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Electrochemical Impedance Spectroscopy and Cyclic Voltammetry on Ferritin in the Presence of Hemoglobin

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Electrochemical and interfacial behavior of ferritin-immobilized gold electrode in the presence of hemoglobin was investigated using electrochemical impedance spectroscopy and cyclic voltammetry. Working electrode used in this study was a gold disk electrode on which ferritin was immobilized by holding at −0.5 V for 3 mins. Hemoglobin added in phosphate buffer caused the interfacial impedance of the ferritin immobilized gold electrode to decrease significantly. Based on the impedance data, ferric ion reduction at 0.0 V vs. Ag/AgCl and hydrogen evolution reaction at −0.8 V vs. Ag/AgCl appeared to be enhanced by hemoglobin in phosphate buffer. This result indicated that hemoglobin-added electrolyte could modify the ferritin layer immobilized on gold electrode and caused reduction of charge transfer resistance on the ferritin related reactions.

Keywords: cyclic voltammetry; electrochemical impedance spectroscopy; ferritin; gold electrode; hemoglobin

INTRODUCTION

Ferritins are stable biological molecules. They are responsible for metabolic control of iron in living system [1]. Ferritin consists of 24 protein subunits to form a spherical molecule with a hollow shell type structure of about 12 nm in external diameter and about 8 nm in interior diameter. It has been reported that Fe ions in ferritin, which

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consists of 24 protein sub units, had electrochemical activity at the potential of roughly −0.3–0.0 V *vs*. Ag/AgCl [2–4]. Due to the electro-activity of ferritin, biofuel cells and biosensors using ferritin has been proposed as new bio devices [2–4]. However, electrochemistry of ferritin has not been fully discovered and understood thus far.

Cyclic voltammetry (CV) has been an important tool for ascertaining ferritin-related electrochemistry [2–5]. However, it couldn't give the selective signals when the overlapped signals by the unknown and impurities appeared at the adjacent potential [6]. Additionally, cyclic voltammetry depends strongly on electrochemical parameters, including sweep rate and cell configuration. Hence, additional electrochemical tool should be introduced in order to characterize the ferritin in detail.

In this study, electrochemical impedance spectroscopy (EIS) based on 3-electrode configuration was introduced as a new versatile electrochemical tool [7]. EIS has been already introduced in order to analyze the interfacial phenomena of energy devices such as fuel cells, Li ion secondary batteries [8], and ultracapacitors [9]. We intended to characterize ferritin-immobilized gold electrode using both EIS and cyclic voltammetry. As a new approach, hemoglobin, which is a well-known Fe containing biomolecule in living systems, was added in phosphate buffer to modify the ferritin layer immobilized on gold electrode by applying the potential. Effect of hemoglobin addition in phosphate buffer was investigated using EIS and cyclic voltammetry.

EXPERIMENTAL

Ferritin Immobilized Gold Electrode Preparation

Ferritin and hemoglobin used in this study were purchased from Aldrich and Sigma, USA, respectively. Dish type gold electrode was used for working electrode. Ferritin was immobilized electrochemically on the gold electrode in ferritin-added phosphate buffer by holding at −0.5 V vs. Ag/AgCl for 3 mins. Hemoglobin was added in phosphate buffer (pH 7) by 0.125 mM to modify ferritin-immobilized gold electrode.

Analysis of Electrochemical Properties

Cyclic voltammetry (CV) measurement was performed at a sweep rate of 20 mV sec^{-1} using VSP potentiostat/galvanostat (Bio-Logic, France). Electrochemical impedance spectroscopy (EIS) measurement was done using SI 1287 of Solatron as electrochemical interface and

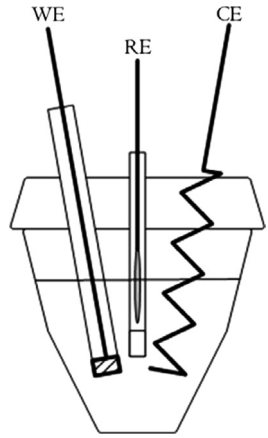


FIGURE 1 The schematic cell diagram for measurement of electrochemical impedance and cyclic voltammetry.

SI 1255 of Solatron as frequency response analyzer. Figure 1 shows the schematic diagram of the electrochemical cell used in cyclic voltammetry. Working electrode used in this study was a gold disk electrode on which ferritin was immobilized by holding at −0.5 V for 3 mins. In order to analyze the electrochemical properties of working electrode with respect to potential (equivalent to an energy level), an aqueous Ag/AgCl electrode was selected as reference electrode. EIS and CV measurements were conducted using the same 3-electrode electrochemical cell.

RESULTS AND DISCUSSION

Ferritin is a naturally originated chelating compound which can accommodate up to 4500 Fe atoms as $Fe(OH)_3$ in the cavity. Soluble Fe(II) ions are oxidized to insoluble Fe(III) ions for incorporation into the ferritin core and the stored insoluble Fe(III) ions are released as Fe(II) by biological redox processes. Since hemoglobin also takes iron-containing groups, it can interact with ferritin molecules on the gold electrode when biofuel cells and biosensors are running. In this study, the change of electrochemical and interfacial behavior of ferritin-immobilized electrode when hemoglobin presented in the electrolyte was investigated.

First, we carried out CV measurements at applied potentials in the range of −0.8 to 0.8 V using two different electrolytes, which were a phosphate buffer (pH 7) and a hemoglobin-added phosphate buffer

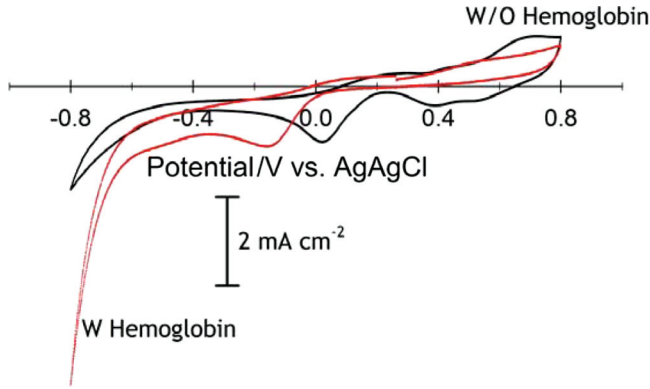


FIGURE 2 Change of cyclic voltammogram by hemoglobin of ferritin-immobilized gold electrode.

(pH 7). The reduction peak positions in the cyclic voltammograms for the two set of experiments were appeared to be different each other as shown in Figure 2. In order to explain the peak shift, the ionic conductivity of the two electrolytes was measured. It was found that the ionic conductivity was changed by the addition of hemoglobin to the phosphate buffer from about 8.9 to about 7.7 mS cm^{-1}. Such reduction of ionic conductivity was thought to cause the peak shift observed in our experiments. The reduction peak shift in a hemoglobin-added phosphate buffer was further analyzed using a different electrochemical technique such as electrochemical impedance spectroscopy. Electrochemical impedance can provide some important information on interfacial reactions, including charge transfer and diffusion.

The same electrochemical cell as described in Figure 1 was used for the EIS measurement. Figure 3 shows nyquist plots of impedance of ferritin-immobilized gold electrode in phosphate buffer (pH 7) measured at different holding potentials. It was found that electrochemical impedance values decreased when electrochemical reactions took place, which were detected by a reaction peak at 0.0 V of holding potential in CV or by current evolution in CV obtained with −0.8 V of holding potential. This impedance decrease at 0.0 V could be caused by electrochemical reactions because electrochemical reactions at 0.0 V are closely related to charge transfer through immobilized ferritin layer. When hemoglobin was added to the phosphate buffer, EIS spectra trend with respect to the applied potential were observed to be similar to those of hemoglobin-free phosphate buffer as appeared in Figure 4. However, the magnitude of impedance was greatly changed.

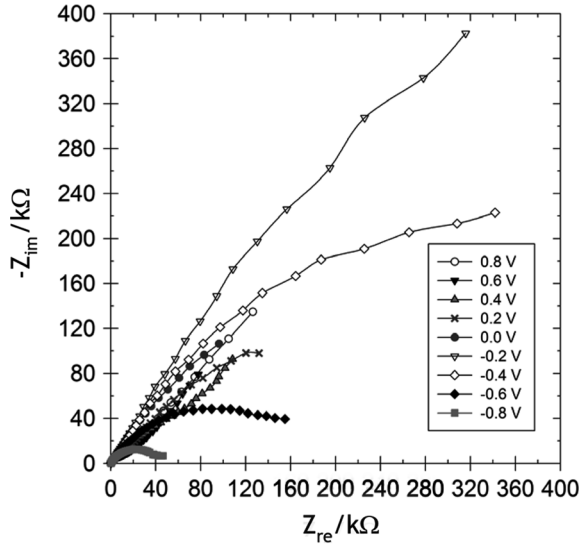


FIGURE 3 Electrochemical impedance spectra of ferritin-immobilized gold electrode, with respect to the holding potential (from −0.8 to 0.8 V *vs*. Ag/AgCl).

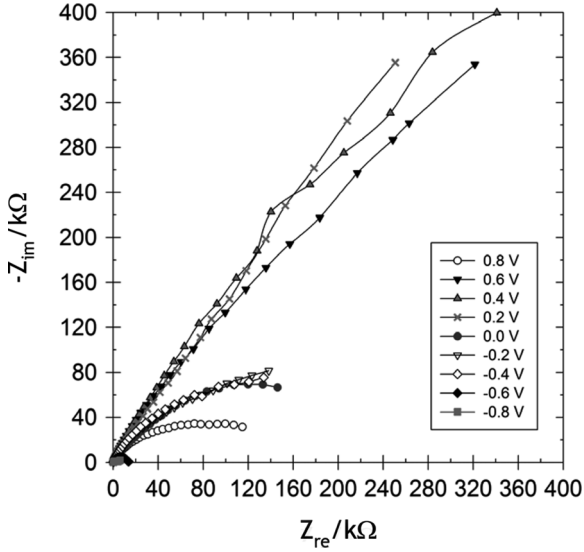


FIGURE 4 Electrochemical impedance spectra of ferritin immobilized gold electrode in the presence of hemoglobin (0.125 mM), with respect to the holding potential (from −0.8 to 0.8 V *vs*. Ag/AgCl).

The magnitude of electrochemical impedance increased with a reduction of applied potentials (from initial 0.8 V to 0.4 V), and then decreased abruptly at 0.0 V, indicating that the electrochemical reaction at 0.0 V or higher was terminated and thereby a semi-circle, buried at other larger electrochemical elements, re-appeared. This result matched well with the appearance of reduction peak in Figure 2. On the other hand, the semi-circle was in a coherent shape at the potential of 0.0 V to −0.4 V, indicating that the charge transfer reaction occurred at 0.0 V continued through −0.6 V of applied potential.

In order to discuss the effect of hemoglobin addition, the impedances at 0.0 V for the two electrolytes were compared as represented in Figure 5. It has been known that reduction peak at 0.0 V is attributed to reduction of Fe(III) in ferritin core to Fe(II), which is then released into electrolyte solution because Fe(II) is soluble. Despite of lower conductivity of hemoglobin-added buffer (7.7 mS cm^{-1}) than original buffer (8.9 mS cm^{-1}), hemoglobin was appeared to cause charge transfer through ferritin layer on the gold electrode to occur readily. It was, therefore, thought that hemoglobin cooperated into

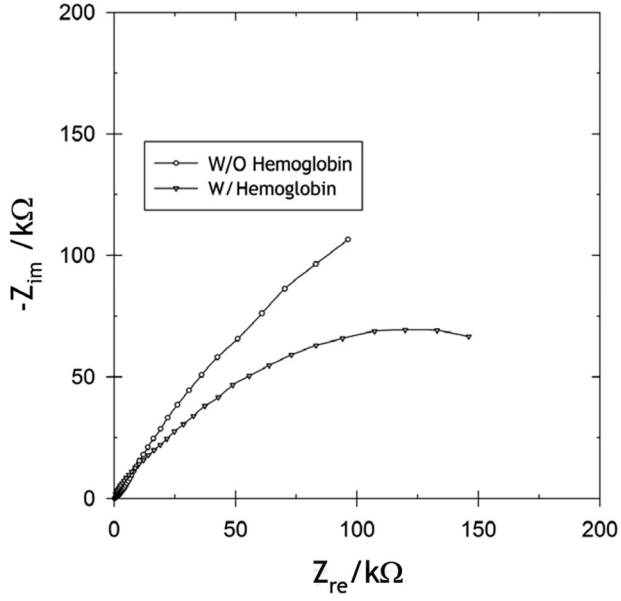


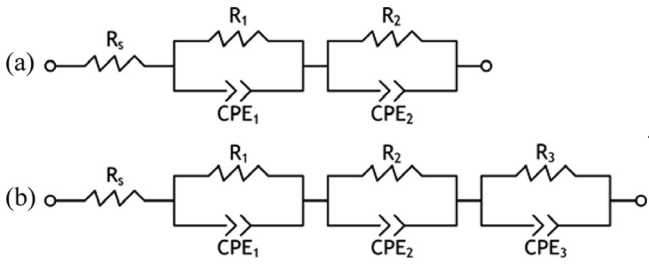
FIGURE 5 Comparison of electrochemical impedance spectroscopy spectra with (0.125 mM) and without hemoglobin in phosphate buffer at 0.0 V of holding potential.

ferritin layer directly without interaction with phosphate buffer because hemoglobin decreased the ionic conductivity of the original buffer.

In order to further discuss the effect of hemoglobin addition, the equivalent circuit fitting using the data obtained at 0.0 V was carried out as summarized in Table 1. A series of pseudo RC (a resistance and a couple of constant phase element) was used in this equivalent circuit fitting study considering surface roughness and porous layer of the ferritin immobilized gold electrode. Accumulated resistance for hemoglobin-added buffer decreased as compared to that of original phosphate buffer in spite of lower ionic conductivity of the hemoglobin-added buffer. Resistance related to constant phase element, as shown in Table 1, could be explained as charge transfer through immobilized layer. Figure 6 shows EIS spectra of ferritin in phosphate buffer with and without hemoglobin at −0.8 V of a holding potential. Current evolution at <-0.8 V of holding potential represents the hydrogen evolution caused by hydrolysis of aqueous electrolyte, that is a charge transfer reaction. Hemoglobin-added buffer

TABLE 1 Equivalent Circuit Elements and Parameters of Two Comparative Cases Obtained From Impedance Data Measured at 0.0 V of Holding Potential

Phosphate buffer (a)		Hemoglobin-added phosphate buffer (b)	
R_s	255.1	R_s	214.5
R_1	1.55×10^3	R_1	2.57×10^3
CPE-T_1	2.07×10^{-6}	CPE-T_1	2.76×10^{-6}
CPE-P_1	0.84	CPE-P_1	1.015
R_2	5.48×10^5	R_2	2.05×10^5
CPE-T_2	1.32×10^{-5}	CPE-T_2	7.9×10^{-6}
CPE-P_2	0.686	CPE-P_2	0.749
Chi^2	0.000505	R_3	1.28×10^4
Sum of Sqr	0.058	CPE-T_3	5.38×10^{-6}
		CPE-P_3	0.934
		Chi^2	0.000505
		Sum of Sqr	0.058



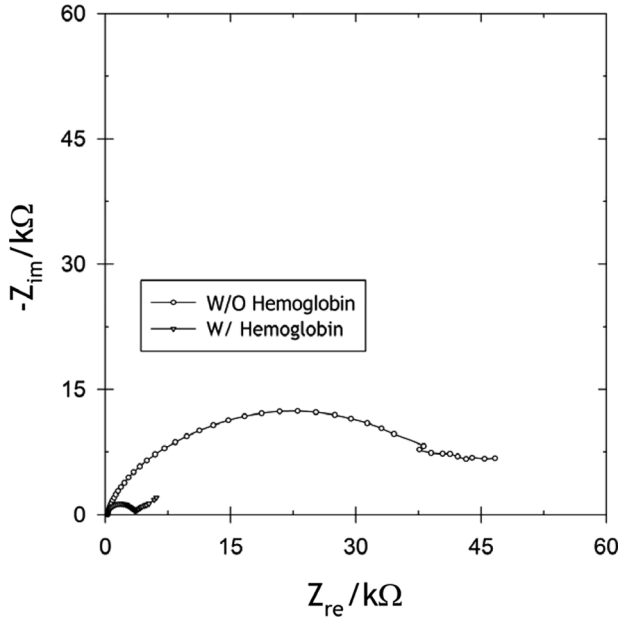


FIGURE 6 Comparison of electrochemical impedance spectroscopy spectra without hemoglobin and with hemoglobin (10 ml) at −0.8 V of holding potential.

showed better performance in terms of impedance value, which was measured by semi-circle radius. This result indicated that charge transfer reaction related to hydrogen evolution was enhanced by hemoglobin in the phosphate buffer. It was, therefore, thought that hemoglobin-added electrolyte could modify the ferritin layer immobilized on gold electrode and caused reduction of charge transfer resistance on this type of immobilized electrode. For better understanding of the effect of hemoglobin on charge transferreaction in ferritin-immobilized gold electrode, further study is ongoing using EIS method.

CONCLUSIONS

The effect of hemoglobin-addition in a phosphate buffer on electrochemical properties of ferritin immobilized on gold electrode was analyzed using CV and EIS techniques. Based on EIS analysis, hemoglobin added in phosphate buffer caused the interfacial impedance of the ferritin immobilized gold electrode to decrease significantly. It was

thought that hemoglobin made the ferritin layer on gold thinner than the untreated sample, resulting in an enhancement of charge transfer reactions in the ferritin system used in this study.

The results in this study indicated that EIS technique along with equivalent circuit model could be used in characterization of interfacial phenomena of ferritin immobilized gold electrode and the mechanism of ferritin-related electrochemical reaction.

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