredoxin. Thus, we used a SPDP as a bridging molecule for the adsorption and orientation of ferredoxin on the 4-ATP surface. The 4-ATP adsorbed layer was immersed into the SPDP dissolved solution for 24 h, and then rinsed with ethanol and deionized water. Finally, SPDP/4-ATP-modified Au substrate was immersed into the ferredoxin solution for 12 h, and then rinsed with deionized water to remove other salts. The fabrication method for the ferredoxin/SPDP/4-ATP-structured hetero SA layer is shown schematically in Fig. 1.

The topographies of the hetero SA layer were obtained by STM (Easy Scan STM, Nanosurf, Swiss). In all experiments, STM was operated in constant-height mode under ambient condition. The typical set point, servo gain, and scan rate used were 1 nA, 9–10, and 0.25 sec/line, respectively. SPR measurements of the 4-ATP,
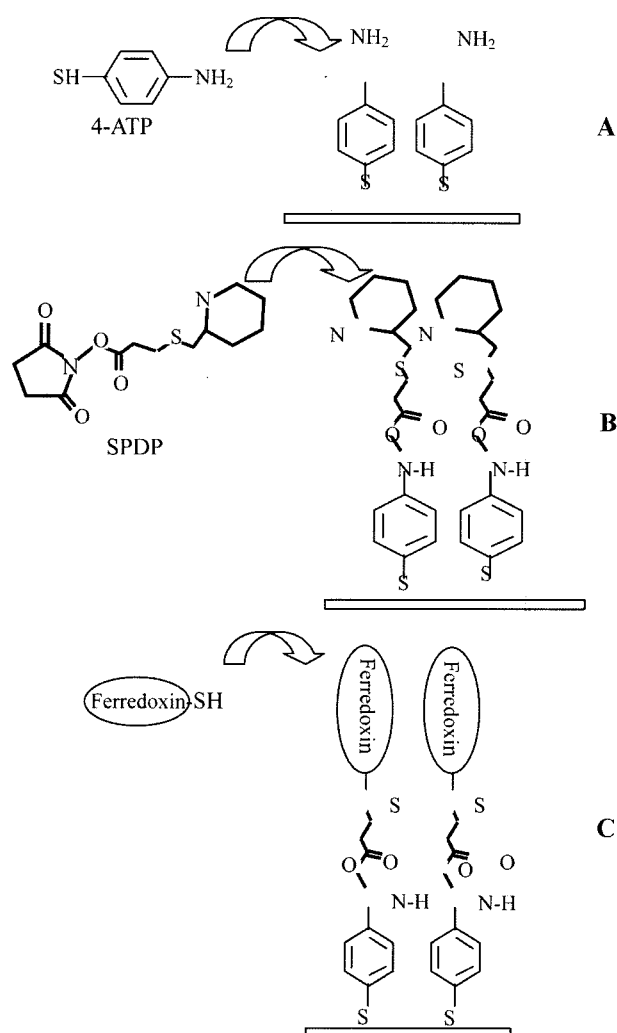

Fig. 1. Schematics of the ferredoxin immobilization onto gold substrate. A. 4-ATP SAM on gold substrate; B. deposition of SPDP interfacial layer; C. immobilization of ferredoxin.

SPDP, and ferredoxin-adsorbed SA layers were carried out using a Multiskop SPR spectrometer (Optrel GbR, Germany).

Current-voltage characteristics were obtained using an STS mode of STM. A STM tip (99.99% purity Au wire, Aldrich Chemical Co., U.S.A.) was used as the upper electrode for conductivity measurements. The set point for the STM tip approach was 1 nA and the scan range used for the electrical property measurement was -1.0~1.0 V. STS-based current-voltage measurement was schematically illustrated in our previous report [14].
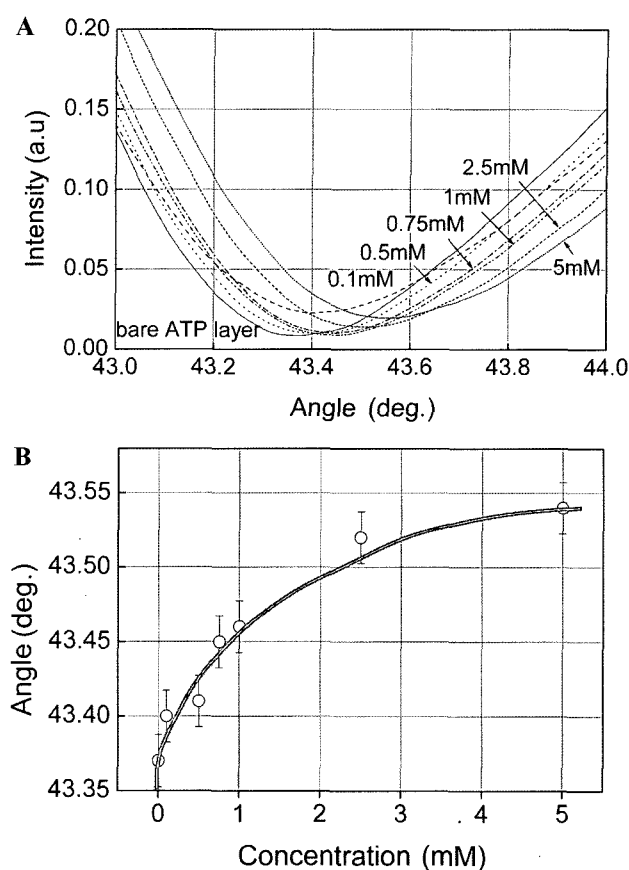
RESULTS AND DISCUSSION

For the optimal deposition concentrations of the 4-ATP and SPDP SA layers, SPR spectra at various concentrations
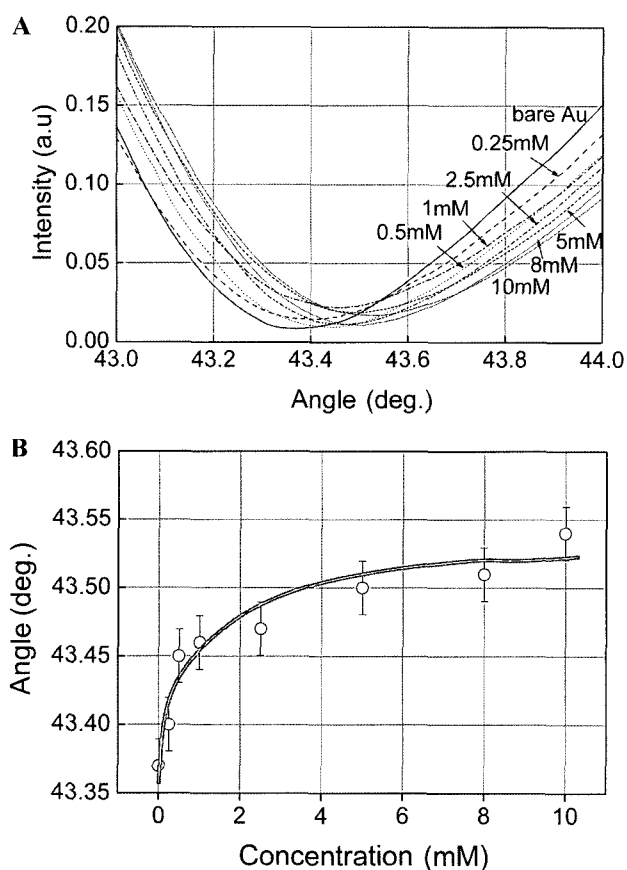
Fig. 2. A. SPR curves of the 4-ATP SA layer for the effect of increasing concentration in 4-ATP; B. Angle shift versus 4-ATP concentration.

Fig. 3. A. SPR curves of SPDP interfacial layer for the effect of increasing concentration in SPDP; B. Angle shift versus SPDP concentration.
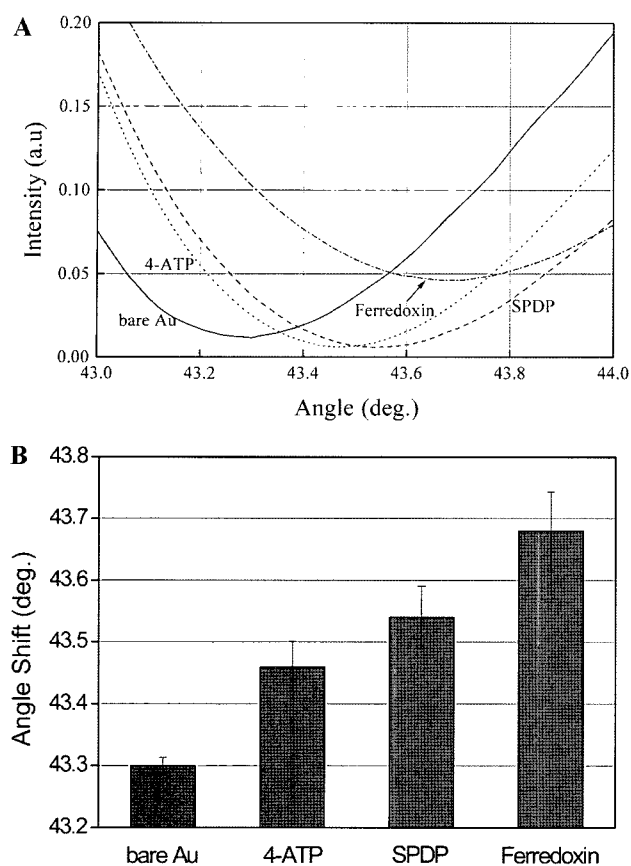
were obtained. Figure 2 shows the change of SPR minimum position by increasing the concentration of 4-ATP deposited on Au substrate. As the concentration of 4-ATP increased, the value of the SPR minimum position shifted to higher angles and was saturated at the concentration above 6 mM. Since SPR angle changes depending on the measurement conditions, we used the SPR minimum position of bare Au plate as the reference angle. Figure 3 also shows the change of SPR minimum position by increasing the concentration of SPDP deposited on the 4-ATP SA layer: Deposition of SPDP produced shifts in the SPR minimum positions by increasing the concentrations. The SPR minimum position of the SPDP layer shifted to higher angles and was saturated at above 4 mM. From these results, the concentrations of 4-ATP and SPDP for the fabrication of the ferredoxin-adsorbed hetero SA layer were determined to be 6 mM and 4 mM, respectively SPR shifts of the ferredoxin, SPDP, and 4-ATP layers are shown in Fig. 4. Adsorption of 6 mM 4-ATP, 4 mM SPDP, and the subsequent deposition of 0.2 mM ferredoxin produced a shift in the SPR curves to high angles. As shown in Fig. 4B, the SPR minimum reflectivity position shifted significantly from 43.30° to 43.47° by the

chemical binding of 4-ATP on the Au substrate and, upon subsequent deposition of SPDP on the 4-ATP layer, the SPR minimum reflectivity position shifted from 43.47° to 43.54°. The SPR minimum reflectivity position was also shifted significantly from 43.54° to 43.68° by the adsorption of ferredoxin on the SPDP layer. Typically, SPR angle change means the thickness of film and roughness of its surface. Therefore, the SPR angle changes of each film indicate the change of film thickness and roughness [15]. We used 6 mM ATP and 4 mM SPDP for optimal concentration to minimize the roughness effect of SPR intensity. This result suggests that the ferredoxin molecules were well immobilized on the SPDP interfacial layer.

To develop molecular electronic devices with functional protein films, film formation is generally considered to be one of the most important factors for device performance. Ferredoxin film formation was done by chemical modification using the SA technique as described in Materials and Methods. The SA technique using alkanethiol provides a high degree of orientation without loss of activity, well-ordered array of molecules, and strong binding to substrate. The surface morphologies of the 4-ATP SA layer, SPDP-
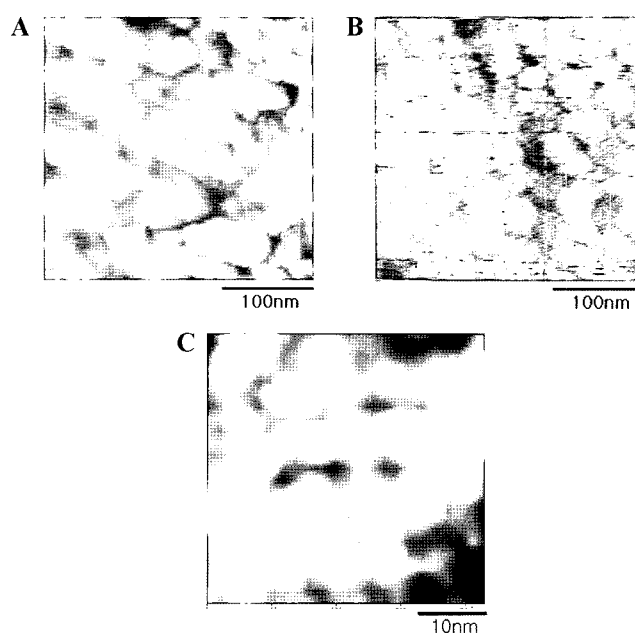
A

B

Fig. 4. SPR shifts of ferredoxin, SPDP, and 4-ATP hetero SA layers.

A   **B**

100nm   100nm

C

10nm

Fig. 5. STM image of (A) 4-ATP SA layer on gold, (B) SPDP SA layer on 4-ATP, and (C) immobilized ferredoxin layer on SPDP/4-ATP layer.

adsorbed layer, and ferredoxin-adsorbed SPDP/4-ATP layer by STM are shown in Fig. 5.

As shown in Fig. 5A, the size of the 4-ATP molecules was about 10–20 Å, and the height was about 5 Å. The head groups of the 4-ATP molecules were observed as bright spots and no holes were shown, indicating that the 4-ATP completely covered the Au surface. Thus, the 4-ATP molecules were successfully adsorbed onto the Au substrate and formed an SA monolayer. As shown in Fig. 5B, SPDP adsorption was observed with sizes of 10–15 nm, suggesting that the SPDP molecules were well adsorbed on the 4-ATP surface. In Fig. 5C, the surface of the ferredoxin-bound SPDP/4-ATP layer is shown at 50 nm scan size. As shown in Fig. 5C, ferredoxin single molecules formed a closely packed array, and the size of each molecule was about 5–7 nm. This indicates that the ferredoxins did not form aggregates on the SPDP surface.

By approaching the STM tip onto the ferredoxin-adsorbed hetero SA layer, electrical properties were determined under ambient conditions. As shown in Fig. 6, the current flow was determined from the measurement of STS characteristics. When a forward bias was applied in the range of 0~+1.0 V, a current was generated with an appropriate bias voltage.

On the other hand, current was generated on reversing the polarity in the range of 0~–1.0 V. When +1.0 V of forward bias was applied to the ferredoxin-adsorbed SA layer, a current of 30 nA was generated, and the current was –25 nA when –1.0 V was applied.

This result indicates that the current flow in forward bias was slightly greater than that in reverse bias. This unsymmetrical property (rectifying property) is due to the redox potential difference between the ferredoxin and 4-ATP: The negative redox potential value of ferredoxin was exhibited between –400~–600 mV [16], and the 4-ATP was exhibited at around –230 mV. Therefore, the electron flow from the ferredoxin layer to the 4-ATP layer was generated because of the redox potential difference that causes the unsymmetrical property.

A hetero SA layer, composed of ferredoxin/SPDP/4-ATP, was investigated. Based on the STM topologies, well-ordered ferredoxin immobilization was verified. Adsorption of the ferredoxin, SPDP, and 4-ATP on the Au substrate was verified by SPR angle shifts. The directional nature of the current occurred efficiently in the proposed molecular array. Thus, the proposed molecular layer, composed of ferredoxin, should be used as a molecular electronic device. The ferredoxin SA layer on the Au substrate, which is modified by the SPDP interfacial layer and the alkane thiol, provides a better orientation of the molecular SA layer than the method of electrostatic layer-by-layer. Thus, we used the SPDP interfacial layer and the alkanethiol-based technique to apply the ferredoxin used for a molecular scale electronic device.
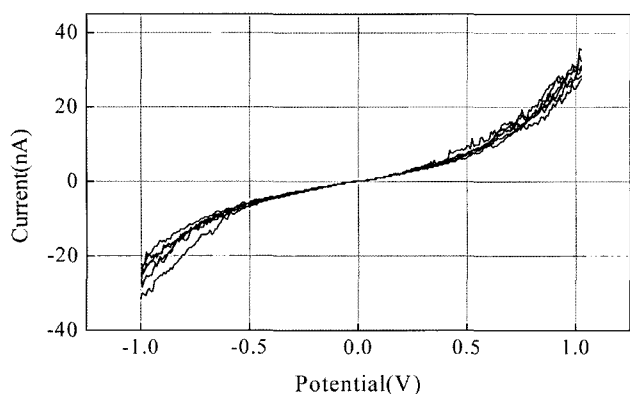
Fig. 6. STS-based current-voltage characteristic of the ferredoxin/SPDP/4-ATP hetero SA layer.

Acknowledgments

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