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Electrochemical Property of Immobilized Spinach Ferredoxin on HOPG Electrode

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Abstract The stability and electrochemical properties of a self-assembled layer of spinach ferredoxin on a quartz substrate and on a highly oriented pyrolytic graphite electrode were investigated. To fabricate the ferredoxin self-assembly layer, dimyristoylphosphatidylcholine was first deposited onto a substrate for ferredoxin immobilization. Surface analysis of the ferredoxin layer was carried out by atomic force microscopy to verify the ferredoxin immobilization. To verify ferredoxin immobilization on the lipid layer and to confirm the maintenance of redox activity, absorption spectrum measurement was carried out. Finally, cyclic-voltammetry measurements were performed on the ferredoxin layers and the redox potentials were obtained. The redox potential of immobilized ferredoxin had a formal potential value of -540 mV. It is suggested that the redox-potential measurement of self-assembled ferredoxin molecules could be used to construct a biosensor and biodevice.

Key words: Spinach ferredoxin, dimyristoylphosphatidylcholine, self-assembled layer, redox potential, cyclic-voltammetry

Some metalloproteins such as the ferredoxins have iron atom, located not in heme but associated in inorganic sulfur atoms in Cys residues. Ferredoxins participate in one-way electron transfers through the oxidation and reduction of iron atoms in photosynthesis [9]. Ferredoxin, extracted from plants, exhibits comparatively low negative redox potentials and acts as an electron carrier (acceptor) in photosynthetic processes [10, 12]. In a previous report [6], electrochemical measurements of spinach ferredoxin were investigated on a viologen (an electron transfer mediator)-coated glassy-carbon electrode. Promoter-immobilized functional electrodes that exhibit well-defined redox potentials of ferredoxin have also been investigated [1, 14]. Recently, Halagdjian *et al.* [7, 13] reported a direct electron transfer reaction between metalloproteins and electrodes using lipid-modified electrodes. In addition, Nassar *et al.* [10] reported on the electrochemical properties of cast layer of *Chlorella* ferredoxin and on lipid bilayer on the basal plan of a pyrolytic graphite (BPG) electrode [10]. However, electrochemical property measurement of immobilized spinach ferredoxin into a lipid layer has not yet been reported since electrochemical measurement of immobilized ferredoxin onto a metal substrate is difficult due to adsorption of ferredoxin molecules at negative potential. The purpose of this research is to fabricate the ferredoxin self-assembly (SA) layer and then to determine its redox-potential by cyclic-voltammetry (CV) measurement. We investigated many types of biodevices such as a biosensor, a biochip, and a bioelectronic device [2–4, 11]. The fabrication and reduction-oxidation state control of ferredoxin molecules should be applied to construct a bioelectronic device for information storage.

In this study, the immobilization and redox-potential measurements of a ferredoxin SA layer into a lipid-layer-coated highly oriented pyrolytic graphite (HOPG) electrode were investigated. Analysis of the bilayer surface was performed using an atomic force microscope (AFM) to verify the ferredoxin immobilization. To verify ferredoxin immobilization on the lipid layer and to confirm the maintenance of redox activity, absorption spectrum measurement was then carried out. Finally, CV measurements were performed on the ferredoxin bilayer and the redox potential value was obtained.

Ferredoxin extracted from spinach was purchased from Sigma (St. Louis, U.S.A.). Ferredoxin was dissolved in 50 mM Tris-HCl at pH 7.2, and degassed with nitrogen gas. The purity of ferredoxin was checked by determining the ratio of absorbances (A_{422}/A_{276}), by absorption spectrum, and it was found to be 0.33. This ratio agreed with the literature value of 0.35 [5]. The biological lipid,

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dimyristoylphosphatidylcholine (DMPC), was also purchased from Sigma Chemical Co. and used without further purification. All experiments were performed at 25°C under nitrogen atmosphere. One hundred μl of 5 mM DMPC dissolved in 50 mM Tris-HCl (pH 7.2) was deposited on a HOPG electrode (SPI, U.S.A.) and a quartz substrate. One hundred μl of 1.67 mM ferredoxin was then added to the lipid layer on the substrates, and air-dried at 25°C for 24 h. Ferredoxin bilayers obtained by the above described method were dried under vacuum for about 24 h. All solutions were degassed with high purity nitrogen gas before use. CVs were measured in 50 mM Tris-HCl (pH 7.2) with a potentiostat (CHI 600, CHI instrument, & BAS 100W, BioAnalytical Systems, U.S.A.). An Ag/AgCl electrode and a coiled platinum wire were used as the reference and counter electrodes, respectively. All experiments were performed at 25°C in a nitrogen glove box (HE-243-2, MO-20-SSG purifier, Vacuum Atmosphere Co.). Absorption spectrum measurements of the immobilized bilayer were performed at 25°C in air atmosphere. Figure 1 shows the structure of the ferredoxin immobilized bilayer. Surface analysis of a bare HOPG electrode and of the immobilized ferredoxin bilayers was performed by atomic force microscopy (XE-100, PSIA, Korea). Scan size, set point, and scan rate for the AFM were 3 μm, 20 nA, and 1 Hz, respectively.

To verify the ferredoxin immobilization, absorption spectra of the ferredoxin solution and of the bilayer were measured. Ferredoxin absorbs visible light in the region from 400 to 460 nm, and in this region, the absorption intensity decreases about 50% when ferredoxin is reduced [12]. The absorption spectra of the ferredoxin solution and that of the bilayer are shown in Fig. 2. The absorption spectrum of the ferredoxin solution after 100 min showed no peaks at 400–320 nm. It was indicated that the absorbances of oxidized and reduced ferredoxin were not present after 100 min. Some absorbance changes at these ranges were occasionally observed at 60 min. In the case of immobilized ferredoxin after 24 h under vacuum drying, the absorption characteristic was similar to native ferredoxin in solution. It is suggested that the ferredoxin molecules were successfully immobilized on the lipid layer through the absorption spectrum compared with ferredoxin in solution state. When a protein SA layer was constructed by lipid layer, proteins tended to form aggregates. In Fig. 3, the surface morphologies of bare HOPG electrode, and ferredoxin immobilized layer, determined by AFM,

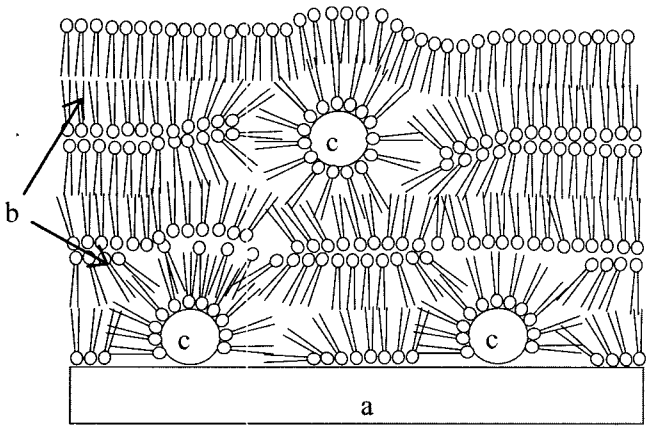

Fig. 1. Schematic structure of the immobilized ferredoxin bilayer on a HOPG electrode. a, Substrate; b, lipid layer; c, immobilized ferredoxin molecules.

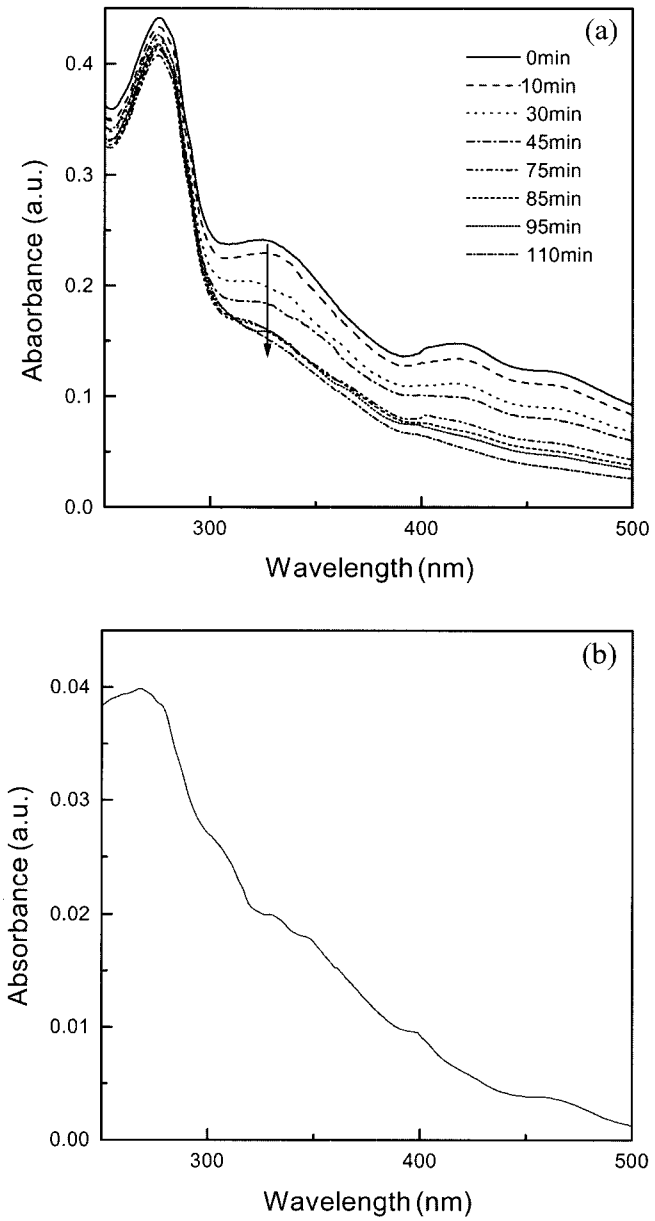

Fig. 2. Absorption spectrum of (a) a ferredoxin-containing solution with time dependence, and (b) immobilized ferredoxin bilayer on quartz substrate.

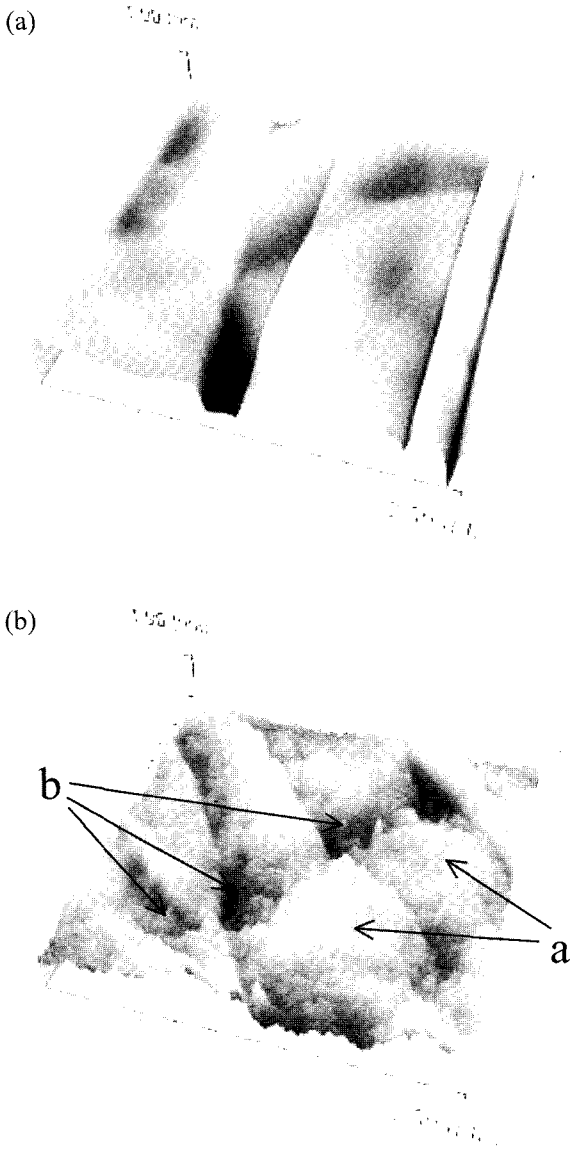

Fig. 3. Surface morphologies by AFM. (a) Bare HOPG electrode; (b) immobilized ferredoxin lipid bilayer on HOPG: a, protein cluster; b, lipid layer.

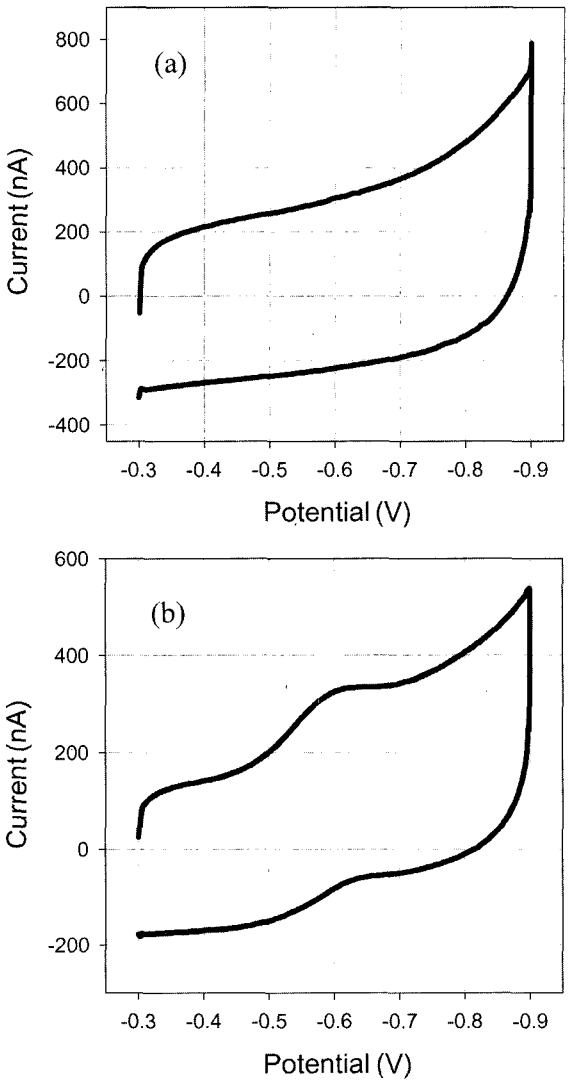

Fig. 4. Cyclic-voltammetry measurements of (a) a bare HOPG electrode and (b) an immobilized ferredoxin bilayer.

are shown. As in Fig. 3(b), the ferredoxin layer was well deposited and formed aggregates on HOPG electrode. In the AFM image, the diameter of the ferredoxin aggregates was about 30–40 nm. These results indicate that the ferredoxin molecules were well immobilized on the lipid-layer-coated HOPG surface.

When a metal electrode was used as a substrate for protein immobilization, it was found that the proteins tended to aggregate and then become denatured on the electrode surface, which should impede the electron transfer from proteins onto the electrode surface [8]. Since the supporting layer between the protein and metal substrate acts as a supporter for protein immobilization, impediment to electron transfer would become smaller if the supported molecule was located in the protein-immobilized electrode. In the present study, the lipid bilayer was used as the supporting layer to immobilize ferredoxin molecules and to reduce denaturation [10]. For protein immobilization on metal electrodes, SA methods are generally used by thiol adsorption, which form the strong chemical bonding between protein and Au substrate onto the Au substrate. However, in electrochemistry, the adsorbed thiol molecules can be easily desorbed from the substrate in the negative potential range. Thus, electrochemical experiments involving some metalloproteins with negative redox potentials are difficult. In this study, the HOPG electrode was used as the working electrode for the redox potential measurements, and a lipid bilayer was used to immobilize the ferredoxin

molecules onto the electrode to prevent molecular desorption at negative potential range. Figure 4 shows a CV of a bare HOPG electrode and of a ferredoxin-immobilized lipid bilayer on HOPG in a nitrogen atmosphere. The CV measurement of the ferredoxin-immobilized bilayer was usually set up in a potential range from -900 mV to -300 mV at a scan rate of 100 mV/s. In Fig. 4(b), the oxidation and reduction potentials of immobilized ferredoxin are highly negative at -470 mV and -610 mV, respectively. The -540 mV value for the midpoint potential determined from CV indicates the redox-potential of the immobilized ferredoxin. Since ferredoxin in solution exhibits a negative redox-potential range from -310 mV to -455 mV, the redox-potential value of the immobilized ferredoxin layer was higher than its value in solution. It is concluded that the ferredoxin molecules were successfully immobilized onto the HOPG electrode, and redox activity of the immobilized ferredoxin was preserved through reduction-oxidation. In conclusion, this study showed that spinach ferredoxin can be easily immobilized onto a metal substrate by a lipid layer. Immobilized ferredoxin was found to be optically and electrically stable. Redox potential in the CV of immobilized ferredoxin had a formal potential value of -540 mV, which is close to the previously reported value in solution. These results provide suitable environments for the direct electrochemical measurement of immobilized biomolecules with negative redox-potential ranges. Based on the results, the next study is to fabricate a ferredoxin-immobilized SA monolayer and a molecular electronic device for the biostorage device using reduction-oxidation of ferredoxin molecules.

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