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Electrochemical Reaction of Cytochrome c on Polyaspartic Acid Modified Gold Electrodes

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Gold electrodes were modified with a polyaspartic acid thin film as a macromolecular recognition element for electrochemical reactions. The effect of electrode modification on the direct electron transfer reaction of cytochrome c was examined. Cytochrome c exhibited quasi-reversible redox reaction on the polyaspartic acid modified gold electrode. The ionic strength dependence and reactivity of the charged redox species suggested that the polyaspartic acid layer provided a matrix structure of charge groups which formed an attractive environment for the electron transfer reaction of cytochrome c.

1. Introduction

Molecular recognition is one of the most important aspects of biological reactions. Enzyme-substrate selection and the specific binding of antigens are examples of biological reactions. Similar to biological electron transfer reactions, the electrochemical reactions of proteins are electrode-specific and sensitive to experimental conditions. If surface structure same as its redox partner is made on the electrode surface, an enzyme-like reaction could be introduced into the electrochemical reaction. Therefore, considerable attention has been paid to clarify the major interactions between electrodes and proteins.[1] The direct electrochemical electron transfer reaction of small redox proteins has been investigated at chemically modified metal electrodes,[2] carbon-based electrodes,[3] and metal oxide electrodes,[4] and the electrostatic interaction between proteins and electrode surfaces has been considered as the primary interaction for the binding of protein to the electrode.[5] The factors controlling this reaction are considered to be the adsorption of denatured

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protein,[5] blocking by an oligomer form of protein,[6] electrostatic repulsion of redox active sites and the electrode surface, and surface tension.[7] From these studies, it has been shown that a direct electron transfer reaction of small redox proteins can be controlled by thin-film electrode modifiers. Many electrode modifiers (promoters) have been investigated for the reversible redox reaction, including bifunctional organic compounds,[2] amino acids,[8] and metal ad-atoms.[9] A promoter is adsorbed on an electrode via a covalent bond and forms a stable self-assembled monolayer. The structure of this layer is determined by the lateral interaction and crystal structure of the base metal used. These electrodes could allow a facile electrochemical reaction of redox proteins; however, it is necessary to select a suitable modifier and an electrode material combination for a given protein. The introduction of flexible modifiers has been proposed for ferredoxin by polypeptides,[10] for carbonic anhydrase by alkanethiolates[11] and for cytochrome c by poly(methacrylic acid).[12] In this scheme, the binding site arrangement could be optimized for a specific protein and a strong interaction will fix the protein on the surface.[13,14] From this point of view, we studied the electrochemical reaction of cytochrome c on gold electrodes modified with polyaspartic acid. We used polyaspartic acid because it is expected to have a random structure on an electrode[15] and may provide a flexible binding site for redox protein. We studied the interaction of the modifier with redox protein, and constructed a three-dimensional environment for an electrochemical reaction of redox protein.

2. Materials and Methods

Cytochrome c from horse and bovine heart and poly-L-aspartic acid (molecular weight of 5000–15000) were obtained from Sigma Chemical Co. (St. Louis, MO). EDC (1-ethyl-3(3-dimethylaminopropyl) carbodiimide) was from Pierce Co. (Rockford, IL). Other chemicals were of analytical grade and purchased from local distributors. The carboxyl group in the polyaspartic acid was modified with ammonium perchlorate using EDC.[16] The gold electrodes were prepared by RF sputtering on a silicon wafer with a SiO_2 layer. The thickness of the gold film was 300 nm. X-ray diffraction analysis revealed that this film had (111) orientation. The wafer was cut into 1×2 cm^2 rectangular chips for electrochemical experiments. A surface-modified electrode was prepared by adding polyaspartic acid solution dropwise to the exposed area (3.1×10^{-2} cm^2) of the gold electrode.

A three-electrode system was used in the electrochemical experiments. Cyclic voltammetry was carried out using a HECS-964 potentiostat (Fuso, Kawasaki, Japan) and a PARC 730 potential scanner (EG&G, Princeton, NJ). The current and the potential were recorded with a PRO20 digital storage scope (Nicolet, Madison, WI). The counter electrode was platinum wire and the reference electrode was saturated KCl Ag/AgCl. The reference electrode was separated from the sample vessel by an agar salt bridge. Electrochemical experiments were performed at 25°C and under anaerobic conditions that were maintained by an argon stream.

3. Results and Discussion

The electrochemical reaction of cytochrome c on the polyaspartic acid modified gold electrode was quasi-reversible, as shown in Fig. 1. The adsorbed polyaspartic acid layer worked as a promoter for the redox reaction of cytochrome c in a solution of low ionic strength. This promotion was not observed in a solution of high ionic strength (Fig. 1(b)). After the modified electrode was exposed to the cytochrome c solution with low ionic strength, the protein was adsorbed on the electrode and showed redox activity without protein in the solution, as shown in Fig. 1(c). The adsorption took about one hour to reach a steady state. When the electrode was rinsed with 1M $NaClO_4$, the current peak in the CV (cyclic voltammogram) disappeared. Then the electrode was placed back into the solution with low ionic strength, and the electrochemical reaction of cytochrome c was restored. Therefore, it is thought that the carboxylate ion in polyaspartic acid interacts with the amino groups of cytochrome c.

Unlike promoters based on self-assembled monolayers such as pyridine thiol, the structure of adsorbed polyaspartic acid may be determined not solely by the interaction between the adsorbate and the metal underlayer, but also by the intrachain interaction of the polymer. The ionic polymer (polyaspartic acid) modifier layer will swell depending on the ionic strength of the solution and will become thicker in a lower ionic strength solution,

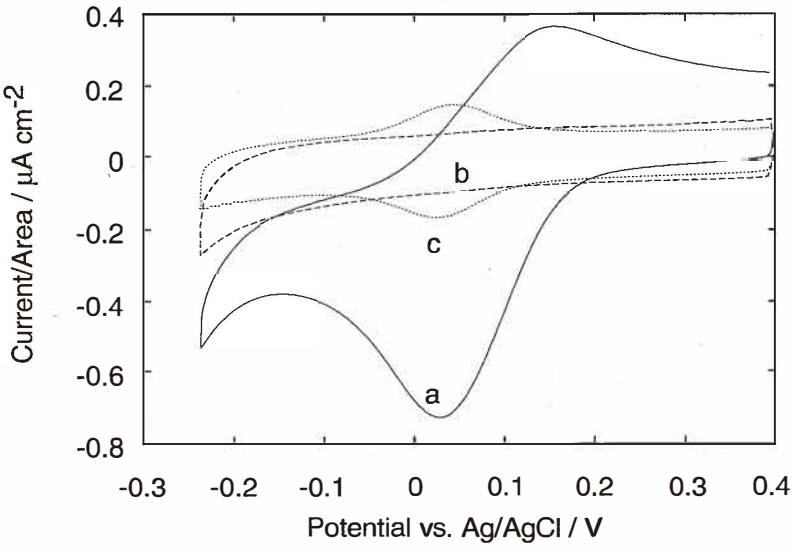

Fig. 1. Cyclic voltammograms of cytochrome c on polyaspartic acid modified gold electrode. Scan rate is 10 mV/s. (a) 17 μM of cytochrome c in 7.6 mM $NaClO_4$, (b) 23 μM of cytochrome c in 1 M $NaClO_4$, and (c) adsorbed cytochrome c in 7.6 mM $NaClO_4$ without cytochrome c in the solution.

because of the hydration of the charged group. This is also confirmed from the fact that the electrochemical reaction of cytochrome c became irreversible at pH 5. The protein may be trapped in this randomly structured layer. Therefore, the polyaspartic acid layer is expected to have a varying degree of electrochemical reactivity with cytochrome c.

The peak current dependence on protein concentration is shown in Fig. 2. The CV peak current is proportional to protein concentrations below 10 μM and the slope decreases in the higher concentration range. At concentrations higher than 10 μM, the protein binding sites of the modified layer became saturated, and the electrochemical current was controlled by adsorbed proteins.

In order to determine the interaction between the electrode modifier and cytochrome c, we employed charged molecules to characterize the electrostatic properties of the polyaspartic acid layer and carried out cyclic voltammetry, the results of which are shown in Fig. 3. Rutheniumhexaammine has a 2+/3+ charge and ferri/ferrocyanide has a 3–/4– charge. Both positively and negatively charged redox species reacted at the polyaspartic acid modified electrode. The electrochemical processes were reversible and fully diffusion-controlled. From these results and the pH-dependent behavior, it was found that the immobilized polyaspartic acid was dissociated, and polyaspartic acid chains were sufficiently separated for the free access of small molecules. Negatively charged, small ferri/ferrocyanide molecules could freely diffuse to the gold surface, and bigger cytochrome c interacted with the polyaspartic acid matrix.

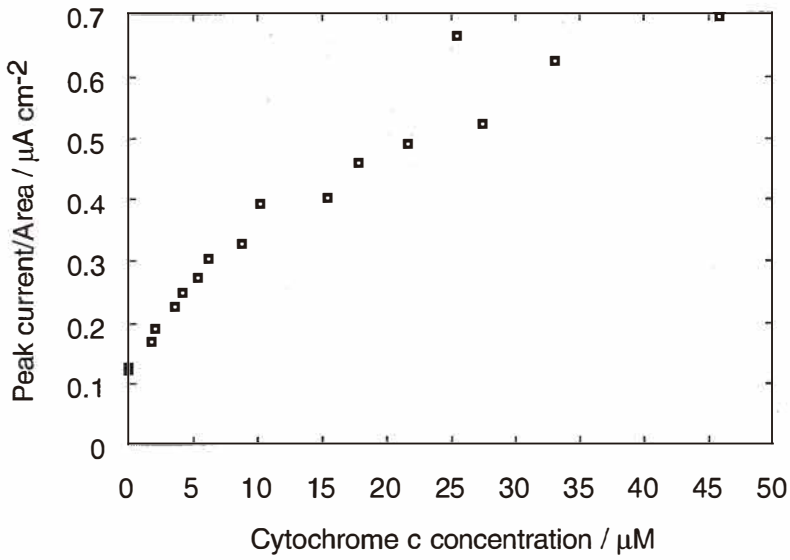

Fig. 2. CV peak current dependence on protein concentration at polyaspartic acid modified gold electrode. Scan rate is 10 mV/s.

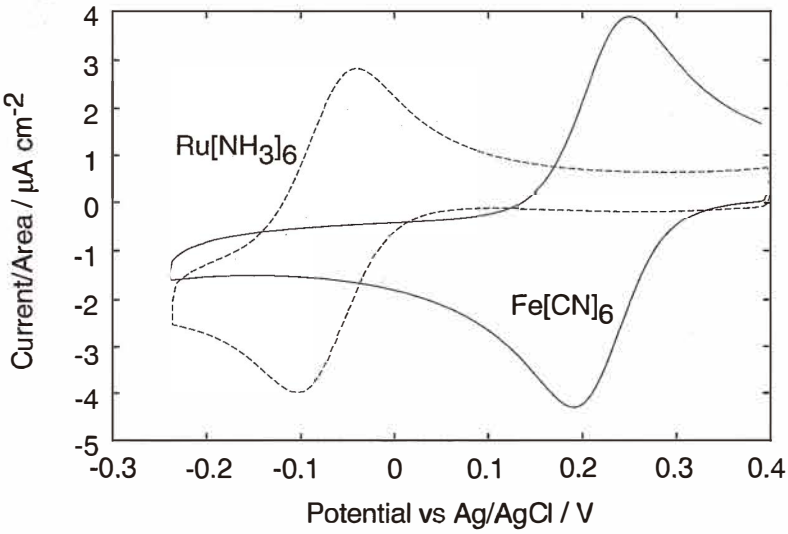

Fig. 3. Cyclic voltammograms of ferricyanide (47 μM) and rutheniumhexaammine (63 μM) on polyaspartic acid modified gold electrode (10 mV/s).

To test the effect of the negative charge of polyaspartic acid, an amidated polymer was used as a modifier. Figure 4 shows CVs of cytochrome c on a polyasparagine modified gold electrode. The electrochemical process was irreversible and only reduction current was observed. This suggests that the negative charge of polyaspartic acid is important to the reversible electrochemical process of cytochrome c. Because the reduction-limiting current was proportional to protein concentrations of up to 240 μM on this modifier, cytochrome c may be directly adsorbed on the gold electrode and a catalytic reaction between adsorbed and bulk species may occur.[6] Polyaspartic acid would prevent this kind of direct adsorption via a stronger adsorption on gold.

Although polyaspartic acid is soluble in water, it is expected that the polymer is anchored on gold and has free volume in a low ionic strength solution. Cytochrome c may interact with polyaspartic acid and be oriented so that it becomes suitable for the redox reaction. An anchored modifier may provide a variety of environments for electrochemical processes. This is the unique feature of this modifier. Self-assembled monolayer-type promoters interact with proteins two-dimensionally and the polymer provides a three-dimensional binding structure. This will be controlled by the partial modification of carboxyl group and can be optimized for specific proteins.

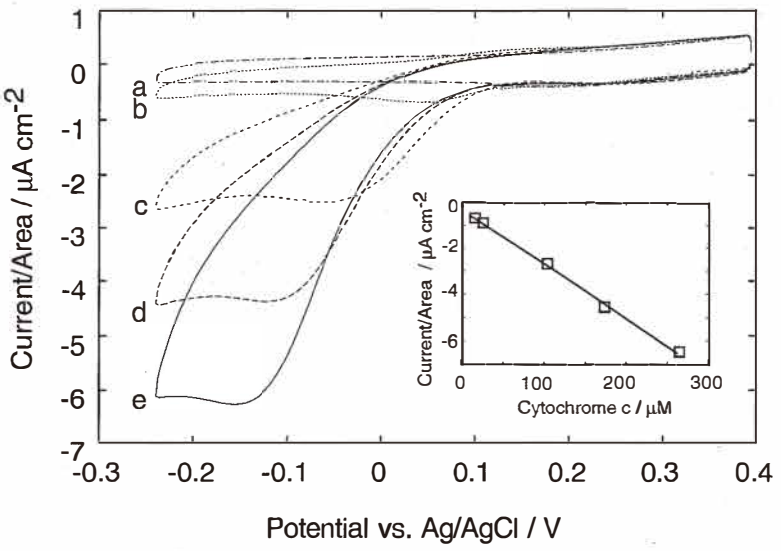

Fig. 4. Cyclic voltammograms of cytochrome c on polyasparagine modified gold electrode in 7.6 mM $NaClO_4$. Scan rate is 10 mV/s. Protein concentrations a = 0 M, b = 21 μM, c = 100 μM, d = 160 μM, e = 240 μM and dependence of peak current on protein concentration (inset).

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