Phase Separation of an Unsymmetrical Disulfide SAM at an Evaporated Au(111) Electrode Monitored by Reductive Desorption and the Electrochemical Response of Cytochrome *c*

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The surface structure of an unsymmetrical disulfide (hexadecyl 4-pyridyl disulfide) modified Au(111) evaporated electrode has been evaluated using the redox behavior of cytochrome c and the electrochemical reductive desorption reaction. Controlling the modification time, either a homogeneous SAM or a phase-separated SAM was prepared.

The self-assembled monolayers (SAMs) of various thiols and sulfides on gold electrodes have received active attention in surface electrochemistry for preparing functional modified electrodes.[1] Typical examples are the bis (4-pyridyl) disulfide and the 4-mercaptopyridine modified gold electrodes, which have no redox moiety, but promotes cytchrome c electrochemistry at electrodes.[2] Because the electrochemical response of cytochrome c is very sensitive to the chemical structure of the promoter itself[3] and the coverage of the modifier,[4] the structures of the mixed SAMs prepared from mixed solutions of promoters and inhibitors for cytochrome c have been evaluated using cytochrome c voltammetry at polycrystalline gold electrodes[4,5]

When the mixed SAMs were prepared from the solutions of two kinds of thiols or symmetrical disulfides with different hydrophobic interaction,[1] it is usually difficult to prepare the homogeneously mixed SAMs at the molecular level. Two different components tend to form phase-separated structures, as shown by scanning probe microscopy,[6] cyclic voltammetry for reductive desorption,[7] and by using the different desorption potentials.[5,6] In contrast, when the substrate was modified with an unsymmetrical disulfide having different properties,[8-14] the homogeneously mixed SAMs can be prepared and the ratio of the surface concentration of the two components is fixed at 1:1. However, phase separation of SAMs of unsymmetric disulfides by heat treatment has been recently reported under some conditions.[13,14]

In the present study, the structure of the SAM of an unsymmetrical disulfide with one thiol moiety acting as a cytochrome c promoter and the other as an inhibitor has been evaluated by the voltammograms of cytochrome c at these SAM modified electrodes and reductive desorption of the SAM. By controlling the modification conditions, we succeeded in preparing homogeneously mixed SAMs and phase-separated SAMs.

Bis (4-pyridyl) disulfide (PySSPy) (Aldrich) was used as received. Hexadecyl 4-pyridyl disulfide (PySSC16) was synthesized from the mixture of 4-mercaptopyridine (Sigma) and hexadecylthiol (C16SH, Tokyo Kasei) by bromide oxidation.[15] The final product was analyzed by ^{1}H NMR (400 MHz).[16] Gold was evaporated on mica with a thickness of ca. 100 nm and annealed above a hydrogen flame for 30 s for use as working electrodes. Cyclic voltammograms of the evaporated gold electrode thus prepared were very similar to that of an Au (111) single crystal electrode in 0.1 M H_2SO_4.[17] A platinum plate and an Ag/AgCl (saturated KCl) electrodes were used as counter and reference electrodes, respectively. All potentials in this study refer to the Ag/AgCl. Horse heart cytchrome c (Sigma) was purified using an ion exchange Whatman CM-52 column.[2-4] In order to evaluate the structure of the modifier on the electrode surface, cyclic voltammetry for the electrochemical reductive desorption[7] and for cytchrome c[2-6] were carried out in 0.1 M KOH solution and in phosphate buffer solution (pH 7.0) containing 0.1 M $NaClO_4$, respectively. A Toho Giken PS-06 potentiostat was used for electrochemical measurements. All electrochemical measurements were carried out at room temperature (25 ±1 °C) under N_2 atmosphere. PySSC16 or PySSPy was modified on gold electrodes by immersing the electrode into an 0.1 M KOH aqueous solution containing 2% EtOH and 20 μM of the disulfide of interest and the electrodes were rinsed with pure ethanol followed by pure water (Milli-Q) to remove excess modifier on the electrodes.

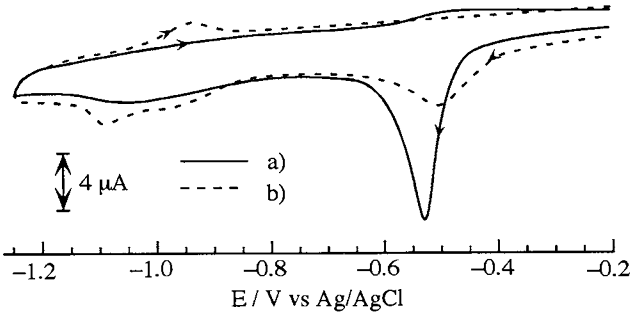

Figure 1. Cyclic voltammograms for the reductive desorption of the SAMs at Au evaporated electrodes recorded at 50 mV/s. Modification was carried out in 0.1 M KOH containing 20 μM PySSC16 and 2% of EtOH for a) 30 s and b) 20 min.

Figure 1a shows the voltammogram for the reductive desorption of the gold electrode in 0.1 M KOH solution for a surface modified with PySSC16 for 30 s. When the modification was made by immersing the electrode for 30 s in the mixed solution of PySSPy (20 μM) and C16SH (1 μM), the voltammogram of the resulting electrode taken in 0.1 M KOH aqueous solution showed two waves at −530 mV and −1130 mV corresponding to the desorption of PySSPy and C16SH domains, respectively. However, only one peak around −530 mV was observed at the electrode modified with PySSC16 for 30 s. This result suggests that the 4-pyridylthiolate (PyS^-) and hexadecylthiolate ($C16S^-$) of PySSC16 were homogeneously mixed at the molecular level. The surface concentration of the PySSC16 evaluated from the area of desorption wave of the voltammogram was 5.9 x 10^{-10} moles / cm^2, meaning that the

PySSC16 forms a relatively packed SAM on the electrode after 30 s of modification. At the modified surfaces only showing this reductive desorption peak around –530 mV no redox response of cytochrome c was observed as shown in Figure 2a. This means that the PyS$^-$ is surrounded by C16S$^-$ with long alkyl chains so the size of PyS$^-$ domain would be small enough that cytochrome c can not access the PyS$^-$ surface as shown schematically in Figure 2a. When the electrode was modified with only the PySSPy, the reductive desorption peak was around –530 mV and well-defined cyclic voltammogram of cytchrome c was observed.

When immersing time for the modification of PySSC16 was extended to 20 min, a second reductive peak around –1100 mV was observed in addition to a broad peak around –505 mV (Figure 1b). The sum of the charge for these two reductive desorption waves (54 μC / cm^2) was almost equal to that obtained from the cyclic voltammogram for the modified electrode with PySSC16 for 30 s (56 μC / cm^2), the surface excess of PySSC16 was independent of the modified time under the conditions. Time-resolved ***in situ*** FTIR spectra (not shown) of the modified electrodes surfaces showed both signals due to PyS$^-$ (pyridine ring stretching at 1557 cm^{-1}) and C16S$^-$ (CH stretching, at 2889 cm^{-1}).[18] Therefore, two reductive desorption waves could be explained by the phase separation at the electrode surface within 20 min. The peak potentials of the first and second reductive desorption waves were shifted by +25 mV and + 30 mV compared to those of pure PySSPy and C16SH SAMs, respectively. Judging from the reduction peak potential, the both domains are mixed with each other to some extent.

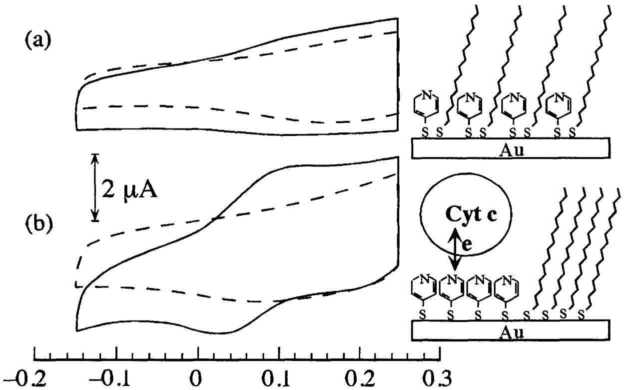

Figure 2. Cyclic voltammograms of 100 μM cytochrome c at SAM modified Au electrodes in a phosphate buffer solution (pH 7.0) containing 0.1 M $NaClO_4$, together with schematic representation of a) homogeneously mixed SAM formed after 30 s-modification and b) phase separated structure after 20 min-modification, allowing electron transfer of cytochrome c. Modification was carried out 0.1 M KOH containing 20 μM PySSC16 and 2% EtOH. Scan rate: 50 mV/ s.

At modified surfaces showing two reductive desorption waves a well-defined cyclic voltammogram for cytochrome c was observed as shown in Figure 2b. This phenomenon suggests that by phase separation of the SAM, C16S$^-$ rich and PyS$^-$ rich domains were formed. Cytchrome c can access the PyS$^-$ rich domain permitting electron transfer to take place at the PyS$^-$ domain (Figure 2b). Using the microelectrode array model,[4] where the size of active site (PyS$^-$ rich domain) is assumed to be uniform and cytochrome c was considered to react only on the PyS$^-$ domain, the observed voltammogram was analyzed. The maximum size of the PyS$^-$ domain obtained by the simulation program was 1.5 μm.[19] In order to ensure how the phase separation takes place at the surface, after the electrode was modified with PySSC16 for 1 min, the electrode was kept in 0.1 M KOH solution containing 2% EtOH without modifier for 20 min. In this case the voltammogram of reductive desorption and cytchrome c was essentially identical to those of 20 min-modification, respectively, which means that the phase separation took place without excess of modifiers in solution.

In conclusion, the structure of the SAM of unsymmetrical disulfide (hexadecyl 4-pyridyl disulfide) modified Au electrode was controlled by the modification time for the first time to prepare homogeneously mixed in molecular dimension and phase-separated binary mixed SAM.

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19 The simulated cyclic voltammograms using our program were independent of the size of the domain up to 1.5 μm.