# VII-C Electrochemical Analysis of Biological Functions of Metalloproteins and Their Mutated Molecules and Its Applications to Coordination Chemistry for Catalysis

Using surface-functionalized electrodes, biological functions and bioelectrochemical properties of metalloproteins and their mutated and redox-center modified molecules have been analyzed electrochemically to develop new bioelectrocatalytic systems and bioelectro-functional devices.

#### VII-C-1 Simple Methods for Preparation of a Well-Defined 4-Pyridinethiol Modified Surface on Au(111) Electrodes for Cytochrome c Electrochemistry

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#### [Electrochim. Acta 45, 2843 (2000)]

A very small amount of sulfide impurity in 4pyridinethiol (4-PySH) modifier solution was found to interfere with the proper formation of the 4-PySH modified surface for cytochrome c electrochemistry on an Au(111) electrode. When the modification was conducted in an alkaline (e.g., 0.1 M KOH) solution, in aqueous solutions under applying a potential more positive than 0.3 V vs. Ag/AgCl, or at a low modifier concentration (e.g., 20 µM), the proper 4-PySH modified surface was obtained even using 4-PySH as received, which contained a small amount of sulfide. The selective adsorption of 4-PySH in the presence of a small amount of sulfide under these conditions was due to the rapid formation of proper 4-PySH modified surface, which prevented the sulfide from reacting with the electrode surface.

## VII-C-2 Voltammetric and In Situ STM Studies on Self-Assembled Monolayers of 4- and 2-Mercaptopyridines and Thiophenol on Au(111) Electrodes

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[Electrochim. Acta 45, 2861 (2000)]

Voltammetric and in situ STM studies were carried out for self-assembled monolayers of 4-mercaptopyridine (4-PySH), 2-mercaptopyridine (2-PySH) and thiophenol (PhSH) on well-defined single-crystal Au(111) electrodes in aqueous solutions. A reversible voltammetric response for cytochrome c was clearly observed only at the 4-PyS/Au(111) electrode, showing that only the 4-pyridinethiolate monolayer promotes facial electron transfer reaction between the Au(111)

and cytochrome c. On the basis of reductive desorption, the surface coverages of the three aromatic thiolate monolayers were found to be similar to each other; 4.6  $\times 10^{-10}$  mol/cm<sup>2</sup> for 4-PyS/Au(111), 4.7  $\times 10^{-10}$ mol/cm<sup>2</sup> for 2-PyS/Au(111), and  $4.4 \times 10^{-10}$  mol/cm<sup>2</sup> for PhS/Au(111). High-resolution STM images in perchloric acid solutions revealed p (5 ×  $\sqrt{3}R$ –30°) and  $p (4 \times \sqrt{7R-40.9^{\circ}})$  structures for the 4- and 2-pyridinethiolate monolayers on Au(111), respectively. No structure order was observed for the PhSH monolayers. While the pyridine units of both 4- and 2-pyridinethiolate monolayers were found to be oriented normal to the surface, 2-pyridinethiolates adsorbed through not only sulfur but also nitrogen atom of the pyridine ring. From these STM images, the orientation of the N atom of the pyridine moiety must face to the bulk solution, as in the case of 4-PyS/Au(111), in order to obtain a facile electrochemical reaction for cytochrome c.

# VII-C-3 Formation of the "Nanotube" Structure of $\beta$ -Cyclodextrin on Au(111) Surfaces Induced by Potential Controlled Adsorption

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[Colloids Surf., A 169, 27 (2000)]

The self-organization of  $\beta$ -cyclodextrin ( $\beta$ -CyD) into a nanotube structure, similar to that of CyDpolyrotaxane, was found to be induced by potential controlled adsorption on Au(111) surfaces in sodium perchlorate solution without a threaded polymer. In situ scanning tunneling microscopy (STM) revealed that the cavities of  $\beta$ -CyD faced side ward not upward in the tube. This ordered structure can form only under conditions where the potential is controlled (-0.45 V to -0.25 V vs. SCE).  $\beta$ -CyD molecules were in a disordered state on bare Au(111) surfaces without potential control (+0 V vs. SCE). In addition, the desorption of  $\beta$ -CyD from Au surfaces was observed at a negative potential of less than -0.60 V. In the range -0.45 to -0.25 V,  $\beta$ -CyD molecules formed ordered array on Au(111) surfaces. Furthermore, the discontinuity of potential control led to disordered phases and the destruction of the tube structure. This indicates that by controlling the electrode potential a delicate balance of various interactions can be achieved, resulting in the self-organization of molecules on the surface.

VII-C-4 Direct Observation of Perchlorate Induced by Redox Reaction of Ferrocene Terminated Self-Assembled Monolayer Studied by in situ FT-Surface Enhanced Raman Spectroscopy

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[Chem. Lett. 930 (2000)]

The incorporation of perchlorate anion into 8ferrocenyloctanethiol (8FT) self-assembled monolayer (SAM) induced by the redox reaction at a gold wire electrode was demonstrated using in situ FT Raman spectroelectrochemistry. Upon oxidation of 8FT SAM, a band attributed to perchlorate anion was clearly observed and the band disappeared in its reduced state. On the other hand, when nitric acid was used as a supporting electrolyte, the band attributed to nitrate anion was not observed in the oxidized form of 8FT.

## VII-C-5 Novel "Wet Process" Technique Based on Electrochemical Replacement for the Preparation of Fullerene Epitaxial Adlayers

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[*Langmuir* **17**, 5 (2001)]

The electrochemical replacement method to form epitaxial adlayers of fullerene on Au(111) surfaces was proposed and demonstrated by in situ electrochemical STM. The new "wet process" method consists of the transfer of Langmuir film of fullerene onto iodinemodified Au(111) surfaces at an air-water interface followed by the electrochemical removal and replacement of iodine adlayers with fullerene adlayers in solution. The fullerene adlayers prepared by this method showed excellent quality and uniformity, and they were essentially the same as epitaxial adlayers prepared by sublimation.

# VII-C-6 Electrochemical and Spectroelectrochemical Studies on Cobalt Myoglobin

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[Electrochim. Acta 45, 2883 (2000)]

The cobalt protoporphyrin-IX reconstituted myoglobin (Co-Mb) was prepared and its electrochemical and spectroelectrochemical properties were studied in comparison with native myoglobin. Unlike native myoglobin, a slow electron transfer reaction for Co(III)-Mb/Co(II)-Mb was detected by cyclic voltammogram and spectroelectrochemical methods. The chemical reduction rate of Co(III)-Mb with dithionite was also slow compared with that of native myoglobin. Direct electron transfer between Co-Mb and an In<sub>2</sub>O<sub>3</sub> electrode was observed, with spectroscopic verification of different redox states of Co-Mb using an optically transparent thin layer electrode (OTTLE) cell. The reversible electrochemical redox reaction of Co(III)-Mb/Co(II)-Mb was observed using azure A, 3-amino-7-dimethylaminophenaza-thionium chloride, as an electron transfer mediator for the first time. The Nernst plot was obtained for Co-Mb with E<sup>0</sup>, of -100 mV vs. Ag|AgCl (sat. KCl) and n = 1 at 25 °C.

#### VII-C-7 Effect of Rapid Heme Rotation on Electrochemistry of Myoglobin

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[*Electrochim. Acta* **45**(**18**), 2903 (2000)]

Myoglobins (Mbs) reconstituted with rotatable octamethylheme and non-rotatable etioheme were prepared and their electrochemical behavior was studied. The redox potential of octamethylheme reconstituted Mb (OMe-Mb), of which heme rotates around iron-histidine (F8-His) bond, shifted negatively by ca. 30 mV compared with non-rotatable etioheme reconstituted Mb (Etio-Mb). On the other hand, the redox potentials of octamethylheme and etioheme themselves were very similar to each other. Due to the similarity of the distal histidine side of the heme of these two reconstituted Mbs, the shifts of the redox potential would be attributable to the drastic change of the orientation of proximal histidine imidazole ring to the heme plane by heme rotation. The dissociation rate constant of cyanide ion from the ferrous heme iron (II) for OMe-Mb form at 5 °C and pH 7.5 was three times faster than that of Etio-Mb. The electron transfer kinetics of these Mbs showed that the heme rotation causes faster electron transfer rates in both electrode reaction and chemical reduction in solution with dithionite. The obtained heterogeneous electron transfer rates constants at an In<sub>2</sub>O<sub>3</sub> electrode and first-order rate constants of the chemical reduction were 12 ( $\pm$  0.5)  $\times$  $10^{-4}$  cm s<sup>-1</sup>, 9.8 (± 1.0) s<sup>-1</sup> for OMe-Mb and 6.0 (± 0.5) ×  $10^{-4}$  cm s<sup>-1</sup>, 4.5 (± 1.0) s<sup>-1</sup> for Etio-Mb under the present experimental conditions.