Electrochemically Regulated Iron Uptake and Release for Ferritin Immobilized on Self-Assembled Monolayer-Modified Gold Electrodes

Masato Tominaga* and Isao Taniguchi†
College of Medical Science, Kumamoto University, 4-24-1 Kuhonji, Kumamoto 862-0976
†*Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555*

(Received April 23, 2001; CL-010366)

Horse spleen ferritin was immobilized on self-assembled monolayers (SAMs)-modified gold electrodes by electrostatic interaction. Electrochemically regulated iron uptake and release for the ferritin immobilized on the electrode was demonstrated.

Ferritin is an iron-storage protein found in most animals, plants and bacteria. Ferritin has been studied extensively with regard to its biochemical characterization.[1–5] Some redox properties of ferritin have also been examined using an electron transfer mediator such as viologen,[4] because the iron uptake and release mechanisms are caused by the oxidation and reduction of iron ions (Fe(II) / Fe(III)) in the protein shell. The redox mechanisms of iron ions in the shell are still unclear. Recently, the direct electrochemistry of ferritin adsorbed onto an indium oxide and bare gold electrodes has been investigated.[6–8] However, in these reports, a stronger ionic solution (μ = 0.4–1.0) is required for the electrode reaction of ferritin, since the adsorption force of ferritin is due to hydrophobic interaction.

In this study, we immobilized ferritin on SAMs-modified gold electrodes in a phosphate buffer solution (μ = 0.1, pH 7) by the electrostatic interaction between ferritin and the terminal functional groups of the SAMs. The iron uptake and release for the ferritin immobilized on the SAMs-modified gold electrode could be regulated electrochemically by changing the electrode potential.

Horse spleen ferritin (Sigma) was purified by size exclusion chromatography to remove free iron ions using a Sephadex G-25 column.[4] The concentration of purified ferritin was determined by the BCA-protein reaction (using BCA protein assay kit, PIERCE Chem. Comp., USA) against an albumin standard curve.[9]

The SAMs-modified gold electrodes were prepared according to the following procedures.[10–12] A single crystal gold working electrode (Au(111)) was prepared by the method of Clavilier et al.,[13] and was pretreated by flame-annealing and quenching in ultra-pure water saturated with hydrogen. The pretreated electrode surfaces were modified with one of four SAMs: 6-amino-1-hexanethiol (AHT, Dojin Chem. Co., Japan), 5-carboxy-1-pentanethiol (CPT, Dojin Chem.), 6-hydroxy-1-hexanethiol (HHT, Dojin Chem.) or 1-hexanethiol (HT, Tokyo Kasei). The SAMs were formed by immersing the gold electrode into a 1 mM (M = mol dm^{-3}) aqueous solution of AHT, CPT or HHT, or an ethanol solution of HT, respectively, for 25 min. The surface coverage of each SAMs on the electrode was estimated to be (7.4–7.6) $\times$ 10^{-10} mol cm^{-2} by the reduction waves on voltammograms observed in 0.5 M KOH solution,[12] indicating that full coverage monolayers had been formed on the gold surface. To investigate the immobilization of ferritin on the SAMs, the SAMs-modified gold electrodes were immersed into a phosphate buffer solution (pH 7, μ = 0.1) of 2 μM ferritin for 60 min. Prior to the cyclic voltammetric measurements, this electrode was rinsed with the buffer solution.

The cyclic voltammograms were performed, using a Fuso HECS 972C potentiostat with a function generator, in phosphate buffer solution (pH 7, μ = 0.1) under an argon gas atmosphere. An Ag|AgCl (saturated KCl) electrode and a platinum electrode were used as the reference and the counter electrodes, respectively. All potentials are reported with respect to the Ag|AgCl (saturated KCl) electrode.

Figure 1 shows cyclic voltammograms of AHT and CPT-modified gold electrodes in the buffer solution not containing ferritin after immersion into a ferritin solution. Well-defined redox waves representing the ferritin immobilized on the AHT and CPT-modified gold electrodes were observed. Ferritin was immobilized on the AHT-modified electrode approximately 5-fold greater than on the CPT-modified electrode, because of the approximately 5 times higher current. The isoelectric point of horse spleen ferritin has been reported to be 4.1–5.1,[5] indicating that the whole ferritin molecule has a negative charge in the buffer solution (pH 7) used. Therefore, ferritin was immobilized on the AHT-modified electrode by electrostatic interactions between the ferritin molecule and the amino terminal group of AHT. On the other hand, the ferritin molecule has positively charged amino acid residues locally.[1] Thus, an electrostatic force of attraction would also occur between the posi-

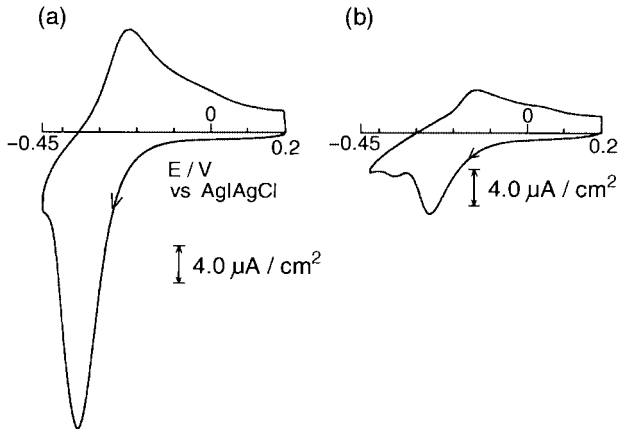


Figure 1. Typical cyclic voltammograms of ferritin immobilized on AHT (a) and CPT (b) -modified gold electrodes in a phosphate buffer solution (pH 7, μ = 0.1). Potential sweep rate: 50 mV s^{-1}. Temperature: 25 °C.

tively charged amino acid residues of ferritin and the carboxyl terminal group of CPT, which could explain how the ferritin was also immobilised on the CPT-modified electrode. In the case of HHT and HT-modified electrodes, no well-defined redox response for ferritin was observed. Furthermore, no redox response was observed at the Au(111) electrode in buffer solution following immersion into the ferritin solution. These results also suggest that ferritin can be immobilized on AHT and CPT-modified electrodes by electrostatic interaction.

The anodic and cathodic peak potentials for the ferritin immobilized on AHT were observed around –0.22 and –0.35 V, respectively. These values are not significantly different from the values reported for ferritin adsorbed onto an indium oxide electrode.[6] However, the values obtained are more positive than the reported redox potential (approximately –0.41 V) for a solution of ferritin using mediated coulometry,[4] which suggests that a slight conformational change of ferritin may have occurred during the immobilization onto the AHT-modified electrode. The anodic and cathodic peak potentials for the ferritin immobilized on the CPT-modified electrode were around –0.14 and –0.27 V, which represents a positive potential shift of approximately 80 mV compared to ferritin immobilized onto AHT-modified electrode. This might reflect the difference in conformation and/or surface orientation of the immobilized ferritin.

The redox peak currents of ferritin immobilized on AHT and CPT decreased gradually with potential cycling in a buffer solution not containing ferritin. The peak current was significantly decreased when the electrode potential was held at –0.4 V for a few minutes. Eventually, no redox response for ferritin could be observed (Figure 2, broken line). These behaviors suggest that reduced Fe^{2+} ions were released from the ferritin (Scheme 1).

We investigated the electrochemically regulated iron uptake for ferritin immobilized on an electrode. The iron ions released ferritin-immobilized electrode (Figure 2, broken line) was immersed into a phosphate buffer solution of 10 μM $FeSO_4$ at an applied potential of 0 V for 30 min, followed by rinsing with the buffer solution. After this procedure, the redox wave of ferritin was observed again in the buffer solution not containing ferritin. The redox wave was also observed even in the presence of 1 mM nitrilotriacetic acid (NTA) as an iron chelator (Figure 2, solid line). This result indicates that the observed redox wave is attributed to iron atoms reincorporated into the ferritin core (Scheme 1), since any free iron atoms adsorbed onto the electrode are immediately chelated by NTA.

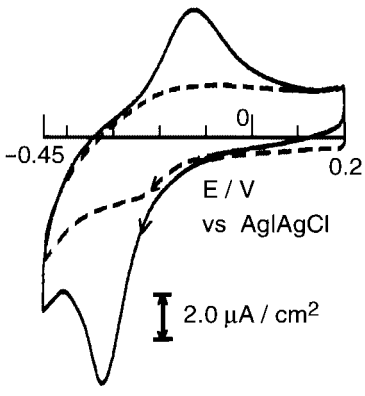
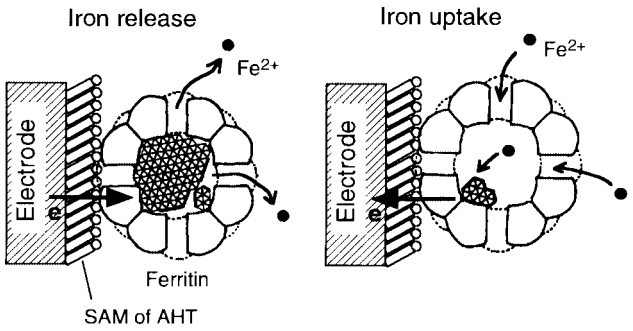


Figure 2. The cyclic voltammograms of ferritin immobilized on AHT-modified electrode in a phosphate buffer solution in the presence of 1 mM NTA after iron uptake (solid line) and that in a phosphate buffer solution before iron uptake (broken line). Potential sweep rate: 50 mV s^{-1}.



Scheme 1. Schematic representations of iron release and uptake for ferritin immobilized on AHT-modified on gold electrode.

In conclusion, we have developed a ferritin-immobilized electrode based on SAMs-modified gold electrodes, using the electrostatic interaction between ferritin and the terminal functional groups of the SAMs. The electrochemically regulated uptake and release of iron ions for the immobilized ferritin on the SAM was demonstrated.

We gratefully acknowledge Professor M. Kunitake of Kumamoto University for his helpful discussions.

References and Notes

1 P. M. Harrison and T. H. Lilley, in "Iron Carriers and Iron Proteins," ed. by T. M. Loehr, VCH Press, New York (1989), Chap. 2.
2 T. G. St. Pierre, P. Chan, K. R. Bauchspiess, J. Webb, S. Betteridge, S. Walton, and D. P. E. Dickson, *Coord. Chem. Rev.*, **151**, 125 (1996).
3 P. M. Proulx-Curry, and N. D. Chasteen, *Coord. Chem. Rev.*, **144**, 347 (1995).
4 G. D. Watt, R. B. Frankel, and G. C. Papaefthymiou, *Proc. Natl. Acad. Sci.*, **82**, 3640 (1985).
5 P. Arosio, T. G. Adellman, and J. W. Drysdale, *J. Biol. Chem.*, **253**, 4451 (1978).
6 R. J. Cherry, A. J. Bjornsen, and D. C. Zapien, *Langmuir*, **14**, 1971 (1998).
7 M.-S. Pyon, R. J. Cherry, A. J. Bjornsen, and D. C. Zapien, *Langmuir*, **15**, 7040 (1999).
8 D. C. Zapien and M. A. Johnson, *J. Electroanal. Chem.*, **494**, 114 (2000).
9 P. K. Smith, R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk, *Anal. Biochem.*, **150**, 76 (1985).
10 A. Ulman, "An Introduction To Ultrathin Organic Films: From Langmuir–Blodgett To Self-Assembly," Academic Press, San Diego (1991).
11 P. E. Laibinis, G. M. Whitesides, D. L. Allara, Y.-T. Tao, A. N. Parikh, and R. G. Nuzzo, *J. Am. Chem. Soc.*, **113**, 7152 (1991).
12 C. A. Widrig, C. Chung, and M. D. Porter, *J. Electroanal. Chem.*, **310**, 335 (1991).
13 J. Clavilier, R. Faure, G. Guinet, and R. Durand, *J. Electroanal. Chem.*, **107**, 205 (1980).