Electron Transfer Reaction of Cytochrome *c* at Poly(ethylene oxide)-Thiolate-Modified Gold Electrode

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Gold electrode was modified with poly(ethylene oxide)s having thiol end (PEO-SH). The electron transfer reaction of cytochrome *c* was evaluated by the gold electrode modified with PEO-SH, having PEO molecular weight of 150, where the adsorption of proteins and resulting denaturation were restrained. The increase of PEO molecular weight conducted suppression of the redox responses at the modified gold electrode.

It is well-known that organosulfur compounds such as alkanethiols were spontaneously adsorbed on the gold surface and formed highly ordered monolayer films.[1,2] Formation of such monolayers, so-called self-assembled monolayers (SAMs), on the gold electrodes has been extensively studied due to their simple preparation method since 1980's.[3,4] The electrochemical redox reaction of proteins is being one of subjects with the SAM-modified electrode. There are a lot of studies on the electrode surface modification for the promotion of electron transfer reaction of proteins.[5,6] Specific interactions with not only electrodes but also proteins were reported to be important for these systems. On the other hand, poly(ethylene oxide) (PEO), which was a typical biocompatible material, was modified onto some substrates to avoid immune response.[7-10] These PEO-modified electrodes are also expected to inhibit the irreversible adsorption of proteins onto the electrode. This type of electrode should be effective for electrochemical reactions only when PEO layer thickness was small enough to allow the electron jump. In this paper, we report preparation of gold electrodes modified with PEO having thiol end (α-methoxy-ω-mercapto-poly(ethylene oxide); PEO-SH), their electrochemical properties, and their effect on the electron transfer reactions of cytochrome *c* (cyt.*c*) in an aqueous buffer solution.

PEO-SH was synthesized *via* a Bunte salt,[11,12] which was prepared by a reaction of sodium thiosulfate and terminal brominated PEO monomethyl ether.[13] PEOs with average molecular weight of 150, 350, 550, 750, and 2000 were used in this study. This Bunte salt was hydrolyzed to obtain PEO-SH.[14] The gold electrode was subjected to successive electrochemical redox cycling between -0.2 and +1.5 V in 1.0 M $HClO_4$ until a clean gold surface was obtained. The gold electrode modified with PEO_m-SH (P_mSAu; here "m" means average molecular weight of PEO part) was prepared by immersing the bare gold electrode in a 10.0 mM ethanolic solution of PEO_m-SH for 1~24 h, generally 4 h. After immersion, the electrode was rinsed with dehydrated ethanol and dried. Cyclic voltammetry measurement was performed in a conventional three-electrode cell with a Pt wire (0.5 mm ø) and Ag/AgCl electrode as counter and reference electrode, respectively. Cyt.*c* from horse heart (type VI from Sigma) was used without further purification.

First, the effect of soaking time of gold electrode into PEO-SH solution on the redox response was analyzed. Gold electrodes were immersed in 10.0 mM ethanolic solution of PEO-SH with PEO molecular weight of 150 (PEO_{150}-SH) for 1 to 24 h. The electron transfer reactions of thus prepared P_{150}SAu were investigated in 1.0 mM potassium ferricyanide aqueous solution (containing 0.1 M $NaClO_4$ as a supporting electrolyte). While the peak current decreased with increasing the immersing time, and it reached a constant when the immersing time was more than 4 h. These results indicated that PEO-SH was adsorbed on the gold electrode slowly but steadily. Similar experiments using PEO-SH with various PEO molecular weight showed clear PEO molecular weight dependence. Longer PEO chains made the peak separation widened and peak current decreased. Sweep rate dependence of electron transfer reaction of the ferricyanide at P_{150}SAu indicated that the electrode reaction was diffusion control. Moreover, the charge was also decreased by the increase of the sweep rate. These results suggested that ferricyanide was not incorporated into the PEO layer. This was confirmed by the following experiments; that P_{150}SAu, rinsed in the buffer, showed no redox response of ferricyanide ions even after cyclic voltammetry measurements.

Next, electron transfer reaction of cyt.*c* at P_{150}SAu was examined in a phosphate buffer solution, and reversible redox wave was clearly observed at +60 mV (vs. Ag/AgCl) with peak separation of 59 mV (Figure 1(b)). In case of a bare gold electrode, cyt.*c* is reported to be adsorbed on the electrode surface and denatured by the strong adsorption,[15] no good redox response was detected (Figure 1(a)). The modification of PEO_{150}-SH on the gold electrode surface prevented both the adsorption and denaturation of cyt.*c*, and allowed the electron transfer reaction between the electrode and cyt.*c*.

A series of experiments was made using P_mSAu (where m = 150~2000). Redox responses of cyt.*c* were also observed in cases of P_{350}SAu (Figure 1(c)) and P_{550}SAu (Figure 1(d)). Redox potential of the cyt.*c* was +60 mV (vs. Ag/AgCl) which was equal to that for P_{150}SAu. However, the peak separation was broadened and the charge was decreased with increasing the PEO molecular weight, similar to the cases of potassium ferricyanide redox reaction. Redox responses could hardly be observed when P_{750}SAu and P_{2000}SAu were used. When the protein approached the PEO surface by diffusion, the PEO layer was reported to be compressed.[16] Even taking this contraction into account, there should be an upper limit of layer thickness for the electron tunneling. When PEO molecular weight was 550 or less, the layer may form a film with thickness of 10 Å or less, through which the electrons can pass.[17] Such a thin layer allows electron transfer reaction between cyt.*c* and the electrode, but the electron transfer process should be suppressed by increasing PEO molecular weight due to the effect of layer thickness.

Recent studies suggest that PEO layer surface has hydrophobic characteristics in some extent, which induces a hydrophobic interaction between PEO and hydrophobic patch on the proteins.[18,19] If this was effective, the hydrophobic interaction between cyt.*c* and PEO might induce the orientation of cyt.*c* on the PEO layer. Sweep rate dependence of electron

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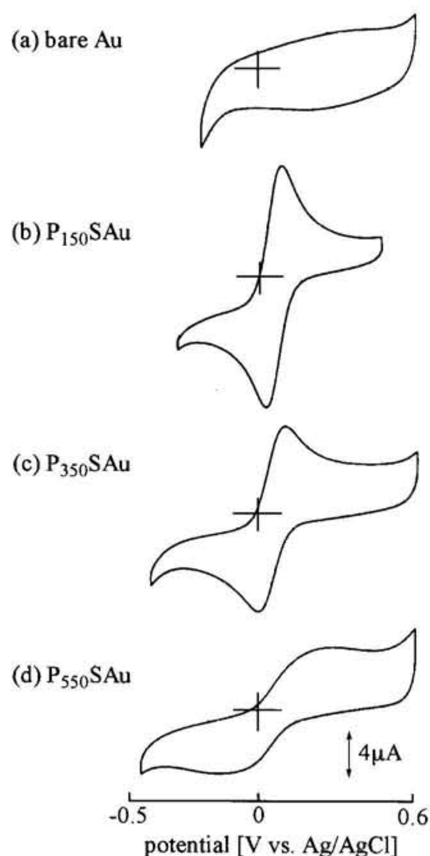

Figure 1. Cyclic voltammograms of 0.2mM cyt.*c* at a bare gold electrode (a), $P_{150}SAu$ (b), $P_{350}SAu$ (c), and $P_{550}SAu$ (d). In phosphate buffer solution (pH 7.0). Sweep rate: 30 mV/s.

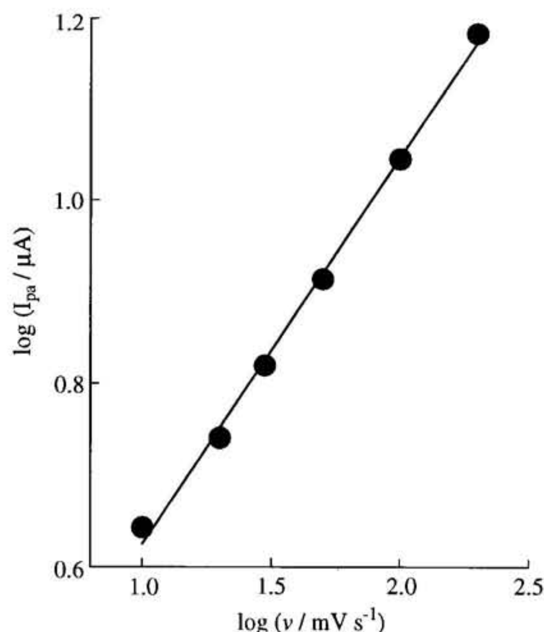

Figure 2. Effect of sweep rate (v) on the peak current (I_{pa}) of cyt.*c* in phosphate buffer.

transfer reaction of the cyt.*c* at $P_{150}SAu$ indicated that an electrode reaction was diffusion control (Figure 2). Moreover, the charge of electron transfer reaction decreased with raising the sweep rate. From these, the electrode reaction of cyt.*c* was diffusion control, in other words, the possibility of adsorption on the surface of $P_{150}SAu$ was denied. After CV measurement, this working electrode showed no redox response when it was rinsed and soaked in a buffer solution containing no cyt.*c*. Therefore, the prevention of direct adsorption of cyt.*c* on the electrode is quite effective to suppress the denaturation of cyt.*c*, and efficient electron transfer was achieved. The PEO monolayer, which has a role for only protection and no interaction with proteins, was effective for reversible redox reaction of the proteins in solution.

At $P_{150}SAu$ reported in this paper, cyt.*c* exhibited the fastest electron transfer. However, it is expected that the P_mSAu electrode with shorter PEO chain length would be more effective for acceleration of the electron transfer reaction of cyt.*c*.

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