Ultramicroscopy 108 (2008) 1342–1347

Contents lists available at ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

# Immobilization of iron storage protein on a gold electrode based on self-assembled monolayers

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ARTICLE INFO

*PACS:*
82.37.Rs
68.37.Ps
82.45.Tv
81.16.Dn

*Keywords:*
Ferritin immobilization
Atomic force microscopy
Cyclic voltammetry
Self-assembled monolayers

ABSTRACT

Ferritin is a globular protein consisting of 24 subunits to form a hollow shell and is capable of storing iron in the cavity. Findings that the naturally existing iron core of ferritin can be readily extracted and replaced with a variety of electroactive materials make ferritin suitable for biosensor and biofuel cell applications. The immobilization of ferritin on the electrode surface is essential for various bioelectronic applications. In this work, based on self-assembled monolayers, ferritin was immobilized on a gold electrode through two different methods: chemisorption of thiolated ferritin onto bare gold electrodes and covalent binding of ferritin to succinimidyl alkanedisulfide-modified Au electrodes. Effects of experimental conditions on the ferritin immobilization were investigated. The ferritin immobilized on the gold electrode was characterized by atomic force microscopy and cyclic voltammetry.



## 1. Introduction

Ferritin is an iron storage protein found in most organisms, which consists of 24 subunits to form a hollow shell with an outer diameter of 12 nm and an inner diameter of 8 nm. This metalloprotein is capable of encapsulating up to 4500 Fe atoms in the cavity [1]. Ferritin protein has hydrophobic and hydrophilic molecular channels, which connect the inside with the outside of the shell. Through the hydrophilic channels, which are lined with aspartate and glutamate residues, soluble Fe(II) ions enter the protein shell and are oxidized to insoluble Fe(III) ions for incorporation into the mineral core [2]. Since the naturally occurring iron core of ferritin can be removed in vitro by chemical and electrochemical reduction, ferritin has been used as a biological template for nanomaterial synthesis [3]. In addition, findings that ferritin can be reconstituted with various electroactive materials (CdS, Co, Mn, Ni, Pd, and Pt) make ferritin suitable for biosensor and biofuel cell applications [4].

The immobilization of ferritin on the electrode surface is essential for various bioelectronic applications. Ferritin was adsorbed on tin-doped indium oxide electrodes and gold electrodes [5,6]. Recently, immobilization of ferritin on a gold electrode has been attempted using a self-assembled monolayer (SAM) [4,7]. In this work, we have extended the ferritin immobilization based on SAMs. Ferritin was immobilized through two different methods using chemical modifiers. One approach is to introduce sulfhydryl (–SH) groups to ferritin molecules and then to attach the thiolated ferritin to a bare gold electrode. The other approach is to modify a gold electrode with succinimidyl alkanedisulfide compounds and then to covalently bind ferritin to the modified Au electrode. Effects of concentration and chemical structure of the modifiers on the ferritin immobilization were examined using atomic force microscopy (AFM) and cyclic voltammetry (CV).

## 2. Experiment

### 2.1. Materials

Horse spleen ferritin was purchased from Sigma (USA) and used without further purification. 2-Iminothiolane hydrochloride (Traut's reagent) and dithiobis(succinimidyl propanoate) (DSP) were also obtained from Sigma (USA). Dithiobis(succinimidyl hexanoate) (DSH), dithiobis(succinimidyl octanoate) (DSO), and dithiobis(succinimidyl undecanoate) (DSU) were from Dojindo (Japan). Fig. 1 shows the chemical structures of the modifiers used for ferritin immobilization. All the chemicals were of analytical grade and used as received.

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doi:10.1016/j.ultramic.2008.04.088

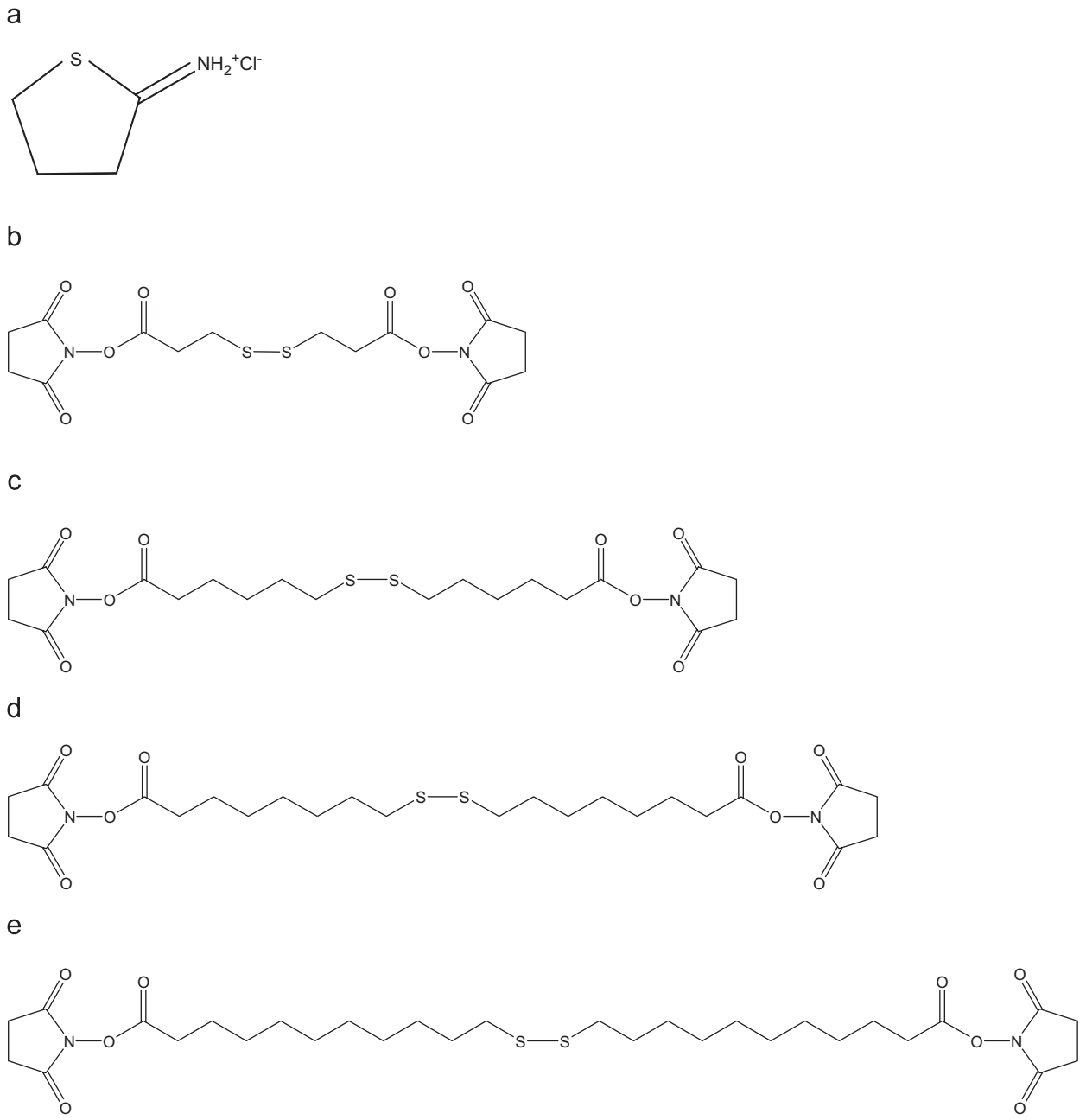



**Fig. 1.** Chemical structures of the modifiers used. (a) 2-Iminothiolane hydrochloride (Traut's reagent), (b) dithiobis(succinimidyl propanoate) (DSP), (c) dithiobis(succinimidyl hexanoate) (DSH), (d) dithiobis(succinimidyl octanoate) (DSO), and (e) dithiobis(succinimidyl undecanoate) (DSU).

### *2.2. Immobilization of thiolated ferritin on a bare gold electrode*

In order to introduce sulfhydryl residues, ferritin molecules were modified with Traut's reagent. To an aqueous 2-iminothiolane · HCl solution (0.3, 0.6, and 1.2 mM) was added ferritin at a concentration of 10 mg/ml. The mixture was incubated for 1 h at room temperature. A gold electrode was immersed in the thiolated ferritin solution for 18 h at room temperature and then rinsed with 0.1 M phosphate buffer.

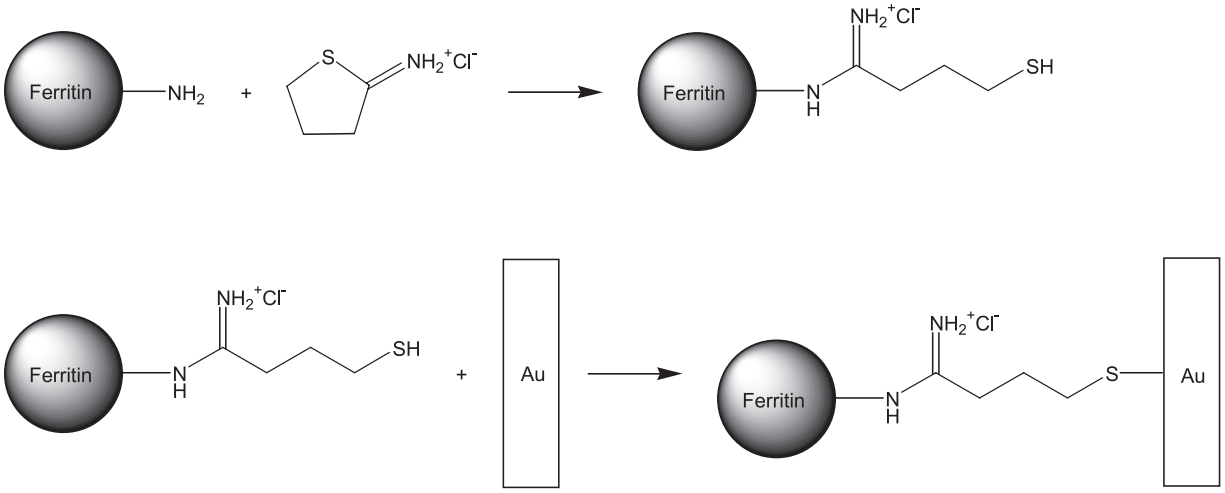
### *2.3. Immobilization of ferritin on a self-assembled monolayer-modified Au electrode*

First a SAM-modified gold electrode was prepared. An electrode with a 2 mm diameter gold disk (Princeton Applied Research, USA) was cleaned with a piranha solution and then immersed in 1,4-dioxane containing 1 mM succinimidyl alkanedisulfide compounds and 10 mM mercaptopropanol for 1 h at room temperature. After being rinsed with acetone and phosphate buffer, the modified gold electrode was immersed in a solution of 2 mg/ml ferritin in 0.1 M phosphate buffer (pH 8.0) for 18 h at 4 °C and then rinsed with 0.1 M phosphate buffer.

### *2.4. Analysis*

Immobilization of ferritin on a gold electrode was examined by AFM and CV. The AFM images were obtained with the XE-150 System (PSIA, Korea) using standard silicon cantilevers. The prepared samples were mounted on the AFM stage and imaged

with tapping mode in the air. The CV of ferritin immobilized on the gold electrode was performed in 0.1 M phosphate buffer (pH 7.0) using the VSP modular potentiostat (Princeton Applied Research, USA). An Ag/AgCl (3 M NaCl) electrode and a platinum wire electrode were used as the reference and the counter electrode, respectively.




**Scheme 1.** Schematic diagram for the immobilization of ferritin on a gold electrode using 2-iminothiolane hydrochloride (Traut's reagent).

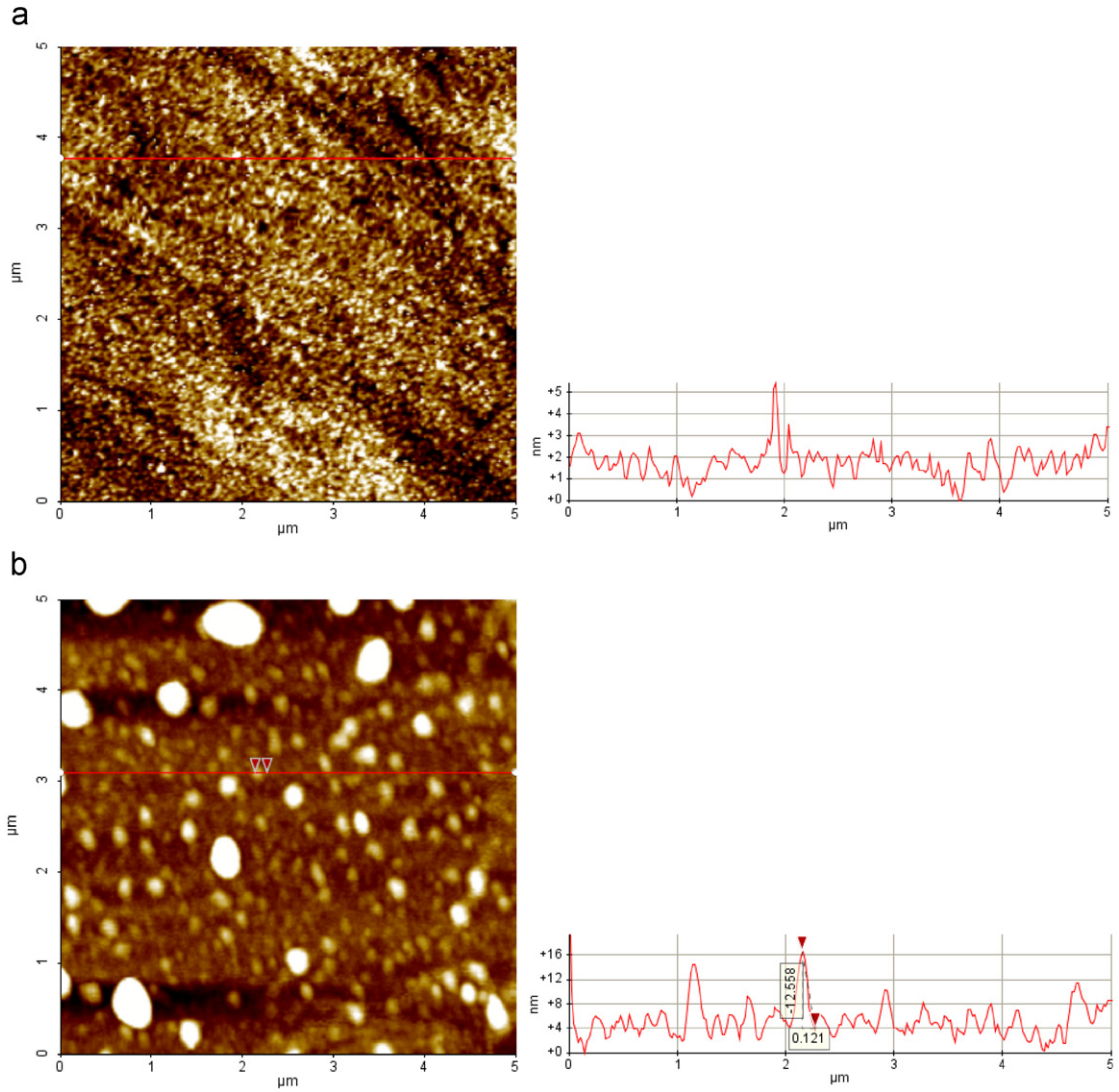



**Fig. 2.** Tapping mode AFM images of (a) the bare gold surface and of (b) the gold substrate that was immersed in a ferritin solution containing 0.3 mM Traut's reagent.

## 3. Results and discussion

First, ferritin was immobilized through chemisorption of the thiol groups, which were introduced to ferritin using 2-iminothiolane hydrochloride (Traut's reagent), onto a bare gold surface. Ferritin molecules have $\alpha$-amines and $\varepsilon$-amines of lysine residues. Traut's reagent reacts with the primary amines on the surface of the ferritin in the pH range 7–10 to create sulfhydryl groups. The thiolated ferritin is then immobilized on a gold electrode (Scheme 1). Effects of the 2-iminothiolane · HCl concentration on the ferritin immobilization were examined using AFM and CV. Recently, AFM has been used for the investigation of protein

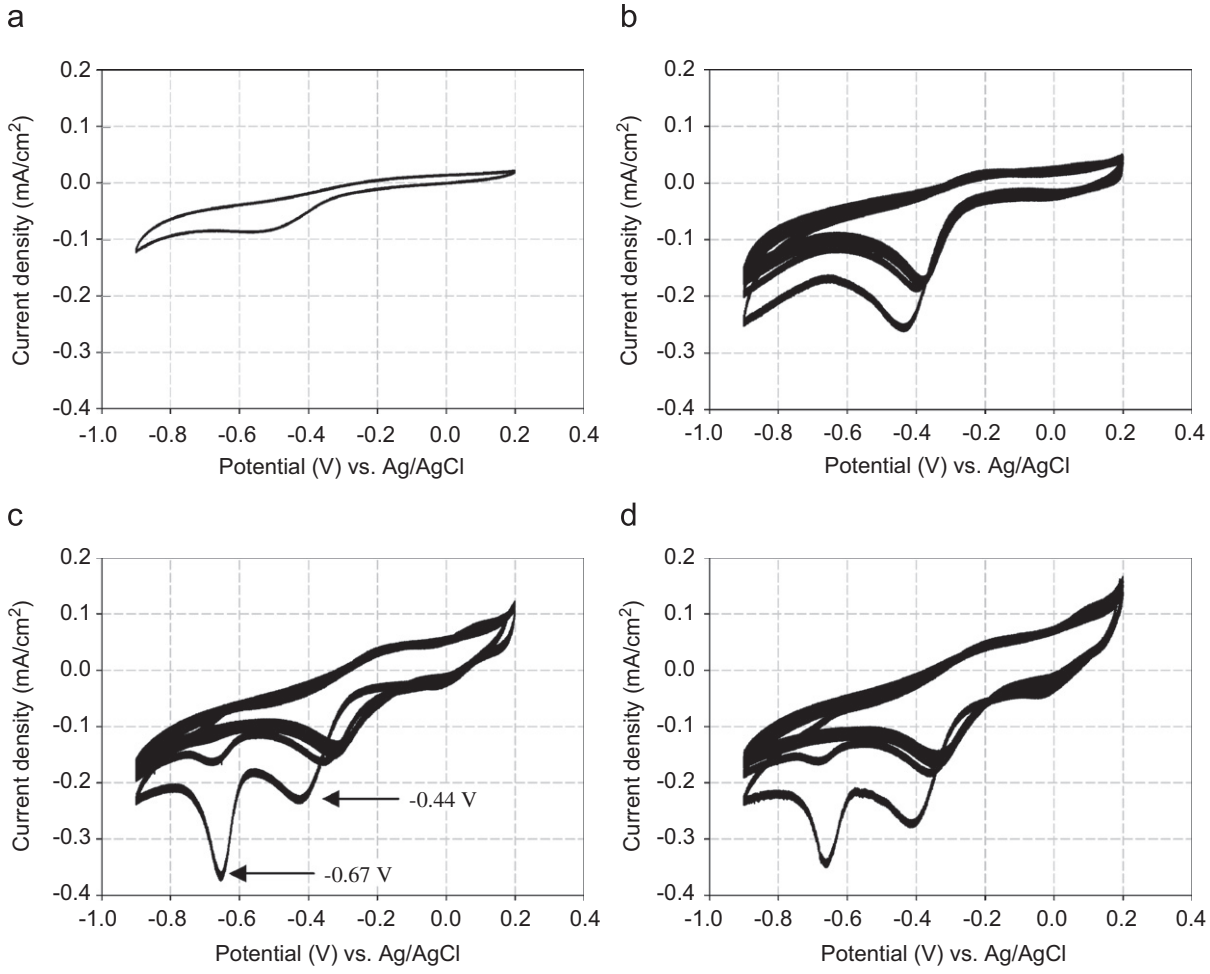
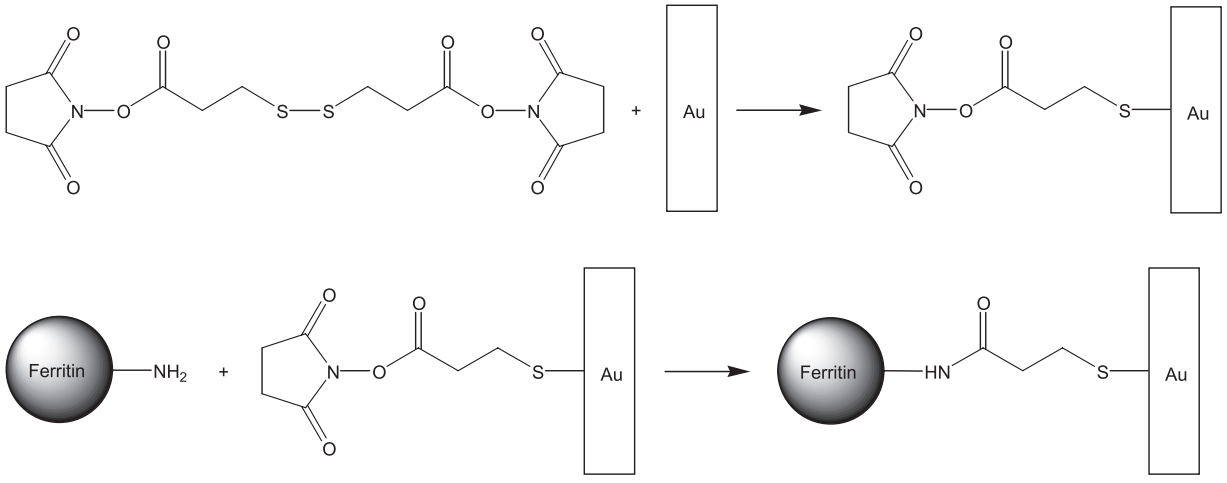



**Fig. 3.** Cyclic voltammograms of (a) the bare gold electrode and of ferritin immobilized on the gold electrode using 2-iminothiolane · HCl concentrations of (b) 0.3, (c) 0.6, and (d) 1.2 mM. The potential was cycled five times between 0.2 and −0.9 V in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 100 mV/s.




**Scheme 2.** Schematic diagram for the immobilization of ferritin on a gold electrode using dithiobis(succinimidyl propanoate) (DSP).

immobilization [7–10]. In Fig. 2(a), tapping mode AFM images of the bare gold surface is shown. Fig. 2(b) exhibits the gold substrate that was immersed in a ferritin solution containing 0.3 mM 2-iminothiolane · HCl. The comparison of Fig. 2(a) and (b) supports the ferritin immobilization on the gold. In Fig. 3(b)–(d), cyclic voltammograms of ferritin immobilized on the gold electrode using different concentrations of Traut's reagent (0.3, 0.6, and 1.2 mM) are shown. In contrast to the bare gold electrode (Fig. 3(a)), the ferritin-immobilized electrodes showed reduction peaks (−0.67 and/or −0.44 V), indicating the electron transfer between the immobilized ferritin and the gold electrode. Ferritin is electrochemically active because of iron stored in the form of hydrous ferric oxide and hydrous ferric phosphate in the protein shell. It has been thought that the cathodic peaks at about −0.65 V (vs. Ag/AgCl) and −0.4 V (vs. Ag/AgCl) are due to the reduction of hydrous ferric oxide and $FePO_4$, respectively [11]. In Fig. 3(b), the reason why only one peak was observed at −0.44 V is not clear for the present. With increasing the number of the cycle, the peak at −0.67 V diminished and disappeared on the third cycle, because the hydrous ferric oxide was reduced and the resultant Fe(II) was released from the ferritin to the solution [4,11]. The other peak at −0.44 V was still observed on the fifth voltammetric cycle. This is because of the reduction of ferric phosphate on the electrode surface, which formed by re-oxidation of the soluble Fe(II) released to the solution [4,11]. Fig. 3(b)–(d) reveals that the 2-iminothiolane · HCl concentration has little effect on the ferritin immobilization in the tested range of 0.3–1.2 mM.

Ferritin was also immobilized on a SAM-modified Au electrode. A gold electrode was modified using succinimidyl alkanedisulfide compounds, which undergo dissociative chemisorption onto the gold electrode. Ferrtin was then covalently attached to the modified Au electrode through the reaction of the terminal succinimidyl groups with amino groups of ferritin molecules (Scheme 2). Effects of the chain length of succinimidyl alkanedisulfide compounds on the immobilization were investigated using DSP (C3), DSH (C6), DSO (C8), and DSU (C11). In Fig. 4, tapping mode AFM images of ferritin immobilized on the gold surface modified with (a) DSP and (b) DSU are shown. Fig. 5 shows cyclic voltammograms of ferritin immobilized on (a) DSP, (b) DSH, (c) DSO, and (d) DSU-modified gold electrode in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 100 mV/s. In all the cases, the reduction peaks were observed and the cathodic currents decreased with increasing the number of the voltammetric cycle. The cathodic peak of ferritin immobilized on the DSU-modified gold electrode was lower compared to the others. This indicates that the electron transfer between the immobilized ferritin and the electrode did not occur easily at the longer chain length [7].

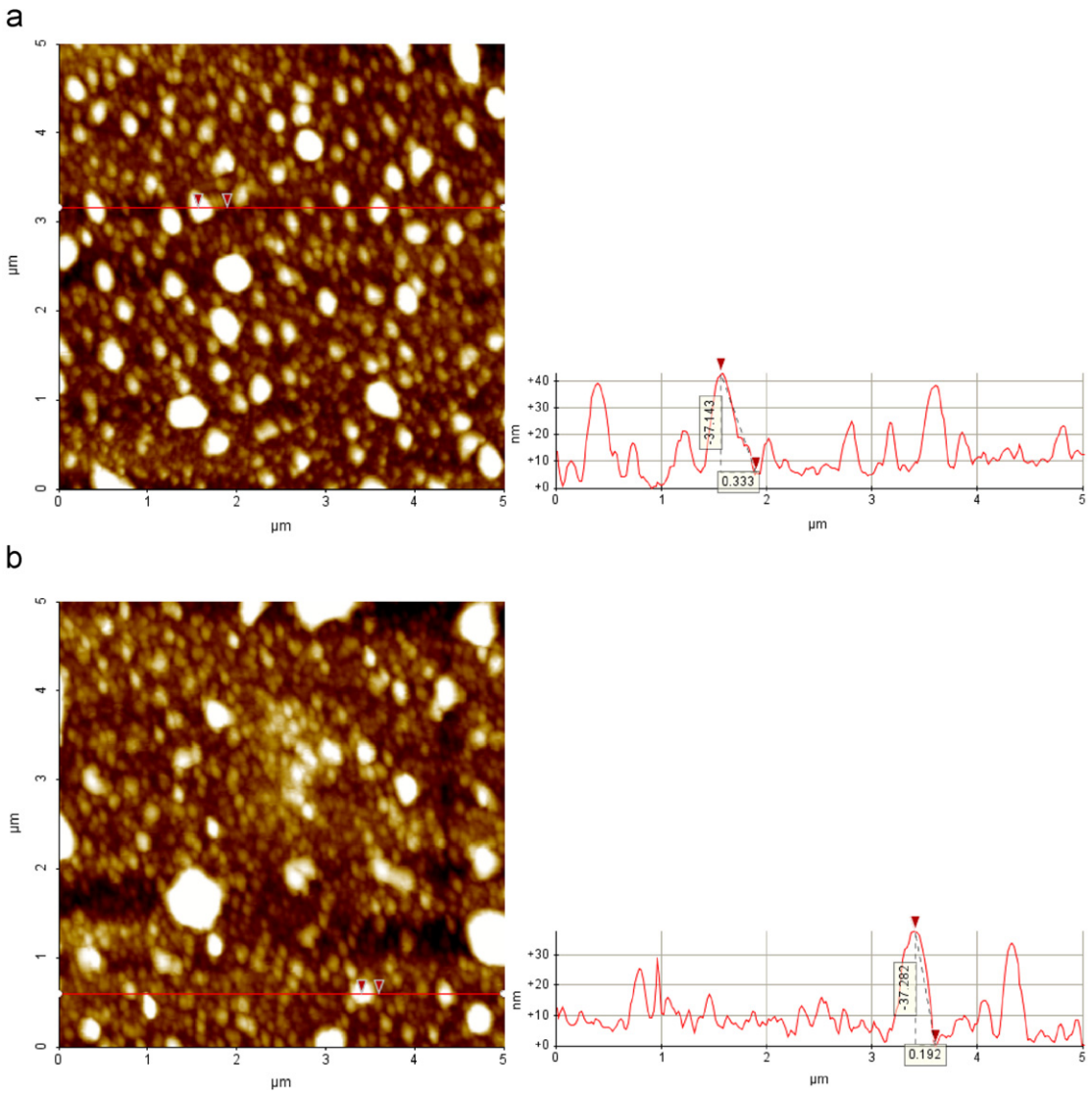



**Fig. 4.** Tapping mode AFM images of ferritin immobilized on the gold surface modified with (a) DSP and (b) DSU.

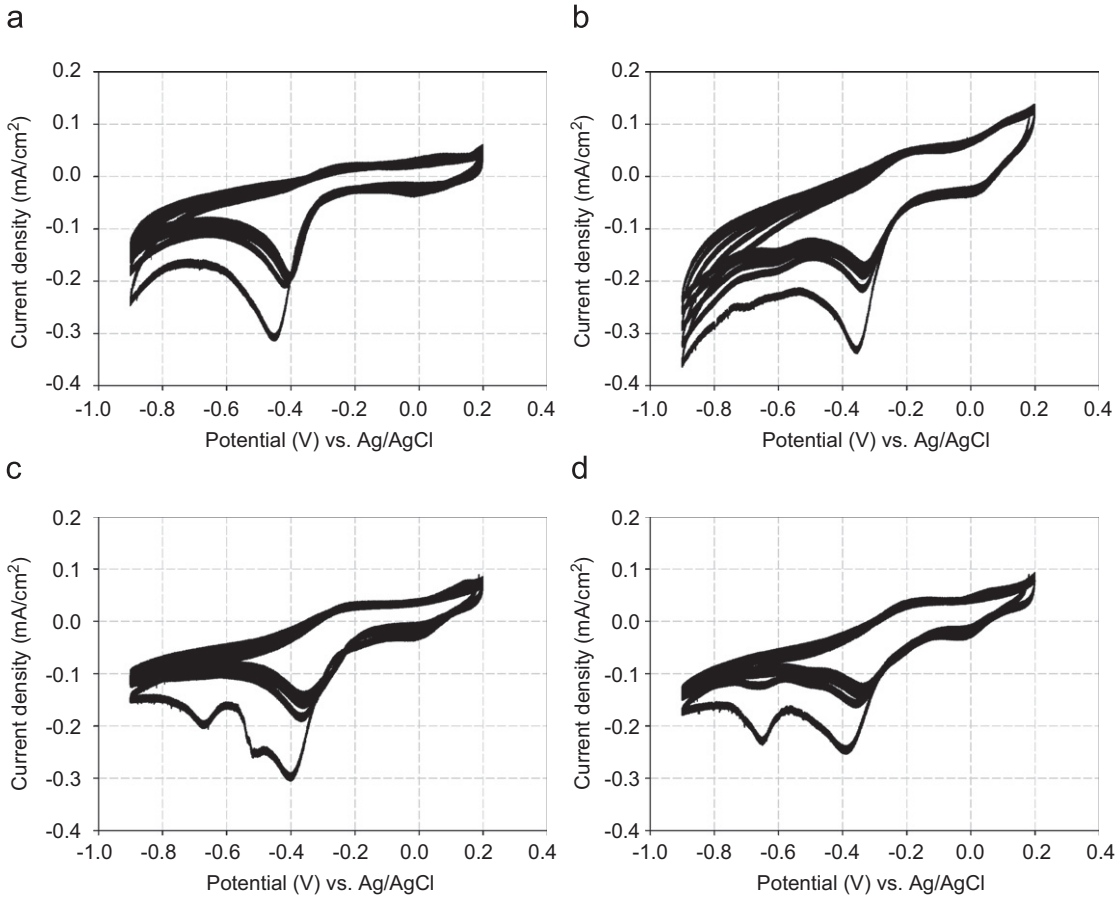



**Fig. 5.** Cyclic voltammograms of ferritin immobilized on (a) DSP, (b) DSH, (c) DSO, and (d) DSU-modified gold electrodes. The potential was cycled five times between 0.2 and −0.9 V in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 100 mV/s.

## 4. Conclusion

Based on self-assembled monolayers, we succeeded in immobilizing ferritin, an iron storage protein, to a gold electrode using two different methods: chemisorption of thiolated ferritin onto bare gold electrodes and covalent binding of ferritin to succinimidyl alkanedisulfide-modified electrodes. The electroactive ferritin immobilized on the Au electrode was characterized by atomic force microscopy and cyclic voltammetry.

## Acknowledgment

This research was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2006-05563).

## References

[1] P.M. Proulx-Curry, N.D. Chasteen, Coord. Chem. Rev. 144 (1995) 347.
[2] E.C. Theil, Annu. Rev. Biochem. 56 (1987) 289.
[3] M. Uchida, M.T. Klem, M. Allen, P. Suci, M. Flenniken, E. Gillitzer, Z. Varpness, L.O. Liepold, M. Young, T. Douglas, Adv. Mater. 19 (2007) 1025.
[4] J.-W. Kim, S.H. Choi, P.T. Lillehei, S.-H. Chu, G.C. King, G.D. Watt, J. Electroanal. Chem. 601 (2007) 8.
[5] R.J. Cherry, A.J. Bjornsen, D.C. Zapien, Langmuir 14 (1998) 1971.
[6] D.C. Zapien, M.A. Johnson, J. Electroanal. Chem. 494 (2000) 114.
[7] M. Tominaga, A. Ohira, Y. Yamaguchi, M. Kunitake, J. Electroanal. Chem. 566 (2004) 323.
[8] S.A. Contera, H. Iwasaki, S. Suzuki, Ultramicroscopy 97 (2003) 65.
[9] M. Tominaga, K. Soejima, M. Matsumoto, I. Taniguchi, J. Electroanal. Chem. 579 (2005) 51.
[10] Y. Lin, J. Wang, L.-J. Wan, X.-H. Fang, Ultramicroscopy 105 (2005) 129.
[11] F. Marken, D. Patel, C.E. Madden, R.C. Millward, S. Fletcher, New J. Chem. 26 (2002) 259.