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Preparation of Au Electrodes Modified with Self-assembled Monolayers of Functional Metal Complexes

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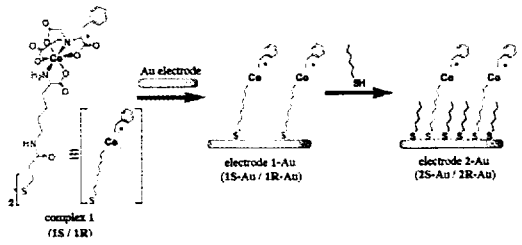
In this paper, we review our recent works on the preparations of some Au electrodes modified with self-assembled monolayers of functional metal complexes, such as molecular recognition, electron transfer, bacteria immobilization, and dioxygen activation, from the viewpoint of the construction of chemical/energetic conversion devices. (i) A new-classed promoter electrode bearing optically-active Co^{III} complexes containing (*S*)-/(*R*)-phenylalanine derivative ligands as the molecular recognition site has been constructed; the chirality and/or orientation of promoter on Au electrode surface have affected the electron transfer rate of cytochrome *c*. (ii) The Fe^{III}-artificial siderophore-modified Au electrode surface has been prepared as an immobilizing tool of microorganisms toward the bacteria sensor and bio-reactor, whose adsorption ability for microorganisms has been studied. (iii) For a purpose of fixation and activation of dioxygen at room temperature, the self-assembled monolayer of dinuclear iron complex has been modified on Au electrode, and its reaction with dioxygen has been studied.

Key words: self-assembled monolayer, electron transfer reaction, chiral surface, molecular recognition, dioxygen activation

1. Self-assembled monolayers of optically active Co(III) complexes as a new promoter. Electron transfer reaction plays an important role in biological processes such as photosynthesis and respiration.[1] Electron transfer proteins, such as cytochrome *c* (cyt *c*) and azurin, recognize their redox partners through various non-covalent interactions, such as electrostatic, hydrophobic (or hydrophilic), hydrogen-bonding, and steric interactions.[2] These non-covalent interactions are originated from the amino acid residues around the molecular recognition site.

In the electrochemical study of cyt *c*, normal electrochemical method can not be easiy applied, because its redox center (heme) is buried inside of the protein.[3] The Au electrode modified with 4-mercaptopyridine or ruthenium complex, so-called "promoter," has been employed in order to detect a reversible redox behaviour of cyt *c*.[4] However, there is no report that have been investigated from the viewpoint of molecular recognition in the electron transfer of cyt *c*. We have prepared the self-assembled monolayers (SAMs) containing a new-classed promoter that can recognize the electron transfer site of horse heart cyt *c*.[5] As a new promoter, negatively charged Co(III) complexes containing (*R*)-/(*S*)-phenylalanine were newly prepared and fixed on Au electrode as the SAMs; **1S-/1R-**Au (Scheme 1). These SAMs indicated clear promoting ability for the electron transfer and molecular recognition between the electrode and cyt *c*.

In cyclic voltammograms of cyt *c* as measured with **1S**-Au and **1R**-Au, the peak separation (ΔE_p) for **1S**-Au is different from that of **1R**-Au. The difference in ΔE_p between **1S**-Au and **1R**-Au (ΔE_p(**S**) – ΔE_p(**R**)) was plotted against scan rates (Fig.1). The difference in ΔE_p between **1S**-Au and **1R**-Au was found out in the



Scheme 1. Schematic view of Co(III) complexes of (*R*)-/(*S*)-phenylalanine derivatives (**1**) and Au electrodes modified with them (**1**-Au and **2**-Au)

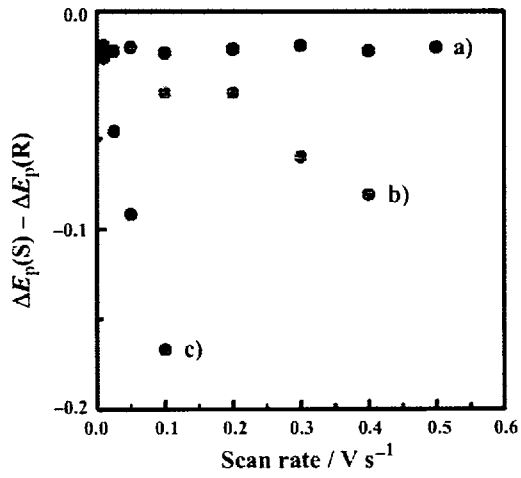


Fig. 1. Relationship between ($\Delta E_p(S)$ – $\Delta E_p(R)$) and scan rate of CV. (a) $[Ru(NH_3)_6]^{2+/3+}$ with **2**-Au , (b) cyt *c* with **1**-Au,(c) cyt *c* with **2**-Au.

case of cyt *c*. The difference in ΔE_p indicates that the electron transfer rate between cyt *c* and Au electrode is distinct between them,[6] indicating that cyt *c* recognizes the difference in the chirality of Co(III) complexes. From the values of ΔE_p(S)-ΔE_p(R), it has been found out that the rate of electron transfer with where the defect site of **1**-Au was complemented with hexanethiol, indicated the same redox behaviour as $[Ru(NH_3)_6]^{2+/3+}$, while **2**-Au caused cyt *c* recognition induced by the chirality of Co(III) complexes as well as the case of **1**-Au. However, the difference in ΔE_p between **2S**-Au and **2R**-Au is larger than that of **1**-Au (Fig. 1). This result suggests that the electron transfer rate between cyt *c* and **2**-Au was slower than that of **1**-Au. The slow rate in **2**-Au is explained as follows: **2**-Au has more rigid structure as compared with **1**-Au. Hexanethiol on **2**-Au provides well-ordered monolayers, so that the Co(III) complex units are difficult to move on Au electrode surface.

From the above results, we propose the electron transfer process of our system as follows: (i) positively charged cyt *c* approaches to negatively charged Co(III) units on Au electrode surface through electrostatic interaction, (ii) adsorbed cyt *c* rotates on the surface and forms the association state, (iii) the electron transfer reaction occurs between cyt *c* and electrode. The second process is the rate determining step of the electron transfer between modified Au electrode and cyt *c*. The difference in the chirality of Co(III) unit, that is, the absolute configuration of phenylalanine, affected on the interaction with heme crevice of cyt *c*.

In this study, we demonstrated that the Au electrode modified with negatively charged Co(III) complexes containing amino acid derivative promoted the electron transfer between cyt *c* and Au electrode. Moreover, this electrode recognized the surface structure of the redox center of cyt *c*. The metal complexes with amino acid derivative ligand are available as a promoter, because its metal center and amino acid are changeable systematically. Thus, this typed electrode should be one of powerful tools for investigating the electron transfer path of various electron transfer proteins.

2. Preparation of Fe^{III}-artificial siderophore-Modified Au electrode surface and its immobilization of microorganisms. The detection of microorganisms is one of the important problems in food and health industry [7-9]. Currently, the quick and high sensitive detection method is required. Various microorganisms-detection methods have been developed and used in the field of environment sanitation. The detection and identification methods for numerous microorganisms, bacteria, and fungi include gene analysis and interactions with specific peptides, sugar chains, and proteins on the cell surface. The interaction between siderophore and its receptor protein on the cell surface is one of binding forces between them.

Siderophores, which are secreted by microorganisms for iron uptake, are one of the most important bio-related materials [10] and bind Fe^{III} ion in the environment to form very stable Fe^{III}-siderophore complex in aqueous media. The receptor and/or binding proteins in the cell membrane recognize their

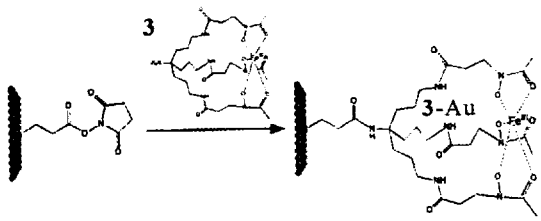


Fig. 2. Schematic views of NH_2-terminated artificial siderophore (**3**) and the Au electrode modified by **3** (**3**-Au).

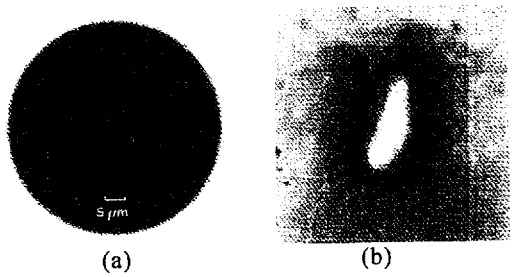
Fe^{III}-siderophore complexes, and then are permeated into the cell. The complex is reductively decomposed and the obtained Fe^{II} ion is used for various reactions in biological systems.

Previously, we have studied hydroxamate-type artificial siderophores as functional models of native siderophores [11-13]. On the basis of this design concept, we synthesized the original siderophore as an adsorbing and detecting probe of microorganisms. Our siderophores are able to change their structure systematically, so that we can modulate their properties freely. To modify the artificial siderophores on the surface of substrate, new artificial siderophore-Fe^{III} complex, **3**, was synthesized (Fig. 2).

Herein, we describe the stepwise modification of hydroxamate-based artificial siderophore on the Au electrode and its absorption behavior of microorganisms.[14]

The hydroxamate-typed artificial siderophore, **2**, was synthesized on the basis of previous method [13,15-18]. Terminal -NH_2 group of tripodal ligand was introduced to modify it on the Au electrode. **3** was easily obtained by ligand exchange reaction [18]. **3**/Au was prepared by the stepwise modification of **3** on a Au electrode. The surface coverage of **3** on **3**/Au, *ca.* 9.0×10^{11} mol cm^2, was estimated from its redox wave.

Fig. 3a shows optical microscopic image of **3**/Au after incubating *M. flavescens* on its surface. Many black spots were observed on **3**/Au. Although similar experiments were carried out on the DTSP-modified Au electrode as control, no spot was observed on it. To clarify these black spots, scanning electron



(a) (b)

Fig. 3. Optical microscopic (a) and AFM images (b) of **3**-Au.

microscopy (SEM) and atomic force microscopy (AFM) were carried out. The SEM image (Fig. 3b) clearly shows the adsorption of *M. flavescens* on **3**/Au. The same result was also obtained by AFM measurement. These results suggest that **3**/Au can adsorb *M. flavescens*.

The measurement of quartz crystal microbalance (QCM) was also tried to investigate the details of the adsorption behavior of *M. flavescens* on **3**/Au. The QCM result indicated the significant frequency decrease by the injection of *M. flavescens* broth. When the broth without *M. flavescens* was injected, the decrease in frequency was hardly observed. The decrease in frequency indicates increase of the surface gravity on **3**/Au, suggesting the adsorption of *M. flavescens* on its surface. On the contrary, no decrease in crucial frequency was observed in the case of the DTSP-modified Au electrode as control. This result well corresponds with the observation of various microscopy as discussed above.

In conclusion, we demonstrated the adsorption of microorganisms using the artificial siderophore-modified **3**/Au, which was clearly confirmed by measurements of microscopic and QCM methods. Current detection limit of microorganism by **3**/Au was ca. 10^5-10^6 CFU. However, we expect that this artificial siderophore-modified **3**/Au has a potential for the detection and/or probe device of microorganisms.

3. Au electrode modified with self-assembled monolayer of dinuclear iron complex having dioxygen activation ability. Molecular oxygen (O_2) binding and activation by non-heme iron proteins are key processes in biological systems. A class of proteins such as hemerythrin (Hr) and methane monooxygenase (MMO), which have similar structural motif of two carboxylate-bridged diiron sites each other, has received much interest in the past decade [19-23].

Hr binds O_2 as terminal peroxide group with $2e^-$ reduction by diiron center. Since this reaction is reversible, Hr can bind/release O_2 reversibly. On the contrary, MMO activates O_2 to convert methane to methanol under very mild condition (at normal pressure and room temperature). Mechanism of O_2 activation by MMO is $4e^-$ reduction process with forming diiron(IV) species named compound Q, which is converted from peroxo intermediate [23].

Numerous studies of diiron model complexes have contributed much to the understanding of the structural and catalytic properties of their active sites. However, almost diiron model complexes generated O_2-adduct only in non-aqueous solution at low temperature [24-29].

The self-assembled monolayers (SAMs) on various metal or metal oxide surfaces have recently received much attention as one of the important tools in nanotechnology and molecular engineering [30-35]. This method allows the introduction of desired functionality to the surface at the molecular

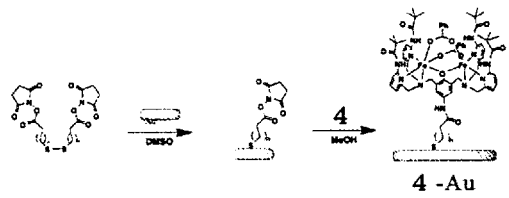


Fig. 4. Preparation of **4**-Au.

level by self-assembling specific molecular groups such as metal complexes and proteins. In addition the molecules modified on an electrode surface generally become more stable than that in homogeneous solution. Thus, we attempted to modify the non-heme model diiron complex on the electrode surface by self-assembling.

Herein, we describe the preparation of diiron(II) complex with new dinucleating ligand containing terminal amino group, and its self-assembled monolayer, **4**/Au (Fig. 4). **4** on **4**/Au was remarkably stable and reacted with O_2 in aqueous solution at room temperature reversibly.[36]

The cyclic voltammogram of **4** in CH_2Cl_2 gave one anodic wave ($2e^-$ process) and two cathodic waves (each $1e^-$ process) at E_{pa} = 0.92 V and E_{pc} = 0.67, 0.40 V *vs.* Ag/Ag^+, assignable to Fe_2(II,II/III,III), Fe_2(II,II/II,III), and Fe_2(II,III/III,III), respectively, which well corresponds to those of the Fe_2 complexes with similar structures reported previously. Each

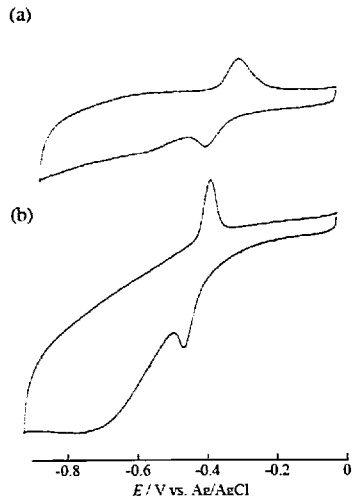


Fig. 5. Cyclic voltammograms of **4**-Au in 0.1 M $NaCF_3SO_3$ aqueous solution under Ar (a) and afer bubbling O_2 (b)

redox process was not reversible, suggesting that **4** in CH_2Cl_2 is not so stable. This instability is probably due to hydrogen bond between $N\text{-}H_{pivalamido}$ and $O_{benzoate}$. The bridging benzoate was easy to release its one O

site by this hydrogen bond, so that several structures of **4** exist in solution.

The cyclic voltammogram of **4**/Au in aqueous solution showed one anodic wave ($2e^-$ process) and two cathodic wave (each $1e^-$ process) at E_{pa} = −0.32 V and E_{pc} = −0.38, −0.55 V *vs.* Ag/AgCl (Fig. 5a). The linear relationship between each peak current and scan rate indicated that this redox process is originated from the species immobilized on the surface, indicating that **4** was modified on the Au surface. Compared to the above result in solution, these waves were assigned to Fe_2(II,II)/(III,III), Fe_2(II,II)/(II,III), and Fe_2(II,III)/(III,III), respectively. Each redox potential of **4**/Au widely shifted to the negative direction compared to **4** in solution. This shift is probably caused by the effect of negatively charged surface by linking to DTSP. Similar negative shift of the redox potential on the complexes immobilized on the surface were reported [35].

Interestingly, the redox potential of **4**/Au in aqueous solution dramatically changed by bubbling of O_2 gas. The waves assigned to Fe_2(II,II/III,III), and Fe_2(II,III/II,III) shifted to negative direction (*ca.* 0.08 V) by comparison with that before O_2-bubbling (Fig. 5b). In addition, these two redox potentials shifted to positive direction by Ar bubbling and finally return to the original potentials. This change was repeated reversibly. Thus, the waves of Fig. 4b were assigned to the waves of μ-1,2-peroxo complex, $Fe_2(O_2)$(II,II/III,III) and $Fe_2(O_2)$(II,III/III,III), respectively. This finding indicates that the reversible O_2 adsorption/desorption occurs on **2**/Au in aqueous solution at room temperature. That is, peroxo complex of **2** is extremely stable by modifying on Au surface. It suggests that **2** on **2**/Au has acted like the solid-state, which prevented degradation of **2** and made its peroxo complex stabilize in aqueous solution at room temperature.

In conclusion, we demonstrated the preparation of the self-assembled monolayer of diiron(II) complex, **2**/Au, its redox behavior and capturing molecular oxygen in aqueous solution at room temperature. We succeeded in observation of its reversible oxygen adsorption/desorption behavior. The immobilization of **4** onto Au electrode surface makes it stabilize extremely. This is rare example that exhibited the reversible adsorption/desorption of the oxygen by the immobilization of the non-heme functional model complex on the electrode.

4. CONCLUSION

Living systems are surrounded with O_2, N_2, CO_2, etc. in air, which are taken up by bacteria, fungi, plants, animals and so on. They are recycled by ingenious biological cyclic systems. Biological systems perform chemical and/or energetic conversion using these systems. In order to construct the devices having higher functionality, we have prepared some Au electrodes having higher functionalities and introduced here. We think that we can construct the high-performance chemical conversion and/or energetic conversion devices through the 'bottom-up' method of the accurate design and synthesis of the functional molecules and their introduction into/onto the surfaces by the self organization method.

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