Dependence of the Electrochemical Response of Ferritin on the Number of Iron Atoms at the Ferritin Core

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The well-defined redox reaction of ferritin was observed at an indium oxide electrode with a fully hydrophilic surface at a low concentration of ferritin solution (0–7 μmol dm^{-3}). This was a diffusion-controlled electrode reaction process. Conversely, at a higher concentration of ferritin (7 μmol dm^{-3} <), ferritin was weakly adsorbed on the electrode surface. The electrochemical redox response of ferritin was strongly affected by the number of iron atoms at the core of the ferritin molecule.

Ferritin is an iron-storage protein, which consists of a protein shell formed from multiple highly symmetrical subunits about 12 nm in diameter and of a mineral core containing up to approximately 4500 iron atoms in the form of hydrated iron and phosphate compounds.[1–3] This biomineral protein, ferritin, has been studied extensively with regard to its biochemical characterization as well as its iron uptake and release functions.[4–6] The iron uptake and release mechanisms are caused by the oxidation and reduction reactions of iron ions (Fe(III)/Fe(II)) in the protein shell.[1–6] Recently, the direct electron transfer reactions of ferritin at the electrodes have been reported.[7–11] Ferritin molecules were adosorbed onto tin-doped indium oxide electrode and bare gold electrode surfaces due to hydrophobic interactions under highly ionic solution conditions.[7–10] The electrochemical behavior of adsorbed ferritin onto the electrodes was investigated. We previously reported the development of a ferritin-immobilized electrode based on self-assembled monolayers (SAMs)-modified gold electrodes, using the electrostatic interactions between ferritin and the terminal functional groups of the SAMs. Furthermore, electrochemically regulated iron uptake and release of ferritin immobilized on the modified-electrode was demonstrated.[11]

We can expect that the electrochemical redox response of ferritin at an electrode is affected by the number of iron atoms at the ferritin core, because the number of iron atoms at the core decreased when the ferritin is reduced on the electrode, and electron transfer is more difficult when the number of iron atoms at the core decreased. However, the effect of the number of iron atoms at the ferritin core on the electrochemical response has not been previously investigated to our knowledge. In the present study, we investigated the electrochemical behaviors of ferritin at an indium oxide electrode in a ferritin solution. A redox reaction involving ferritin was found to occur at an indium oxide electrode. At low concentrations of ferritin solution (0–7 μmol dm^{-3}), this was a diffusion-controlled reaction. Furthermore, the redox peak current of ferritin was strongly dependent on the number of iron atoms at the ferritin core.

Horse spleen ferritin was obtained from Sigma. Ferritin containing a different number of iron atoms at the core was prepared by a dialysis method using a reduction agent.[2,5] The ferritin sample was purified further by size exclusion chromatography to remove free iron ions using a Sephadex G-25 column. The concentration of purified ferritin was determined by measuring absorbance at 562 nm, followed by the BCA-protein reaction (using a BCA protein assay kit, Pierce Chem. Comp.) against an albumin standard curve.[12] The number of iron atoms per ferritin molecule was determined by atomic absorption spectroscopy against an iron standard curve using a Nippon Jarrell Ash AA-845 Atomic Absorption & Flame Emission Spectrophotometer. Cyclic voltammograms were carried out in a phosphate buffer solution (20.0 mmol dm^{-3} NaH_2PO_4 + 26.3 mmol dm^{-3} Na_2HPO_4, ionic strength $\mu = 0.1$, pH 7.0) under an argon atmosphere using a BAS CV-50W electrochemical analyzer system and a Toho Giken PS-6 potentiostat with a function generator. An indium oxide electrode (0.25 cm^2, from Kinoene Optics Corp., Japan) with a fully hydrophilic surface (72 dyn cm^{-1} at 25 °C) was used as a working electrode. The electrode was cleaned by ultrasonication in 1% aqueous New-Vista (AIC Corp.) solution according to methods described elsewhere.[13,14] An Ag|AgCl (saturated KCl) electrode and a platinum electrode were used as the reference electrode and the counter electrode, respectively. All potentials were recorded with respect to an Ag|AgCl (saturated KCl) electrode. Other reagents used were of analytical grade.

Figure 1a shows the typical cyclic voltammogram of 4.5 μmol dm^{-3} ferritin (containing 3300 (±100) iron atoms) at an indium oxide electrode in a phosphate buffer solution (pH

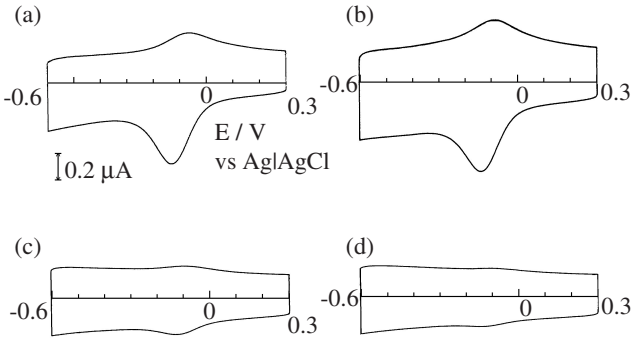


Figure 1. Cyclic voltammograms of 4.5 μmol dm^{-3} ferritin containing various the numbers of iron atoms at an indium oxide electrode in a phosphate buffer solution (pH 7.0, $\mu = 0.1$). The numbers of iron atoms were 3300 (a), 2323 (b), 1671 (c), and 338 (d). Cyclic voltammogram of (d) was the same as that of the background. Potential sweep rate: 50 mV s^{-1}. Temperature: 25 °C.

7.0, $\mu = 0.1$). The well-defined oxidation and reduction peaks were observed around -0.06 and -0.13 V, respectively, at a potential sweep rate of 50 mV s^{-1}. The redox peak was in good agreement with the reported values of ferritin adsorbed onto a bare gold electrode,[9] but not with the reported values of ferritin adsorbed on a tin-indium oxide electrode under highly ionic solution conditions.[7–10] The reduction peak currents observed in cyclic voltammograms in 2.0 and 4.5 μmol dm^{-3} ferritin solutions were proportional to the square root of the potential sweep rate, suggesting that the redox response was a diffusion controlled reaction. On the other hand, ferritin was weakly adsorbed onto the electrode surface when the concentration of ferritin was higher than ca. 7 μmol dm^{-3}. For example 10 μmol dm^{-3}, the redox peak of the adsorbed ferritin was observed, when the electrode was transferred to the buffer solution. The adsorbed ferritin desorbed from the electrode during potential cycling over several cycles in the buffer solution, suggesting ferritin weakly adsorbed onto the electrode surface in higher concentrations of ferritin. No significant difference in the redox peak potentials between the adsorbed ferritin and the ferritin in the buffer solution was observed. This result also supports that ferritin weakly adsorbed onto the electrode surface in higher concentrations of ferritin.

The effect of the number of iron atoms at the ferritin core on the electrochemical response was investigated in 4.5 μmol dm^{-3} ferritin solution. Figure 1 shows the redox responses of ferritin containing various numbers of iron atoms. The number of iron atoms was observed to have a strong influence on the reaction. The redox peak current of ferritin containing ca. 2323 (±100) iron atoms was almost the same as that of ferritin containing ca. 3300 iron atoms. However, the redox peak current of ferritin containing ca. 1671 (±100) iron atoms was significantly smaller than that of ferritin containing ca. 2323 and 3300 iron atoms. Eventually, no redox response was observed when ferritin containing 338 (±100) iron atoms was used. Figure 2 shows the plot of the cathodic peak current vs the number of iron atoms in ferritin. This figure shows that the redox peak currents of ferritin were drastically decreased at iron atom numbers lower than ca. 2300 and were gradually decreased at iron atom numbers from ca. 1700 to 300. The redox reaction was not observed at iron atom numbers lower than ca. 300. These results indicate that the electron transfer reaction

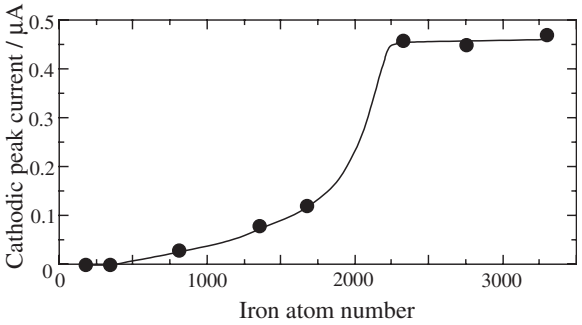
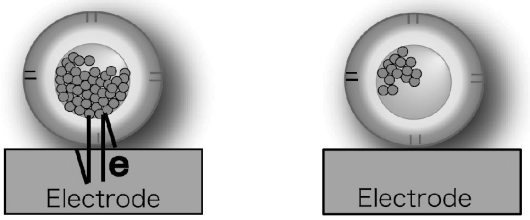


Figure 2. Plot of the cathodic peak currents vs the iron atom numbers. The cathodic peak currents were obtained from cyclic voltammograms of 4.5 μmol dm^{-3} ferritin containing various numbers of iron atoms at an indium oxide electrode in a phosphate buffer solution (pH 7.0, $\mu = 0.1$). Potential sweep rate: 50 mV s^{-1}. Temperature: 25 °C.



Well-defined redox response

No redox response

Scheme 1. Schematic representations for the dependence of the electrochemical response of ferritin at an indium oxide electrode on the number of iron atoms at the ferritin core.

of ferritin at the electrode becomes more difficult with the decrease in the number of iron atoms at the ferritin core, as shown in Scheme 1.

In conclusion, at a low concentration of ferritin solution (0–7 μmol dm^{-3}), the well-defined redox reaction of ferritin was observed at an indium oxide electrode with fully hydrophilic surface. This was a diffusion-controlled electrode reaction process. On the other hand, at a higher concentration of ferritin (7 μmol dm^{-3} <), ferritin was weakly adsorbed onto the electrode surface. The electrochemical redox response of ferritin at the electrode was strongly affected by the number of iron atoms at the ferritin core.

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