Self-Assembled Monolayer-Based Electrodes for Selective Determination of Cu^{2+} and Ag^+ Ions with Antifouling Activity against Protein Adsorption

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Self-assembled monolayers consisting of 3,3'-thiodipropionic acid and *n*-decyl mercaptan formed on gold electrodes have been studied for sensitive and selective determination of Cu^{2+} and Ag^+ ions. The monolayers were found to provide both superior sensitivities and stabilities even in the presence of protein. Various factors, such as solution pH and concentration time, were optimized to obtain detection limits of 2×10^{-9} M and 6×10^{-8} M for Cu^{2+} and Ag^+ ions at an *S/N* ratio of 2 by linear-sweep anodic stripping voltammetry. In the presence of albumin, the currents of the modified electrode, 1.75 times as great as those for the bare electrode, show a significant performance improvement over the previously reported cellulose coated electrode (1.13 times). One of the main advantages of using this electrode is an excellent antifouling activity against protein adsorption, coupled with highly selective metal-ion suppression/concentration capabilities, which can be attained without using a mercury film for preconcentration.

Keywords Self-assembled electrode, heavy metal ion determination, protein adsorption, antifouling activity

The advent of self-assembled monolayers formed by organosulfur compounds on gold and other substrates is opening the possibility of designing the molecular architecture of the electrode-solution interface. One of the primary goals of this research area is to control the reactivity of the electrode so that it becomes selectively responsive to a given species.¹⁻⁷ For example, Rubinstein *et al.* demonstrated that functionalized self-assembled monolayers consisting of 2,2'-thiobisethyl acetoacetate and *n*-octadecyl mercaptan recognized selectively Cu^{2+} ions in the presence of Fe^{3+} .^{8,9} Turyan *et al.* prepared gold electrodes modified with a $\text{HSC}_2\text{H}_4\text{COOH}$ monolayer and with a 4-(mercapto-*n*-alkyl) pyridinium monolayer to concentrate Cd^{2+} and Cr^{5+} from aqueous solutions.^{10,11} It has been suggested that the ionic recognition is based upon selective binding of ions to the ligand molecules in the monolayer membranes with appropriate coordination and geometric requirements being satisfied. However, more fundamental studies from analytical aspects seem to be necessary before applying these ligand monolayers to real sample analyses. We report here some studies to enhance the resolution of anodic stripping voltammetry by using the selectivity of 3,3'-thiodipropionic acid to metal ion as a surface ligand.

These stable organic monolayers could also be useful to block electrode contamination from undesired macromolecule adsorption during analysis. Gold electrodes are very susceptible to poisoning caused by sample contaminants as well as by products of electro-

chemical reactions. This often happens in the determination of biological samples, such as serum, plasma, urine, containing a variety of proteins and other components. Consequently, this kind of strong adsorption attenuates electrode responses and the initial activity and reliability are lost in a short time. In the present work, we have prepared highly selective and sensitive self-assembled electrodes suitable for determination of heavy metal ions; the performance was evaluated in the presence of albumin.

Experimental

Chemicals

Reagent grade 3,3'-thiodipropionic acid [$\text{S}(\text{CH}_2\text{CH}_2\text{COOH})_2$] was purchased from TCI Chemicals. Bovine serum albumin (BSA; assay, minimum 95%), reagent grade *n*-decyl mercaptan, and methanol were supplied from Wako Chemicals. All the metal salts used here were of reagent grade. Milli-Q water was used throughout.

Apparatus and procedures

The monolayers were prepared on gold electrodes (1.6 mm in diameter, BAS) by the following procedures: First, the electrodes were polished with 0.3 μm α -alumina, washed with water and immersed in HCl-HNO_3 aqueous solutions (weight ratio, $\text{HCl:HNO}_3:\text{H}_2\text{O}=3:1:16$) for about 1 min. Afterwards, the electrodes were immersed in methanol solutions containing both 20 mM 3,3'-thiodipropionic acid and

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Fig. 1 IR spectra of (A) surface thiodipropionic acid measured by reflective absorption spectroscopy and of (B) the authentic sample diluted with KBr.

20 mM *n*-decyl mercaptan for 24 h to modify the surfaces by the self-assembly process.

All the electrochemical measurements were referred to an Ag|AgCl|saturated KCl|| electrode at ambient temperature ($25 \pm 1^\circ\text{C}$). Cyclic voltammograms of the metal ions were recorded in 0.10 M KCl or 0.10 M NaNO_3 with a data-acquisition system constructed in our laboratory. The concentration of Cu^{2+} ion was determined by anodic stripping voltammetry (ASV) by the following procedures. The modified electrode was inserted in a 0.10 M KCl solution containing Cu^{2+} ion, which was concentrated on the electrode at -0.3 V for 10 min. The copper was then stripped anodically in the other 0.10 M KCl solution, the pH of which was adjusted to 2.0 or 3.0 with HCl. In the presence of BSA, the concentration and stripping procedures were conducted in the same solution.

Reflective-absorption IR spectroscopy was used to confirm the presence of the monolayer on the gold surface; detailed experimental procedures have been published elsewhere.¹² Scanning tunneling microscope (STM) images were obtained with a Nanoscope II microscope (Digital Instruments). Quartz crystal microbalance (QCM) experiments were conducted with a SEIKO Model 917 Quartz Crystal Analyzer by using a gold-plated 9 MHz AT-cut crystal oscillator (nominal surface area, 0.20 cm^2).

Results and Discussion

Basic characteristics of the modified electrode

Impermeable one-molecule-thick barriers for ions and

Fig. 2 Cyclic voltammograms of (a, b) 3.0 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and (c, d) 3.0 mM CuSO_4 in 0.10 M KCl aqueous solutions at a scan rate of 0.10 V s^{-1} . Curves a and c are for the bare electrode, and curves b and d are for the electrode modified with thiodipropionic and *n*-decyl mercaptan.

molecules have been produced on gold substrates by the monolayer self-assembly techniques.^{8,9} The sulfur bridge used here for the selective determination of Cu^{2+} and Ag^+ was composed of 3,3'-thiodipropionic acid, in which two carboxylic acids work as a didentate chelating center. To confirm the immobilization, surface reflective IR spectra were measured for the monolayer (Fig. 1). The carbonyl stretching vibrations, which appeared at 1690 and 1681 cm^{-1} for the modified surface, agreed well with those found for an authentic sample (1724 and 1698 cm^{-1}) as well as the other spectral features, demonstrating that the ligand molecules were immobilized on the surface.

Figure 2 illustrates the cyclic voltammetric responses of $\text{K}_3\text{Fe}(\text{CN})_6$ and CuSO_4 at the bare and modified gold electrodes. Well-defined redox peaks for the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and $\text{Cu}^{2+}/\text{Cu}^0$ couples were found at the bare electrodes (curves a and c), while the two metal ions behaved differently at the modified electrode (curve b and d). Here, Cu^{2+} ions were allowed to penetrate into the monolayer, followed by electron transfer with the underlying electrode, although $\text{Fe}(\text{CN})_6^{3-}$ remained in the bulk solution because of denied access to the monolayer channels. Similarly, the voltammetric peaks for Fe^{3+} , Fe^{2+} and Co^{2+} were not detected for the modified electrode, while Cu^{2+} , Ag^+ , Cd^{2+} and Pb^{2+} ions showed well-defined peaks (Table 1). It has been suggested that metal ions that are capable of forming 1:1 complexes with the ligand can enter the barrier channel, although the other metal ions that require the octahedral coordination on the complex formation cannot find a suitable position in the monolayer.^{7,8} The pH

Fig. 3 The pH dependence of the barrier energy for reduction of 3.0 mM Cu^{2+} at the modified electrode observed in 0.10 M KCl at 0.10 V s^{-1} . The pH was controlled with HCl.

Fig. 4 Anodic stripping voltammetry of Cu^{2+} at the modified electrode at 80 mV s^{-1} after preconcentration at 0.3 V for 10 min. Inset: the corresponding calibration curve.

Table 1 Electrochemical characteristics for reduction of metal ions in 0.10 M KCl at bare and modified gold electrodes

Metal ion ^a	Bare electrode		Modified electrode	
	$E_{\text{pc}}/\text{V}^{\text{b}}$	$I_{\text{pc}}/\mu\text{A mM}^{-1}\text{c}$	E_{pc}/V	$I_{\text{pc}}/\mu\text{A mM}^{-1}$
$\text{Fe}(\text{CN})_6^{3-}$	0.13	3.3	N.D. ^b	N.D. ^d
Fe^{3+}	0.48	4.0	N.D.	N.D.
Co^{2+}	-0.63	0.36	N.D.	N.D.
Cu^{2+}	-0.18	6.7	-0.28	8.2
Pb^{2+}	-0.5	10	-0.52	16
Cd^{2+}	-0.81	10	-0.87	11
Ag^{e}	0.34	7.0	0.32	11

a. Concentration, 2–3 mM.

b. Peak potential.

c. Normalized peak current.

d. Not detected, less than 10 nA.

e. In 0.1 M LiNO_3 .

dependence of the reduction current for Cu^{2+} ion (Fig. 3) suggests the presence of the competitive equilibria between two Lewis acids, *i.e.* the complex formation of the ligand with Cu^{2+} ion and protonation of the ligand on the gold surface. The complex formation constants of 2,2'-thiodiglycolic acid [$\text{S}(\text{CH}_2\text{COOH})_2$] with Cu^{2+} and Pb^{2+} have been reported to be $10^{4.3}$ and $10^{3.6,13}$.

As is seen in Fig. 3, the penetration of Cu^{2+} through the membrane at low pH values is not energetically favored because of protonation of the acid anion. The direction of the peak shifts in Fig. 3 is opposite to what the voltammetric theory, based on the complex formation between a metal ion and ligand, predicts. The shifts in the peak potential can therefore be attributed to the reaction sites becoming farther away from the Au surface as the pH is decreased.

Table 2 Effects of matrix constituents for the concentration of Cu^{2+} ($n=5$) and Ag^+ ($n=1$) for the modified electrodes

Analyte	Matrix ^a	Stripping current/ μA	Relative error, %
40 nM Cu^{2+}	—	5.1 ± 0.1	—
	40 μM Fe^{3+}	5.2 ± 0.1	1.6
	40 μM Pb^{2+}	5.2 ± 0.04	1.2
	40 μM Ni^{2+}	5.2 ± 0.1	1.7
100 nM Ag^+	—	1.4	—
	1.0 mM Fe^{3+}	1.3	-7.4

a. The base solution contained 0.10 M KCl (or 0.1 M NaNO_3 for Ag^+) and HCl (pH 2.0).

Application to ASV

Figure 4 illustrates the results of the ASV experiments at the modified electrode, where the Cu^{2+} ion, deposited at pH 5.5 for 10 min at -0.30 V, was stripped at pH 2.0. Here, the stripping was conducted at smaller pH, because of slightly sharper and greater stripping peaks recorded in acidic solutions (1.6 μA at pH 2.0 and 1.2 μA at pH 5.5). The calibration curve in the inset shows that the current was dependent almost linearly on the Cu^{2+} concentration in a range of 1×10^{-8} to 6×10^{-8} M, and a detection limit of 2×10^{-9} M was estimated for an S/N ratio of 2 under the linear-sweep ASV condition. We also have studied the interference from other metal ions for the determination of Cu^{2+} . As is listed in Table 2, a 1000-fold excess of Fe^{3+} , Pb^{2+} and Ni^{2+} did not interfere with the determination to show that the monolayer behaves as a highly selective barrier for these metal ions. Generally, Fe^{3+} ion interferes strongly with the voltammetric determination of Ag^+ ion because the redox potentials are close to each other. However, upon using the modified electrode, 1 mM

Fig. 5 STM images of the bare gold surfaces with and without 0.2% BSA treatment for 10 min. (A and C) before the treatment, and (B and D) after the treatment. (A) and (B), top view; (C) and (D) three-dimensional view.

Fe^{3+} did not interfere with the determination of 1×10^{-7} M Ag^+ , and the detection limit of Ag^+ was estimated to be 6×10^{-8} M. Excellent stability and reproducibility of the modified electrode have been found for the determination of Cu^{2+} ; variations of the stripping current on measurements of 10 freshly prepared electrodes were less than $\pm 2\%$, and decrease in the stripping current for each electrode was not appreciable ($< 2\%$) during 10 consecutive stripping experiments over 2 d.

Protein adsorption and voltammetric behavior

Voltammetric determination of metal ions in biological matrixes has been a difficult analytical task, because adsorption of proteins and other biologically related substances fouls the electrode. To overcome this problem, several types of surface-coating techniques have

been presented.¹⁴⁻²³ Wilson *et al.* reported that platinum electrodes modified with cellulose acetate enhanced the current stability for the determination of hydrogen peroxide in the presence of BSA, although the currents observed at the modified electrodes decreased by about 50% because of a small transport rate of hydrogen peroxide through the cellulose films.¹⁴ Nafion coated thin mercury film electrodes also exhibit excellent resistance against surface-active macromolecule adsorption.^{17,18} Whitesides *et al.* demonstrated that self-assembled monolayers consisting of $\text{HSCH}_2(\text{OCH}_2\text{CH}_2)_n\text{OH}$ resist protein adsorption, suggesting that surface hydrophilicity is an important factor for the prevention of protein adsorption.^{20,21} Oxidative coating of glassy carbon electrodes with oligomers of ethylene glycol has also been studied

Fig. 6 STM images of the modified electrode treated (B) with and (A) without 0.2% BSA for 10 min.

based on a similar idea.²² A different way to circumvent the problem has recently been presented by using microporous overoxidized polypyrrole films.²³

Figures 5 and 6 show the STM images observed for gold plates. The images obtained for the bare surface (Fig. 5, A and C) were featureless in the absence of BSA, while changing dramatically after immersion in a BSA solution (B and D). Macroscopic adhesion of protein piling up on the gold surface is clearly seen in B and D, and only the limited areas (black images in B) seem to be available for the metal ion reduction. In contrast, no detectable changes have been observed for the modified gold surfaces after treatment with BSA (Fig. 6). Figure 7 shows mass-related frequency changes for the bare and modified Au-coated quartz microbalances on the BSA addition. It can be seen from the figure that the adsorption became almost steady 400 s after the BSA additions and that the surface modification prevented as much as 90% of protein adsorption. The corresponding mass changes were estimated to be *ca.* 500 ng/cm² and 50 ng/cm² for the bare and modified electrodes.

To see if the modified electrode retains sufficient electroactivity after the adsorption, ASV experiments have been carried out consecutively in the presence of BSA. Here, the bare and modified electrodes were immersed into a Cu²⁺ solution containing 0.2% BSA for the times given in Table 3 before the concentration and then subjected to the stripping analyses in the same solution. The procedures were repeated for a total of 90 min of the immersion time (see footnote b for details). As a result, the modified electrode demonstrated a marked improvement in the current stability, while the unmodified electrode showed wide variations in the current from one measurement to another. The large variations for the unmodified electrode could be assigned to the reorientation and displacement of the adsorbed protein molecules upon depositing and stripping of Cu²⁺. Presumably, less such reorganization

took place on the modified electrode, where the redox perturbation occurred well under the adsorption sites of protein.

Although BSA adsorption on the modified surface was not seen clearly in the STM images, about 80% decreases in the stripping current on adding BSA (Table 3) indicated that the protein blocked up most of the Cu²⁺ channels; this was also suggested by the substantial mass increase in the QCM measurement. However, the steady state in the mass and the stable stripping currents indicate that the surface was not fouled progressively after the initial adsorption had occurred. It should be noted here that the currents for the modified electrode, which were 1.75 times as great as those for the bare electrode, show a significant performance improvement over the cellulose film (1.13 times, *ref.* 14). The peak potentials for the copper stripping at the modified electrode, observed at 0.07 V and 0.05 V in the solutions with and without BSA (estimated accuracy, ± 20 mV), show that the IR drop across the membrane arising from the adsorbed protein is negligible.

In conclusion, our modified electrode can concentrate heavy metal ions in a highly selective manner, featuring an excellent antifouling activity against protein adsorption. Further, different from most polymer-coated glassy carbon electrodes, our ASV procedures do not require any mercury film pre-deposition before concentration of metal ions, so the current environmental waste-material regulations will cause less inconvenience.

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Fig. 7 QCM frequency changes for the (a) bare and (b) modified electrodes on the BSA additions. The arrow mark indicates the time for the BSA spikes into 0.1 M KCl solutions. BSA concentration, 0.2%.

Table 3 Improved reliability for the modified electrode in consecutive ASV determinations of 10 μM Cu^{2+} in the presence of 0.2% BSA^a

No.	Standing time/min ^b	Normalized stripping current ^c	
		Modified electrode	Unmodified electrode
0 ^d	—	1.23	(1)
1	10	0.25 ₆	0.18
2	25	0.25 ₆	0.14
3	40	0.26 ₃	0.068
4	60	0.26 ₁	0.19
5	75	0.26 ₁	0.11
6	90	0.28 ₁	0.21
Average ^e		0.26 ₃ ±0.009	0.15±0.05

a. The stripping currents were monitored after 10-min concentrations of Cu^{2+} in a KCl (0.10 M) – HCl (pH 3.0) aqueous solution at –0.3 V.

b. The total time for which the electrodes were immersed in the BSA solutions; after the given time, Cu^{2+} was concentrated for 10 min, and current recording was then carried out in about 5 min, followed by the next standing-concentration-stripping cycle.

c. Normalized to the stripping current at the bare electrode for data No. 0.

d. No BSA was added.

e. Averages and standard deviations of the currents for the data 1 through 6.

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