Effect of the Structure of Modifiers Adsorbed on Gold Single Crystal Surfaces on the Promotion of the Electrode Reaction of Cytochrome c

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(Received January 9, 1997; CL-970023)

On Au(100) and Au(110) single crystal surfaces, clear electrochemical responses of cytochrome c were observed only at electrodes modified with bis(4-pyridyl)disulfide or 4-mercaptopyridine, but not at all on the surfaces modified with bis(2-pyridyl)disulfide or 2-mercaptopyridine.

Electron-transfer reaction of a metalloprotein is one of the currently most active areas in bioelectrochemistry and related fields. Many functionally modified electrodes have been used to study the electrode reactions of metalloproteins in recent years.[1,2] For cytochrome c electrochemistry, self-assembling monolayers of so-called electron-transfer promoters such as bis(4-pyridyl)disulfide (4,4'-PySSPy) have been well known to be effective.[2-4] However, since many similar promoter molecules have been found[4] to give more or less some electrochemical response for cytochrome c, surface functions of the promoter molecules, such as the interaction between cytochrome c and the surface modifier, have become ambiguous. For example, 2-mercaptopyridine (2-PySH) was also reported[5] to be effective as a promoter molecule like 4-mercaptopyridine (4-PySH) for cytochrome c, but the other group showed[4,6] 2-PySH was much less effective than 4-PySH. Such discrepancy may partly come from that the electrode surfaces so far used are not well-defined in atomic level. In order to give more definite answer on this problem, use of an atomically flat surface is necessary, because the orientation of the promoter molecule on the electrode surface can be controlled more rigorously. In the present study, the promoter activity of 4,4'-PySSPy was compared with 2,2'-PySSPy and diphenyldisulfide (PhSSPh) using Au(110) and Au(100) surfaces, and clear effect of the structure of the promoter molecule on cytochrome c electrochemistry was observed, for the first time.

Gold single crystals were prepared by so-called the flame-annealing-quenching method developed by Clavilier et al.[7] The crystallographic axes of the crystal were determined by laser beam reflection from an Au(111) or Au(100) facet, and the crystal was imbedded in resin for cutting. The surfaces were exposed by polishing mechanically with successively finer grades of alumina paste. In order to remove mechanical damages of the polished surfaces, the single crystal was annealed at ca. 940 °C for at least 12 h in an electric furnace. The electrode was finally annealed in hydrogen flame and then was quickly dipped into ultra pure water saturated with hydrogen. Each surface of the single crystal thus prepared in the present study showed its well-known characteristic voltammograms in both 0.1 M $HClO_4$ and 0.1 M H_2SO_4 solutions[8] (1 M = 1 mol dm^{-3}). An example is shown in Figure 1. Surface modification of the electrode was carried out by contacting with an ethanol solution of 20 μM of a modifier for 1 h. The modified electrode was then sonicated in ethanol for several minutes to remove excess modifier, if any. In the present study, Au(111) surface was not used, because, very recently, promoter molecule such as mercaptopyridine (PySH) has been reported[9,10] to make the structure of the promoter-modified surface complicated due to the surface reaction such as the pyridine-S bond cleavage. On the other hand, on Au(100) and Au(110) surfaces, such reaction was confirmed to be negligibly slow under the same conditions.[11] Electrochemical reductive desorption of the modifier from each single crystal surface was performed in a 0.1 M KOH solution, as reported in the literature,[12] to evaluate the modified surface structure. Horse heart cytochrome c (from Sigma) was purified by cation exchange chromatography using a Whatman CM-52 column as was described elsewhere.[13] Cyclic voltammetry was carried out using a Toho Giken PS-06 potentiostat with a function generator by using the meniscus (or hanging electrolyte) method.

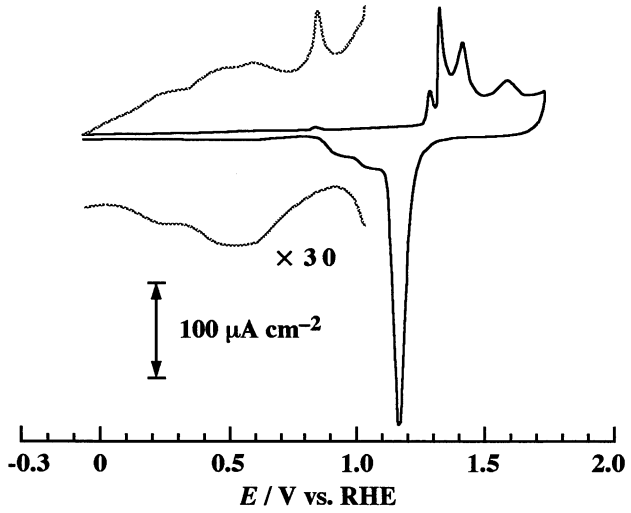

Figure 1. Cyclic voltammogram of the prepared Au(100) electrode in a 0.1 M $HClO_4$ solution at 25 °C, where RHE was used as a reference electrode. Scan rate: 50 mV s^{-1}.

Figure 2 shows the voltammograms for the reductive desorption of adsorbed 4,4'-PySSPy and 2,2'-PySSPy on Au(100) surface. The PhSSPh modified electrode also showed similar voltammogram. The structures of assembled monolayers are suggested to be essentially the same independent of the modifiers from the similarity of the observed voltammograms for these modified electrodes. The reductive desorption current in the voltammogram is due to the reaction of Au-SPy + e = Au + PyS^- as is known for many electrodes modified with thiols.[10,12] PySSPy is adsorbed onto an Au electrode with the S-S bond cleavage,[14] because the desorption peak potential of the disulfide at a single crystal surface was the same as that of the corresponding mercaptopyridine modified electrode. Spectral data of the surface[14] also supported the S-S bond cleavage.

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Although at the Au (110) surface the potentials of reductive desorption of the modifiers were a little more negative (by ca. 0.1 V) than those observed at the Au(100) surface, again no significant difference in structure of assembled monolayer was seen from the voltammograms of Au(110) electrodes modified with these three compounds.

The amounts of the surface excess of the modifiers were estimated from the peak areas of the voltammograms for the reductive desorption (see Figure 2) to be similar to each other (ca. 7 x 10^{-10} moles cm^{-2}), suggesting the monolayer adsorption.

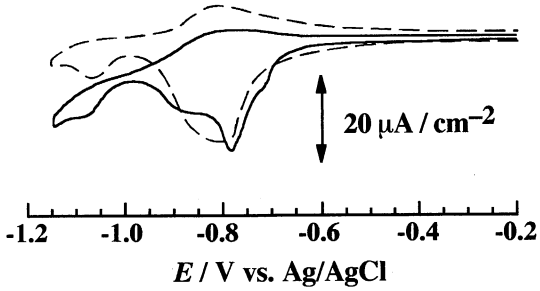

Figure 2. Cyclic voltammograms for reductive desorption of (——): 4,4'-PySSPy and (– – –): 2,2'-PySSPy modified Au(100) electrodes in a 0.1 M KOH solution at 25 °C. Scan rate: 50 mV s^{-1}.

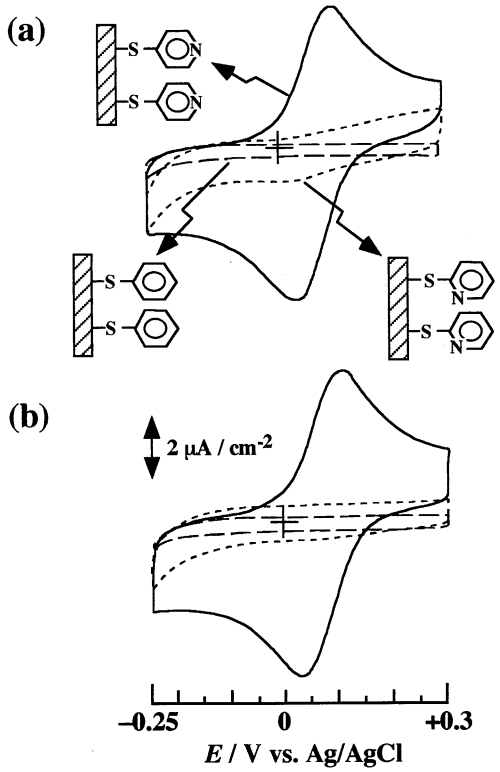

Figure 3. Cyclic voltammograms of 100 μM horse heart cytochrome c at a) Au(100) and b) Au(110) electrodes modified with (——): 4,4'-PySSPy, (-----): 2,2'-PySSPy, and (– – –): PhSSPh in a 0.1 M phosphate buffer solution containing 0.1 M $NaClO_4$ (pH 7.0) at 25 °C. Scan rate: 50 mV s^{-1}.

Interestingly, for the modified Au(100) and Au(110) electrodes, clear dependence of the electrode reaction of cytochrome c on the structure of the modifier was observed as shown in Figure 3. Only 4,4'-PySSPy and 4-PySH modified electrodes gave well-defined voltammetric response of cytochrome c, but no response was seen at all on 2,2'-PySSPy and 2-PySH modified electrodes. No response was also observed from diphenyldisulfide and mercaptophenol modified electrodes. The present results clearly indicate that an interaction between cytochrome c and the modifier is essential for the rapid electron transfer reaction of cytochrome c at the modified electrodes. In the present case nitrogen atom of pyridine ring faced to the solution interacted with cytochrome c at the electrode. On a 4,4'-PySSPy modified Au(110) electrode the redox potential, $E^{0'}$, the heterogeneous electron transfer rate constant, $k^{0'}$, and the diffusion coefficient, D, of cytochrome c were estimated to be 0.061 V vs. Ag/AgCl (Sat. KCl), 6 x 10^{-3} cm s^{-1} and 1.0 x 10^{-6} cm^2 s^{-1}, respectively, at 25 °C by applying the curve fitting method using a digital simulation technique for the voltammogram after subtraction of the background current, where the apparent geometrical area of the electrode (0.061 cm^2) was used.

This work was supported in part by a Grant-in-Aid for Scientific Research (Nos. 08232266/ 0845399/ 08355024) from the Ministry of Education, Science, Sports and Culture.

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