Self-assembled monolayers of optically active Co(III) complexes: a new promoter electrode recognizing the electron transfer site in cytochrome *c*†

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A new-class of promoter electrode bearing a molecular recognition ability has been constructed; the chirality and/or orientation of promoter on the Au electrode surface have affected the electron transfer rate of cytochrome *c*.

Electron transfer reactions play 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. In fact, steric, electrostatic and hydrogen bonding interactions have been observed in the crystal structure of the association complex between cyt *c* peroxidase and iso-1 cyt *c* from yeast.[2] These non-covalent interactions originate from the amino acid residues around the molecular recognition site.

In an electrochemical study of cyt *c*, normal electrochemical methods cannot be applied easily, because its redox center (heme) is buried inside 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, an investigation from the viewpoint of how molecular recognition affects the electron transfer of cyt *c* has not been reported.

Herein we report the preparation of self-assembled monolayers (SAMs) containing the new class of promoter that can recognize the electron transfer site of horse heart cyt *c*. As a new promoter, negatively charged Co(III) complexes **1** containing amino acid derivatives were prepared and fixed as SAMs (Scheme 1). These SAMs indicated clear promoting ability for the electron transfer and molecular recognition between the electrode and cyt *c*. Since a side chain of this ligand is changeable systematically, it will be a usable method that can probe around the electron transfer site.

Optically active ligand, *N*,*N*-bis(carboxymethyl)-(*S*)-/(*R*)-phenylalanine ((*S*)-/(*R*)-BCMPA), and its Co(III) complexes **1** were prepared according to methods described previously.[5] The dithio derivative **2**, containing **1**, was synthesized *via* a coupling reaction of **1** and dithiosuccinimidylpropionic acid (DTSP).‡ In Scheme 1, **1***S* and **1***R* denote absolute configuration of phenylalanine for their ligands. The resulting complexes were identified by various spectroscopic and elemental analyses.§ SAM of **2** (**2**–Au) was prepared by dipping poly-Au electrode in an aqueous solution containing **2** for 3 days. Then **2**–Au was treated with hexanethiol to fill the defect site on the Au surface, which (**3**–Au) was prepared by dipping **2**–Au in MeOH containing hexanethiol for a few hours.

In an electrochemical study of complex **2**, the irreversible reduction wave assigned to Co(III/II) and was observed at −0.30 V *vs.* Ag/AgCl.¶ **2**–Au also showed an irreversible reduction wave (*ca.* −0.36 V *vs.* Ag/AgCl) and its electrochemical behaviour was similar to that of complex **2**. A surface coverage of **2**–Au was calculated to be 3.7×10^{-11} mol cm^{-2} from its reduction wave. This value is quite small in comparison with its identical one (5.2×10^{-10} mol cm^{-2}; as estimated from the projected size of **2**). It suggests that **2**–Au was not well ordered among Co(III) complexes because of the electrostatic and steric repulsions of each Co(III) unit. The SAM **3**–Au that was filled with the thiol showed a decrease of background current in cyclic voltammograms as

† Electronic supplementary information (ESI) available: Table S1: Second-order rate constants for the oxidation of ferrocyt *c* by Ru^{III} complexes. Fig S1: Cyclic voltammograms in (a) buffer solution (black) and (b) cyt *c* solution (red), as measured with 3*S*–Au. See http://www.rsc.org/suppdata/cc/b4/b412009d/

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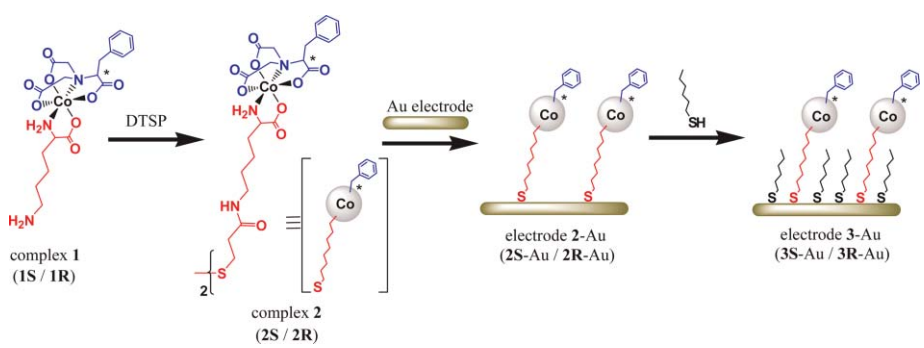

Scheme 1 Schematic view of Co(III) complexes **1**, **2** and Au electrodes modified with **2** (**2**–Au and **3**–Au).

compared with **2**–Au. This decrease clearly suggests that the defect site in **2**–Au was complemented with hexanethiol.[6]

In cyclic voltammograms of $[Ru(NH_3)_6]^{2+/3+}$ with **2**–Au, the redox wave assigned to Ru(II/III) was observed at −0.17 V *vs.* Ag/AgCl. It is evident that **2**–Au can promote the electron transfer between Ru complex and Au electrode. Furthermore, there was no significant difference in redox behaviour of Ru complex between **2***S*–Au and **2***R*–Au. This result indicates that the chirality of **2**–Au does not affect the electron transfer of $[Ru(NH_3)_6]^{2+/3+}$.

Fig. 1 shows the cyclic voltammogram of cyt *c* solution as measured with **2***S*–Au, which is in contrast with that of buffer solution. The more negative region was not measured, because the charge of the Co complexes was changed by the Co reduction. When a bare gold electrode was used, there was no wave in this range. Therefore, it is clearly indicated that **2***S*–Au promoted the electron transfer between cyt *c* and the Au electrode. **2***R*–Au also promoted the redox reaction of cyt *c*. A most interesting point is that the value of the peak separation (ΔE_p) for **2***S*–Au is different from that of **2***R*–Au. The difference of ΔE_p between **2***S*–Au and **2***R*–Au ($\Delta E_p(S) - \Delta E_p(R)$) was plotted against scan rates (Fig. 2). The difference of ΔE_p between **2***S*–Au and **2***R*–Au was determined in the case of cyt *c*, although it was not determined in the case of $[Ru(NH_3)_6]^{2+/3+}$. The difference in ΔE_p indicates that the electron transfer rate between cyt *c* and Au electrode is distinct between them,[7] indicating that cyt *c* recognized the difference in the chirality of Co(III) complexes. From the values of $\Delta E_p(S) - \Delta E_p(R)$, it is found that the rate of electron transfer with **2***S*–Au is faster than **2***R*–Au. In our previous study,[8] we have reported in detail the electron transfer reaction between cyt *c* and similar Ru complexes with amino acid derivatives by using a stopped-flow method in homogeneous solution, and have obtained the interesting result that the second-order rate constant of (*S*)-configurated complex is larger than that of the (*R*)-configurated one. There was no difference in the second-order rate constants when the enantiomers bearing the sterically smaller side chain, the methyl residue, were used (See ESI† for details). These data show that the absolute configuration of phenylalanine has been recognized by cyt *c*.[8] Thus, similar molecular recognition occurs between **2**–Au and cyt *c*.

Both **3***S*–Au and **3***R*–Au, where the defect site of **2**–Au was complemented with hexanethiol, possessed the same redox behaviour as $[Ru(NH_3)_6]^{2+/3+}$, while **3**–Au caused cyt *c* recognition

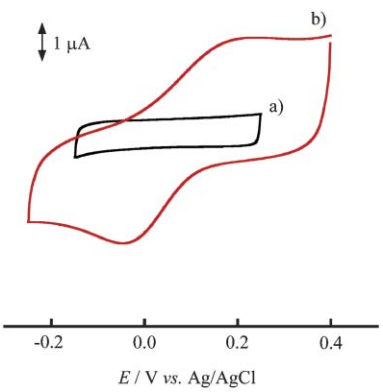

Fig. 1 Cyclic voltammograms in (a) buffer solution (black) and (b) cyt *c* solution (red), as measured with **2***S*–Au. Scan rate is 0.05 V s^{-1}.

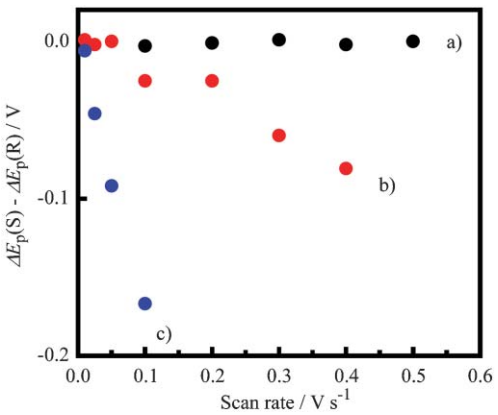

Fig. 2 The relationship between ($\Delta E_p(S) - \Delta E_p(R)$) and scan rate of cyclic voltammetry. (a) $[Ru(NH_3)_6]^{2+/3+}$ with **3**–Au (black), (b) cyt *c* with **2**–Au (red), (c) cyt *c* with **3**–Au (blue).

induced by the chirality of Co(III) complexes as well as in the case of **2**–Au. However, the difference in ΔE_p between **3***S*–Au and **3***R*–Au is larger than that of **2**–Au (Fig. 2). This result suggests the electron transfer rate between cyt *c* and **3**–Au was slower than that of **2**–Au (see ESI† for details). The slow rate in **3**–Au is explained as follows: **3**–Au has a more rigid structure as compared with that of **2**–Au. Hexanethiol on **3**–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 the 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 the Co(III) unit, that is, the absolute configuration of phenylalanine, affected the interaction with heme crevice of cyt *c*.

In summary, we demonstrated that the modified electrode 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 environmental structure of the redox center of cyt *c*. The metal complexes with amino acid derivative ligand are available as promoters, because the metal center and amino acid are changeable systematically. Thus, this type of electrode should be a powerful tool for investigating the electron transfer path of various electron transfer proteins.

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Notes and references

‡ Preparation of **2**. **1** (0.1 mmol), DTSP (0.05 mmol) and Et_3N (0.1 mmol) were stirred for 3 h in DMSO (20 cm^3). The resulting solution was evaporated completely. The residue was recrystallized from MeOH. Yield 46% (**2***S*) and 31% (**2***R*).

§ Selected data for **2**. UV-vis (H_2O) λ (ε): 372 (380), 509 nm (350 dm^3 mol^{-1} cm^{-1}); *m*/*z* (ESI-MS): $[M - 2K]^{2-}$ 569.0; **2***S*·$4H_2O$ anal. calc. for $C_{52}H_{56}Co_2N_6O_{22}K_2S_2$: C, 40.99; H, 5.00; N, 6.52. Found: C, 40.83; H, 5.14; N, 6.40. **2***R*·$5H_2O$ anal. calc. for $C_{54}H_{56}Co_2N_6O_{23}K_2S_2$: C, 40.43; H, 5.09; N, 6.43. Found: C, 40.34; H, 4.81; N, 6.34.

¶ All cyclic voltammograms were observed in 0.1 M phosphate buffer (pH 7.0) containing 0.1 M $NaClO_4$. Counter and reference electrodes were Pt coil and Ag/AgCl, respectively. The concentrations of complex **2**, $[Ru(NH_3)_6]$ and cyt *c* were 1 mM, 1 mM and 100 μM, respectively.

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