Removal of Aggregates of Protein from Its Self-assembled Monolayer on Metal Substrates

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Abstract: Elution ability of 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) was found to segregate readily the aggregates of ferredoxin from its self-assembled monolayer onto metal substrates without a loss of the intrinsic property of ferredoxin: a high-resolution AFM image showed that the self-assembled ferredoxin monolayer on the (100) surface of the silicon substrate consisted of ferredoxin monomers or small clusters when the surface was treated with CHAPS. The electric property of the monolayer of ferredoxin on the gold surface was also confirmed to remain intact by the cyclic voltammetry measurements. Those results offer a new technique for the elimination of nonspecific adsorption of proteins on inorganic surfaces, which has been a long-term problem in the fabrication of biomolecular electronic devices.

Keywords: biomolecular electronic device, ferredoxin, self-assembled monolayer, CHAPS

Introduction

Currently, study on the fabrication of the artificial molecular devices based on organic molecules has been extensively made [1-7]. In particular, some model systems for the molecular photodiodes and the electronic diodes have been investigated by Choi and coworkers [8-10] using redox proteins including cytochrome c, green fluorescent protein and bacteriorhodopsin. The pattern formation of biomolecules in their model devices was made by a self-assembly technique, and the current-voltage characteristics and the photo-switching effect of the proposed devices were successfully demonstrated. However, when proteins are adsorbed onto metal substrates, aggregation of proteins due to their nonspecific affinity is most problematic, because those aggregates reduce the electronic efficiency of the devices [11,12] and thus this issue should be resolved for the long-term stability of the biomolecular devices.

At a solution with the higher pH than isoelectric point of a protein, the protein surface in the solution is charged negatively and accordingly the proteins are dispersed in the solution by the repulsive force between them. However, they are not completely dissolved and exist as aggregates. The interaction between the surfactant and the protein in the bulk solution has been extensively studied. Harder and coworkers [13] and Sigal and coworkers [14] have studied the interaction between proteins and the solid surface self-assembled with specific functional groups. They reported that a number of functional groups have an ability to prevent nonspecific adsorption of sample proteins on the solid support. The removal of the preadsorbed protein aggregates on the solid/liquid interface by using surfactants in food industry is also a practical interest for the cleaning of food processing equipments [15,16]. However, the deformation of three-dimensional structure of protein and the loss of biological activity have been often observed due to the strong affinity between proteins and surfactants.

Recently, we have found that ferredoxin can exist as monomers without a change in inherent properties when the protein aggregates in the solution or on the solid surface are treated with a zwitterionic surfactant, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate). In this paper, we report the fabrication method for the self-assembled ferredoxin monomolecular layer on metal substrates by eliminating the nonspecific adsorption of ferredoxin with CHAPS.

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Experimental

Materials
Ferrodoxin from Spinacia oleracea (lot number 71K7008), 11-mercaptopoundecanoic acid (MUA), 3-aminopropytri-
methoxysilane (APTMS), succinic anhydride and 1-
ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
from Aldrich and ethanol from Hayman were used as
received. N,N-Dimethylformamide (DMF) from Aldrich
was distilled with Linde type 4 A molecular sieve.
CHAPS was purchased from Fluka. Trizma buffer (pH =
7.5) and phosphate buffer (pH = 7.4) were used. Gold-
coated glass plates from Inostek were used after cleaning
by dipping into a piranha bath consisting of the 3:1
mixture of sulfuric acid and hydrogen peroxide for
10 min to remove organic residues on the surface. Those
plates were washed with copious amount of deionized
distilled water (DI water).

Pattern Formation of Ferrodoxin on Metal Substrates
Ferrodoxin (Figure 1) in Spinacia oleracea, a Fe-S redox
metalloprotein, is composed of 97 amino acids, its
molecular weight is 11000 Da, and has optimal activity
in pH = 7.0 [17,18]. Ferrodoxin molecule has an ellip-
soidal shape with 5 nm in size [19] and its isoelectric
point is 4.0. Two amine groups (Lys 50 and Lys 52) on
the bottom surface of ferrodoxin were considered suitable
to be coupled with an inorganic substrate through the
reaction between the amide group and the carboxylic
acid group and were expected to be arranged with a
configuration in which the active site is close to
perpendicular to the planar metal surface.

The scheme for the preparation of self-assembled
ferrodoxin monomolecular layers onto silicon or gold
substrates is given in Figure 2. The formation of the
self-assembled monolayer of protein on the silicon
substrate is well known and thus only the preparation
procedure of the self-assembled monolayer of ferrodoxin
on the gold substrate will be introduced briefly here. In
Figure 2, the attachment of the thiol group of MUA
tethered with the EDC moiety was considered to be
immobilized in the hollow site on Au (111) surface and
was confirmed by X-ray photoelectron spectroscopy
(XPS). XPS spectra of the samples were recorded on an
ESCALAB 250 XPS spectrometer (VG Scientifics) with
AlKα excitation (1486.6 eV). The XPS spectra (not
shown here) were found to show clear evidence that the
reaction was done efficiently. Also it was confirmed that
CHAPS does not react with the gold substrate. EDC-
activated gold or silicon substrates were inserted into 9.8
µM ferrodoxin solution prepared using 10 mM phosphate
buffer and they were reacted for 10 h at 25°C. The
substrates expected to be self-assembled with ferrodoxin
molecules were washed with DI water and were dried
with a gentle stream of high purity N₂.

Segregation of Ferrodoxin in Solution and Metal
Substrates
Without addition of any surfactant, the size distribution
of ferrodoxin molecules in the solution at pH = 7.5 was
measured using a dynamic light scattering spectrometer
(BJ-200SM Goniometer, Brookhaven Instrument). It was
found that the size of ferrodoxin monomolecules was
about 10 nm and that of the most aggregates was about
120 nm. As an amount of CHAPS added into the solution
was increased, the number of monomolecules was found
to increase rapidly and that of aggregates to decrease.
When molar ratio of CHAPS to ferrodoxin (C/F) was
equal to 200, only ferrodoxin monomers were found to
exist in solution [20].

Based on the results given above, ferrodoxin aggregates
on the gold substrate were treated with CHAPS
(C/F=200). However, it was very difficult to distinguish
between the gold grain and protein molecules in the
atomic force microscope (AFM) image below 1 µm ×
1 µm of the ferrodoxin monolayer on the gold substrate.
On the other hand, it has been known that better images
of protein on a silicon wafer can be obtained [21] and
thus in order to demonstrate indirectly the size of
ferrodoxin clusters self-assembled on the gold substrate,
the self-assembled ferrodoxin monolayer on a silicon
substrate was prepared with the CHAPS treatment by the
method shown in Figure 2 and the AFM images of
ferrodoxin aggregates on the silicon substrates were
obtained from Autoprobe CP (Park Scientific instru-
ment). To confirm directly the formation of the
ferrodoxin monolayer on the gold substrate, the redox
behavior of the ferrodoxin was measured by a cyclic
voltammetry system. In those experiments, a ferrodoxin/
EDC/MUA-modified gold plate, an Ag/AgCl electrode
and a platinum electrode were used as a working electrode, a reference and a counter, respectively.

Results and Discussion

Figure 3(A) shows the AFM image of a bare silicon wafer. Figure 3(B) is the image of ferredoxin aggregates on the silicon substrate of 1 μm × 1 μm pixel without the treatment of surfactant. It is shown that the size of ferredoxin aggregates is on the order of 100 nm. Figure 3(C) is the AFM image of the ferredoxin monolayer self-assembled on the silicon wafer after the CHAPS treatment. The size of ferredoxin clusters is clearly shown to be about 5-10 nm which is on the order of the ferredoxin monomer. From those results, it is concluded that the CHAPS can remove the nonspecifically adsorbed ferredoxin aggregates and the surface of the substrate is expected to be covered by chemically adsorbed ferredoxin molecules. Therefore, we expect that the morphology of ferredoxin monolayer on the gold substrate with the CHAPS treatment would be similar with that shown in Figure 3(C).

Figure 4 shows a cyclic voltammogram for a ferredoxin monolayer-coated gold surface in the 100 mM Trizma buffer solution (pH = 7.5, 0.1 M KCl). Applied voltage in the present experiments was from -0.8 V to -0.3 V with a scan rate of 20 mV/s, considering that the standard electrode potential of ferredoxin was -0.441 eV [22]. When the potential went over the energy state of the redox protein, the reduction potential at -0.59 V was generated between the redox molecule and the gold.
and also indicates that a large potential at the gold electrode needs to overcome this band gap, because the gold electrode does not oxidize readily. In addition, there was no difference in the cyclic voltammograms obtained from the electrodes with the ferredoxin monolayer and with the ferredoxin aggregates. From this result, it is known that the CHAPS treatment does not degrade the electronic property of the ferredoxin molecule.

Conclusions

It was shown that segregation of protein aggregates nonspecifically adsorbed on the metal substrates with the CHAPS treatment is possible by providing a high-resolution AFM image of the self-assembled monolayer with 5-10 nm sized ferredoxin molecules on the (100) surface of the silicon substrate. The electric property of the ferredoxin monolayer self-assembled on the gold surface with the CHAPS treatment was also shown to remain intact by the cyclic voltammetry measurements. Those results demonstrate a new way for the fabrication of a self-assembled protein monomolecular layer using a surfactant without degradation of the inherent properties of a protein. Experiments on the segregation phenomenon of redox proteins with various surfactants are underway for the fabrication of a biomolecular device with diverse protein monomers.

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References


