CHEM. RES. CHINESE U.
2007, 23(3), 339—342
Article ID 1005-9040(2007)-03-339-04

Interaction of Biologically Active Molecules with Sulfur-modified Gold Surface*

DING Xue-feng[1], YANG Gui-fu[2], WANG Xiao[3], WANG Zi-chen[1] and LIN Hai-bo[1]**

1. *College of Chemistry and Key Laboratory of Surface & Interface Chemistry of Jilin Province, Jilin University, Changchun* 130021, *P. R. China*;
2. *Department of Computer, Northeast Normal University, Changchun* 130024, *P. R. China*;
3. *Jilin Province Product Quality Supervision Test Institute, Changchun* 130012, *P. R. China*

Received Sep. 5, 2006

The immobilization of cytochrome c or horseradish peroxidase at the sulfur-modified gold electrode exhibits a rapid electron transfer behavior because of its specific orientation on the electrode surface and the interaction between cytochrome c or horseradish peroxidase and sulfur-modified on the surface of the Au electrode.

Keywords Sulfur-modified electrode; Electron transfer; Bioactive molecules

Introduction

Sulfur adsorption and its effects on metal surface have been extensively reported[1—3]. From these reports, it has been concluded that the sulfur, which is covalently adsorbed onto the transition metal surface, is ordered and negatively charged. The results from the core-level electron-energy-loss spectroscopy suggest that sulfur adatoms retain some of the negative charges. The *ab initio* SCF calculation also shows the same result[2]. Therefore, the modified surface can easily immobilize some positively charged molecules or positively-charged moiety of dipole molecules *via* the electrostatic interaction.

The immobilization of biological molecules is highly beneficial to understand the protein interaction in a biological system. Of all the biological molecules studied, redox active cytochrome c[4—10] and horseradish peroxidase[11—16] have been the focus of research interests. The asymmetric distribution of the uncompensated lysine residues of cytochrome c results in large dipole moment, and the end near the exposed heme edge possesses positive charges[11]. Similarly, horseradish peroxidase holds a net charge of +5 in the middle third residues 100—215 along the peptide chain[12]. These positive charges easily facilitate their adsorption onto the negatively charged sulfur surface *via* the electrostatic interaction. Peripheral peptide chain around the heme kernel contains several amino residues and lead to multiple interactions between the target biomolecules and sulfur adatoms *via* N—H···S hydrogen bonds and specific N···S bond. These factors effectively promote the immobilization of such biological molecules on sulfur-modified metal surface and thereby, a stable and oriented monolayer modification is obtained.

This article deals with the immobilization of cytochrome c and horseradish peroxidase at sulfur-modified gold electrode. Such modification exhibites excellent ordering and rapid electron transfer. Results from the photoelectron spectroscopy confirm the specific coordination interaction between the modified biomolecules and the electrode substrate.

Experimental

Horse heart ferricytochrome c (Type Ⅵ) and horseradish peroxidase(HRP, 90 units/mg) were purchased from Sigma Co. and used without further purification. Cyclic voltammetric measurement and chronoamperometric measurement were carried out in a conventional three-electrode cell. The Au electrode(1 mm in diameter) was used as the substrate of the working electrode, which was polished with 1.0, 0.3, and 0.05 mm α-Al_2O_3 powders, respectively. After washing, it was scanned in 1.0 mol/L H_2SO_4 solution until a stable cyclic voltammogram was obtained. A Pt wire was used as the counter electrode and an Ag|AgCl(saturated KCl) electrode was used as the reference electrode.

All the potentials were measured with respect to the reference electrode. The electrochemical area of the gold disk electrode was determined using the method described by Rodriguez *et al.*[17] *via* the chemisorption

* Supported by the National Natural Science Foundation of China (No. 20573045) and the "863" Program of China (No. 2006AA06Z321).

** To whom correspondence should be addressed. Email: lhb910@jlu.edu.cn

of iodine.

The clean gold electrode was immersed for 20 min in a solution of 0.1 mmol/L Na_2S adjusted to pH = 11 using 10 mmol/L NaOH. The electrode was subsequently rinsed several times with distilled water. This sulfur-modified electrode was immersed in cytochrome c or horseradish peroxidase aqueous solution for a certain time. Finally, the resulting electrode was thoroughly rinsed with distilled water.

Results and Discussion

Sulfur on the gold surface is very stable, which is similar to that in the thiols/Au chemical adsorption, and an irreversible anodic process will take place in the potential range studied. Therefore, the periodic voltage excursion in 1 mol/L H_2SO_4 can be applied for the determination of the coverage of sulfur *via* the measurements of charge consumed in the oxidation of adsorbed sulfur[1]. Assuming that the hexagonally close-packed Au(111) was used as the substrate, a ratio of 2.7:1 of atom numbers between the surface of Au and sulfur can be obtained. Such a sulfur packing density is consistent to $(\sqrt{3} \times \sqrt{3})R30^0 - S$ pattern, as shown in literature[3]. It indicates that an ordered and compactly packed monolayer has been formed, and the sulfur adlayer is very important to prevent the adsorbed proteins from denaturation.

Fig. 1(A) shows the cyclic voltammograms of cytochrome c immobilized at the sulfur-modified gold electrode after adsorption for a short period of time(*ca.* 30 min) in phosphate buffer solution(pH = 7.02) containing high concentration of cytochrome c(1 mg/mL). A pair of redox peaks was obtained as those in the previous reports[4—10]. At a scan rate of 20 mV/s, E_{pa} = +115 mV, E_{pc} = +55 mV, and ΔE_p = 60 mV were obtained. The formal potential of +85 mV is similar to the result of +62 mV measured in solution by the spectroscopic measurement[18] as well as to those observed at other chemically modified electrodes. The linear relationship between the peak current and the scan rate confirmed the occurance of surface wave process[shown as inset in Fig. 1(A)]. The surface coverage of the adsorbed cytochrome c on S/Au electrode is on the order of a monolayer/submonolayer, with an average value of 1.01×10^{-12} mol/cm^2.

The apparent electron transfer(ET) rate constant of the cytochrome c/S/Au surface was calculated using Laviron's model[19] by the variation of peak separation with scan rate. In the region of scan rates studied, a rapid electron transfer rate was obtained (k_{et}° = 1.56

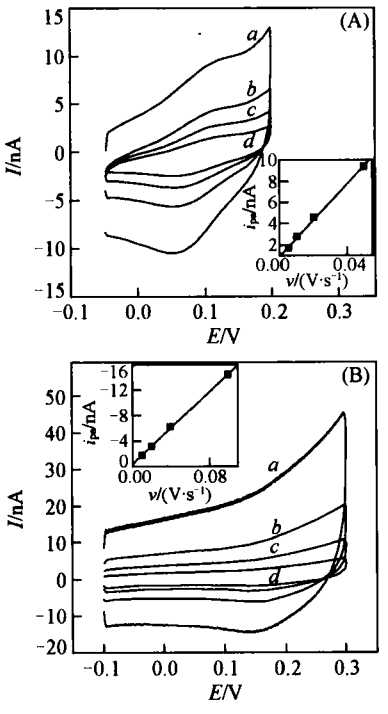

Fig. 1 Cyclic voltammograms at the S/Au electrode modified with cytochrome c in 1.0 mg/mL solution for 30 min(A) and in 10 mg/L solution for 20 h(B)

Scan rate/(mV · s^{-1}): *a*. 50; *b*. 20; *c*. 10; *d*. 5. Inset: plot of anodic peak current *vs*. scan rates.

s^{-1}), which was a fast redox process in comparison with those of other biomacromolecules such as proteins and enzymes on the electrode surface. This value is 10—100 times more rapid than k_{et}° for other cytochrome c/self-assembled monolayer systems[10,20,21]. The fast electron transfer process is because of the advantages of immobilization on sulfur-modified substrate. The immobilization, based on the atom-modified technique, is beneficial for proteins to obtain a favorable orientation, and the small effective electron transfer distance also facilitates the electron transfer.

Furthermore, under the conditions of dilute cytochrome c(10 mg/L) solution and a longer adsorption time(>20 h), the immobilized cytochrome c is irreversibly reduced. The anodic current just appears from *ca.* 0.25 V[Fig. 1(B)]. The slow anodic electron transfer exhibits electron transfer process in single-direction, *i. e.*, it is easy to be reduced and difficult to be oxidized as in the case of biological system[22,23]. After rinsing with buffer followed by potential cycling in buffer several times, almost identical current-voltage traces are resulted, demonstrating that the desorption is negligible.

For further characterizing the bioactivity of the immobilized biomacromolecules, an easy template with