Self-Assembled Monolayer of Ni(II)-Macrocyclic Complex Containing Thiophene for the Determination of Homocysteine

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Received January 20, 2006

Key Words : Homocysteine, Ni(II)-macrocyclic complex, Thiophene, SAM

Homocysteine is an intermediate formed during the metabolism of methionine to cysteine, and high levels of blood plasma homocysteine are considered to be a risk factor for heart attack and stroke. It exists in either its reduced (homocysteine, RSH) or oxidized form (homocystine, RSSR), and the oxidized form the major one in human blood plasma.[1,2] Since it contains sulfur in its structure, it was also used to modify the electrode surface for the electrocatalytic detection of another compound, such as catecholamine.[3]

Homocysteine is usually determined by using fluorescence,[4] UV/Vis[5] and electrochemical[6] detection methods. Spectroscopic detection requires derivatization of homocysteine, and electrochemical detection needs electrodes that enable continuous measurements. However, electrochemical detection has a representative advantage, which is to be able to detect homocysteine without any additional derivatization, if electrodes are free from adsorption. Modified electrodes can solve the problem caused by adsorption and then have high reproducibility for repetitive measurements. A self-assembly technique for electrode modification is simple and has diverse materials for modification. A number of organic compounds containing the thiol group can readily adsorb on the electrode surface, such as gold, and form a self-assembled monolayer (SAM).

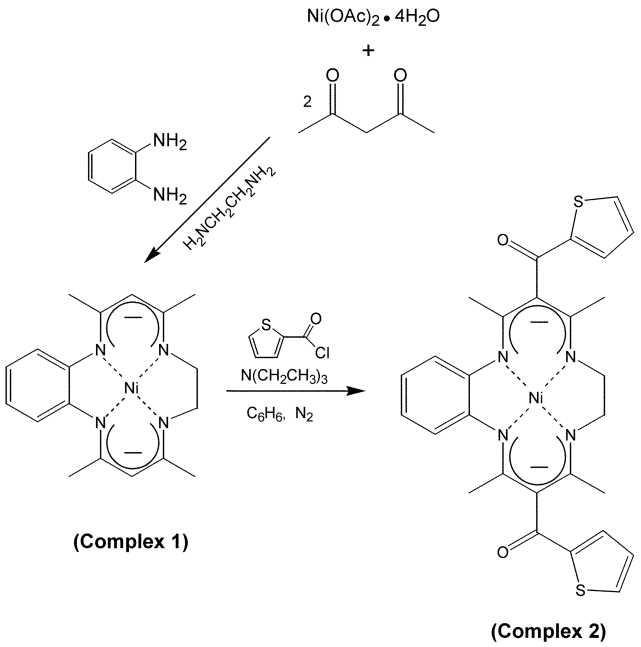
Macrocyclic complexes with an electrocatalytic effect can be used to determine homocysteine for higher sensitivity and reproducibility, if they can form a SAM on the electrode surface. Previous studies have shown that compounds with sulfur, such as thiophene and its derivatives,[7] and disulfide compounds of 6-mercaptopurine[8] and mercaptobenzimidazole,[9] formed a stable SAM. Although it may be difficult to synthesize macrocyclic complexes containing sulfur, using these materials can extend the fields for the determination of homocysteine.

In this work, Ni(II)-macrocyclic complex with thiophene groups (NiMCT) was synthesized and used to form a SAM on a gold electrode surface. A gold electrode modified with the SAM of NiMCT (NiMCT SAM) was characterized spectroscopically and electrochemically. It was used to determine homocysteine by differential pulse voltammetry and its reproducibility was examined for repetitive measurements. The oxidation process of homocysteine at the NiMCT-SAM modified electrode is discussed.

Experimental Section

Ni(II)-macrocyclic complex containing thiophene derivative (3,10-dithiophenecarbonyl-2,4,9,11-tetramethyl-1,5,8,12-monobenzotetraazacyclo[14]annulene nickel(II)) was newly synthesized, purified and characterized by spectroscopic methods. A Ni(II)-macrocyclic complex without the thiophene derivative was synthesized as described previously,[10,11] and the synthesized complex and thiophenyl chloride were dissolved in benzene and refluxed for 24 hours under a nitrogen atmosphere. The structure and synthetic procedure of NiMCT containing thiophene are shown in Scheme 1.

Homocysteine was purchased from Sigma-Aldrich and was used as received. A stock solution of homocysteine was prepared in a 0.1 M phosphate buffer solution, pH 7.0, and was diluted as necessary before use. The other chemicals were of guaranteed grade quality. All aqueous solutions were prepared with deionized water using the Milli-Q water



Scheme 1. Synthetic procedure of Ni(II)-macrocyclic complex containing thiophene derivatives (3,10-dithiophenecarbonyl-2,4,9,11-tetramethyl-1,5,8,12-monobenzotetraazacyclo[14]annulene nickel(II)).

system (Millipore Co, Bedford, MA, USA).

To prepare a NiMCT-SAM modified electrode, gold electrode surfaces were polished with aqueous slurries of alumina of 1.0, 0.3, and 0.05 μm. After rinsing the electrodes thoroughly with water and chloroform, they were sonicated in water for 10 min each. The polished electrodes were activated in 0.5 M H_2SO_4 by cycling the potential 20 times between –0.2 V and +1.5 V versus a saturated calomel electrode (SCE). Gold electrodes modified with the SAM of NiMCT were prepared by dipping the activated gold surface in a 1.0 mM NiMCT solution in chloroform for a day. The electrodes were removed and dried at room temperature. The gold surface coated with the NiMCT SAM was washed with chloroform and then used to make measurements.

All electrochemical measurements were carried out at 25.0 °C using a three-electrode system with a BAS 100W electrochemical analyzer (Bioanalytical Systems, USA). The working electrodes were a bare gold electrode (i.d. 1.6 mm, BAS MF-2012) and a gold electrode modified with the NiMCT SAM. SCE and Pt wire served as the reference and auxiliary electrodes, respectively.

Results and Discussion

Formation of the SAM of NiMCT can be confirmed voltammetrically using 1.0 mM potassium ferricyanide in 0.1 M KCl, and the results are shown in Figure 1. A bare gold electrode had well-defined redox waves in a cyclic voltammogram of potassium ferricyanide, but the waves disappeared at a NiMCT-SAM modified electrode. This indicates that the SAM of NiMCT was formed on a gold electrode surface and that it blocked the diffusion of ferricyanide ion to the gold electrode surface.

The surface of the NiMCT-SAM modified electrode was analyzed by atomic force microscopy (AFM) and scanning

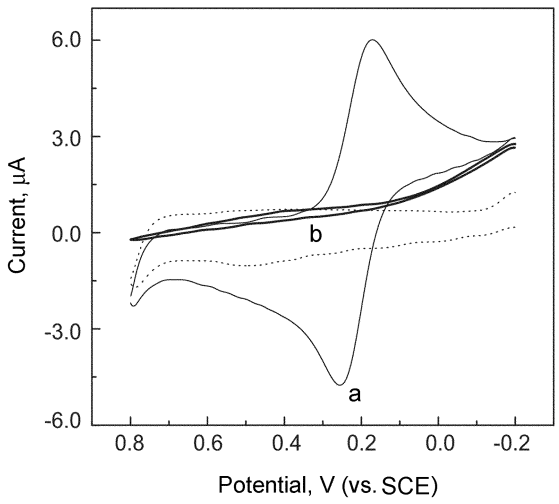
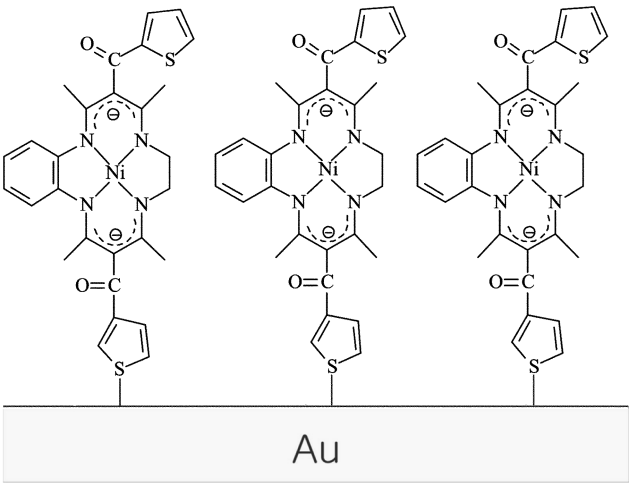


Figure 1. Cyclic voltammograms of 1.0 mM potassium ferricyanide in 0.10 M KCl using (a) bare gold and (b) NiMC-SAM modified electrodes at 25.0 °C and a scan rate of 0.1 V/s. Dotted lines represent a blank solution obtained at the NiMC-SAM electrode.



Scheme 2. The formation of a Ni(II)-macrocyclic complex self-assembled monolayer on the gold electrode surface.

tunneling microscopy (STM). The AFM image of the NiMCT SAM differed from that of bare gold surface, although the images are not shown here. It had a rougher structure with an average height of *ca.* 0.4 nm. The STM image showed that the electrode surface had a regular arrangement, indicating that NiMCT molecules formed a highly ordered layer on the gold surface. The proposed SAM of NiMCT formed on the gold surface is shown in Scheme 2.

To estimate the surface coverage of an electroactive species on a NiMCT-SAM modified electrode, a cyclic voltammogram was obtained in 0.10 M KOH at 0.1 V/s. A reduction wave observed at −0.35 V was caused by desorption of the NiMCT SAM bound to the gold surface. Thus, the surface coverage of the electroactive NiMCT, Γ, can be estimated using the voltammogram and Eq. (1).

$$\Gamma = Q/nFA \quad (1)$$

where Q is the electric charge, n the electron number, F the Faraday constant, and A the electrode surface area. The resulting estimated surface coverage was 4.5×10^{-10} mol/cm^2. The NiMCT SAM has a higher coverage than a derivative of metallophthalocyanine containing eight sulfur moieties.[12] This means that NiMCT containing two sulfur moieties formed a SAM with the orientation connected to the electrode through one sulfur moiety, as shown in Scheme 2.

Homocysteine was oxidized using a NiMCT-SAM modified electrode to evaluate its electrocatalytic effect, and the cyclic voltammogram is shown in Figure 2. The NiMCT-SAM modified electrode had two clear oxidation peaks at 0.60 V and 0.91 V, which were confirmed by the increase in the concentration of homocysteine. In contrast, a bare gold electrode had an unclear oxidation wave. The current at 0.60 V increased by 23 times after modification. Therefore, the NiMCT-SAM modified electrode enhances electron transfer for an oxidation reaction of homocysteine, indicating that it has an electrocatalytic effect on homocysteine oxidation.

As previously reported the two oxidation peaks obtained at NiMCT-SAM modified electrode were also observed at a

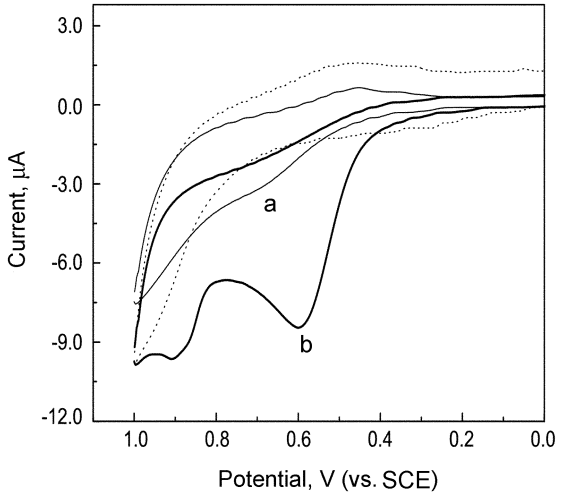


Figure 2. Cyclic voltammograms of 1.0 mM homocysteine obtained using (a) bare gold and (b) NiMC-SAM modified electrodes in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 0.1 V/s. Dotted lines represent a blank solution obtained at the NiMC-SAM modified electrode.

carbon nanotube (CNT)-modified electrode.[13] Although the peaks for the CNT-modified electrode have different potentials of 0.0 and 0.35 V *vs.* Ag/AgCl, the peak separation of *ca.* 0.3 V is consistent with that at the NiMCT-SAM modified electrode. The two peaks for homocysteine at the CNT-modified electrode can be described as redox-mediated oxidation by oxygen-containing moieties of CNT (at 0.0 V) and direct oxidation at the CNT-modified electrode (at 0.35 V). Similarly, the NiMCT-SAM modified electrode had oxygen-containing moieties (carbonyl groups), but those were contained within the structure of NiMCT, not on the electrode surface. This means that a NiMCT-SAM modified electrode has a different oxidation process for homocysteine from a CNT-modified electrode.

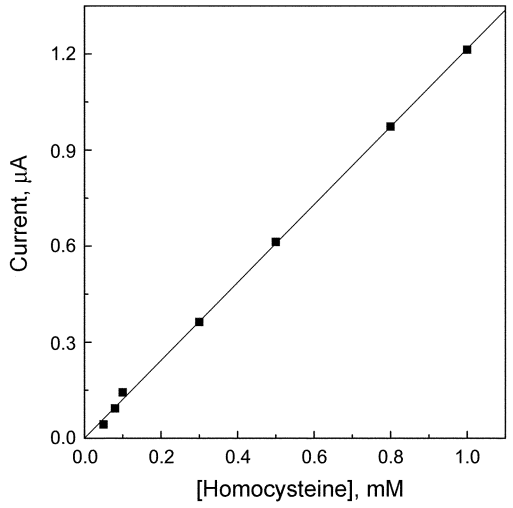
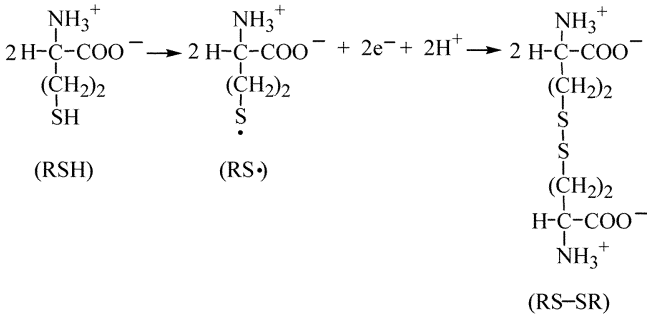


Figure 3. Calibration curve of homocysteine obtained by differential pulse voltammetry, using a NiMC-SAM modified electrode in 0.1 M phosphate buffer (pH 7.0). The scan rate was 25 mV/s; pulse amplitude was 50 mV; pulse interval was 1 s.

The oxidation reaction of homocysteine at NiMCT-SAM modified electrode, which can be explained using Complex 1 (in Scheme 1). The Ni(II) complex (Complex 2) used in this study is a derivative of Complex 1 which contains thiophene moieties. Complex 1 has been found to have an electrocatalytic effect on the oxidation of DA and AA[14] and to be reduced to a Ni(I) complex in non-aqueous solutions.[15] In Ni(II/I) couple of tetraaza-macrocyclic nickel complexes, Ni(I) can be stabilized in aqueous solutions by tetraaza-macrocyclic ligands.[16] In their currents, the first peak current at 0.60 V is approximately three times higher than the second one at 0.91 V. Therefore, the first oxidation of homocysteine might be redox-mediated by the Ni(II/I) couple. Since there was no oxidation wave near 0.91 V at the bare gold electrode, the second one might be attributed to both the direct oxidation on gold surface and the redox-mediated oxidation at NiMCT SAM. Considering the irreversible reduction reaction of homocysteine, the overall oxidation process of homocysteine at NiMCT-SAM modified electrode can be described as follows:



(2)

$$2RSH \rightarrow 2RS\cdot + 2e^- + 2H^+ \rightarrow RS\text{-}SR \quad (3)$$

$$2Ni(II)\ complex + 2RSH \rightarrow 2Ni(I)\ complex + RS\text{-}SR + 2H^+ \quad (4)$$

To determine homocysteine using a NiMCT-SAM modified electrode, its calibration curve was obtained using the first oxidation peak at 0.60 V for higher sensitivity. The result obtained by differential pulse voltammetry is shown in Figure 3. The linear range, i (μA) = 1.22 [homocysteine] (mM) + 3.4 (R = 0.9997, n = 7), was 10 μM to 1.0 mM (1.22

Table 1. Reproducibility of a NiMCT-SAM modified electrode using relative standard deviation (RSD) by repeated cyclic voltammetric measurements of homocysteine[a]

Measured Number	RSD (%)
10	0.79
20	1.51
30	2.79
40	4.11
45	4.94
50	5.12

[a]1.0 mM homocysteine in 0.1 M phosphate buffer, pH 7.0, and a scan rate of 0.1 V/s.

μA/mM), and the detection limit (S/N = 3) was 5.0 μM. The results suggest that a NiMCT-SAM modified electrode can be used for the determination of total homocysteine in blood plasma, since the concentration of total plasma homocysteine in healthy humans is known to be between 5 and 15 μM. On the contrary, free homocysteine in plasma represents approximately 1–2% (0.1–0.3 μM) of total plasma homocysteine.[1,2]

Homocysteine containing a thiol group can decrease the reproducibility of a NiMCT-SAM modified electrode by its adsorption on the electrode surface during electrochemical measurements. The reproducibility was evaluated by repetitive cyclic voltammetric measurements for 1.0 mM homocysteine. The electrode surface was washed with deionized water at each measurement, and the oxidation current was obtained at the first peak of 0.60 V. As shown in Table 1, the relative standard deviation was less than 3% up to 30 times and gradually increased with an increase of the number used. This indicates that NiMCT-SAM modified electrode is stable and reproducible for the repetitive measurements of homocysteine at pH 7.0.

Consequently, NiMCT formed a self-assembly on the gold electrode surface and was used to determine homocysteine. The NiMCT-SAM modified electrode had an electrocatalytic effect on the oxidation of homocysteine probably caused by the redox-mediation of Ni(II/I) couple. The linear range and detection limit for homocysteine obtained by differential pulse voltammetry were appropriate for the determination of total plasma homocysteine. The NiMCT-SAM modified electrode had a stable SAM with which to determine homocysteine electrocatalytically and reproducibly.

Acknowledgements. This work was supported by the Korea Science and Engineering Foundation (Grant 2003-015-C00419).

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