Electrochemical Characterization of an Assembled Monolayer of α-Methoxy-ω-mercapto-poly(ethylene glycol) on Gold and Complex Formation of the Monolayer with α-Cyclodextrin

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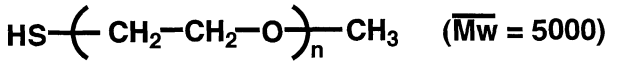
Assembled monolayers of α-methoxy-ω-mercapto-poly(ethylene glycol) on gold were found to provide unique microenvironments for the electrochemistry of small molecules and a heme protein and to form a two-dimensional supramolecular complex with α-CD on the metal electrode surfaces.

Considerable attention is being given for the design and construction of organic nanostructures based on supramolecular chemistry.[1,2] Recently, Harada et al.[3,4] reported the formation of supramolecular inclusion complexes between poly(ethylene glycol) and α-CD, and of the nanoscale molecular tubes derived from the inclusion complexes. We have found that the mixing of an aqueous bilayer of an artificial lipid bearing a poly(ethylene glycol) with α-CD produces a crystalline inclusion complex which possesses the fundamental characteristic of lipid bilayers.[5] One of our interest in this field is to design and fabricate novel electrodes modified with supramolecular organizates. Kaifer and coworkers[6] reported the self-assembly of a 2[catenane] attached to a gold surface. In this paper, we describe protein electrochemistry at an assembled monolayer of poly(ethylene glycol) and then the possible formation of a supramolecular monolayer of the poly(ethylene glycol) with α-CD on a gold electrode which has been examined using an electrochemical technique and the quartz crystal microbalance (QCM).

Methoxy-ω-mercapto-poly(ethylene glycol) (**1**) from Shearwater Polymers Inc. was used as a surface modifier. Two different types of electrodes, a gold disk electrode (geometric area, 0.0201 cm^2) polished with a 1.0 μm and then 0.05 μm alumina slurry and a gold evaporated quartz crystal microbalance (QCM) electrode (9 MHz, AT cut) were immersed in a 10 mmol dm^{-3} ethanol solution of **1** for 12 - 24 h at 25 °C followed by rinsing in Milli-Q water (> 18 MΩcm) to remove any excess adsorbates. For electrochemistry of the modified electrode, an Ag/AgCl (saturated KCl) electrode and a platinum electrode were used as the reference and counter electrodes, respectively. Cytochrome *c* from bovine heart was purchased from Sigma Chem. Co., and was used as received. A Britton & Robinson buffer solution (pH 7, μ=*ca.* 0.03) was used as the buffer solution for electrochemistry.



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The formation of a modified layer of **1** on gold was examined using the QCM.[7,8] The observed frequency change in air was 870 (± 130) $Hzcm^{-2}$, which corresponds to the adsorption of 1.7 (± 0.3) × 10^{-10} mol cm^{-2} of **1** on the gold surface. This value suggests the monolayer formation of **1** on Au. No significant difference in the frequency change for electrode dipping time of 12 to 24 h in ethanol solution was observed.

Figure 1a-c shows cyclic voltammograms of a **1**-modified electrode in the presence of a given redox compound. The observed cyclic voltammograms were very stable for more than 6 h under the given experimental conditions. The observed redox wave indicates that the heterogeneous electron transfer reaction of $Fe(CN)_6^{4-}$ with the electrode is almost inhibited and that there is a significant difference in the voltammograms between $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$. The modified electrode did not block the electrochemical communication of $Ru(NH_3)_6^{3+}$. The hydrate diameter of $Fe(CN)_6^{3-/4-}$ (0.52 nm) is smaller than $Ru(NH_3)_6^{3+/2+}$ (0.62 nm); therefore, as the origin of the large difference in the voltammograms, the charge recognition ability of the monolayer is suggested. The cation-dipole interaction between $Ru(NH_3)_6^{3+}$ and the poly(ethylene glycol) monolayer results in a higher permeability of $Ru(NH_3)_6^{3+}$ into the monolayer. The modified layer on gold is suggested to act as the barrier for the incorporation of the ion into the modified layer.

It is well known that poly(ethylene glycol)s provide suitable microenvironments for a variety of protein enzymatic reactions. The electrochemistry of cytochrome *c*, a spherical (roughly) redox protein with a diameter of about 3 nm,[9] at the modified electrode was examined and found that a well-defined redox wave in the cyclic voltammogram was observed (Figure 1d, solid line). The formal redox potential of cytochrome *c*, $E^{o'}$, evaluated from the midpoint of the anodic and cathodic peak potentials was 60 mV *vs.* Ag/AgCl, which is in good agreement with those of cytochrome *c* in solution at the promoter modified

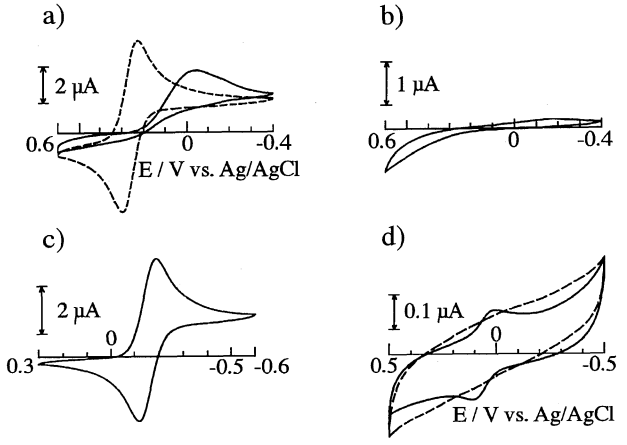

Figure 1. Typical cyclic voltammograms of 2 mmol dm^{-3} $Fe(CN)_6^{3-}$ (a: solid line), $Fe(CN)_6^{4-}$ (b), $Ru(NH_3)_6^{3+}$ (c) at a **1**-modified gold electrode (solid line) or a bare gold electrode (a: dotted line) and of 0.1 mmol dm^{-3} cytochrome *c* in the absence (d: solid line) and presence (d: dotted line) of α-CD at a **1**-modified gold electrode. α-CD concentration is 13.4 mmol dm^{-3}. The solutions are a Britton & Robinson buffer solution (pH 7, μ=*ca.* 0.03). Scan rate, 20 mV s^{-1}. Temperature, 25 °C.

electrodes,[10-12] suggesting that the faradaic current is from native cytochrome *c*.[13,14] The peak current was proportional to the square root of the scan rate, $v^{1/2}$ in the v region of 5 to 100 mVs^{-1}, indicating an electrode reaction with diffusion control. Prime and Whitesides[15] described the adsorption of proteins onto the surface of self-assembled monolayers of oligo(ethylene glycol)end-attached mono(mercaptoundecylethers). In this study, we revealed that the two-dimensional poly(ethylene glycol) on the gold electrode provides a suitable microenvironment for the direct electron transfer reaction of the redox protein.

The formation of supramolecular inclusion complexes of α-CD with an aqueous bilayer membrane of a poly(ethylene glycol)-bearing lipid[5] suggests the possibility of the formation of similar inclusion complexes at two-dimensional monolayers on solid substrates. To explore this possibility, α-CD was added to the solution containing cytochrome *c* at the modified electrode. The faradaic currents in the cyclic voltammograms of cytochrome *c* were found to remarkably decrease with the addition of α-CD and the presence of 13.4 mmol dm^{-3} α-CD in solution resulted in the disappearance in the faradaic currents (Figure 1d, dotted line). The redox wave of cytochrome *c* was fully recovered when the modified electrode was placed in the solution of cytochrome *c* not containing α-CD. β-CD is unable to form crystalline inclusion complexes with poly(ethylene glycol)s.[3-5] In this system, the addition of β-CD instead of α-CD with concentrations up to 16 mmol dm^{-3} caused little decrease in the redox currents. The obtained results together with the fact that poly(ethylene glycol)s form crystalline complexes with α-CD suggest that the current decrease in the voltammograms comes from the inclusion complex formation of the **1** monolayer with α-CD. Excluding cytochrome *c* from the poly(ethylene glycol) atmosphere on the electrode due to the formation of such rigid inclusion complex is understandable.

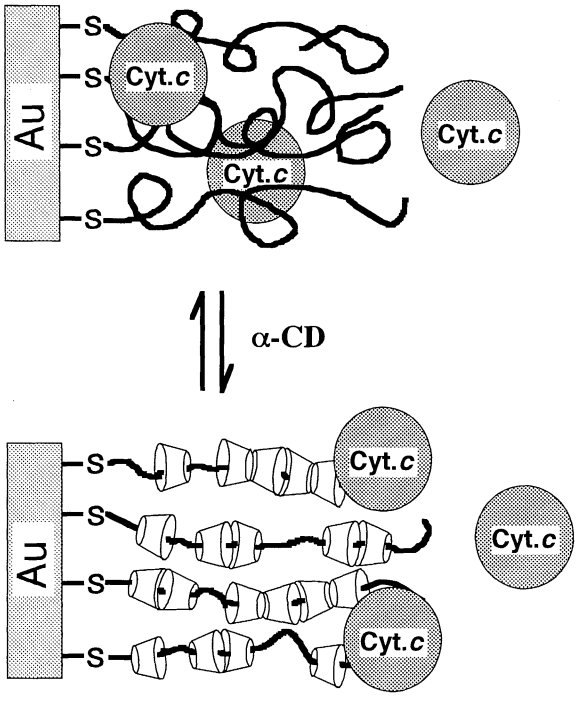

Figure 2. Possible model for the supramolecular inclusion complex between α-CD and the monolayer of **1** on gold.

The stoichiometry of the crystalline inclusion complex between poly(ethylene glycol) and α-CD has been reported to be 2 : 1 (ethylene glycol unit : α-CD).[16] The QCM measurement for the poly(ethylene glycol) monolayer / α–CD complex on the electrode gave the stoichiometry of *ca.* 6 : 1 (ethylene glycol unit : α-CD). Based on these results, we propose a possible model for the two-dimensional supramolecular complex in Figure 2.

In conclusion, we have characterized assembled monolayers of the thiol-terminated poly(ethylene glycol) on gold and propose a possible model for the two-dimensional supramolecular complex between α-CD and the monolayer. An intense effort is currently under way in our laboratory to get direct proof for the structure of the supramolecular complex.

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