Electrocatalysis of a Self-assembled Thiol-modified Electrode Having Immobilized Dopamine for Oxidation of Reduced Nicotinamide Adenine Dinucleotide (NADH)

Toshio FUCHIGAMI*, Toshiro SHINTANI, Akinori KONNO and Tsutomu NONAKA

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1 INTRODUCTION
Modification of electrode surfaces is one of the most challenging fields in recent electrochemistry. Among many modified electrodes reported, self-assembled thiol-monolayer modified electrodes have been of much interest owing to relative ease of their preparation, and potentiality of their wide applications such as electrocatalysis, sensors, and display devices.

On the other hand, the electrocatalytic oxidation of dihydronicotinamide adenine dinucleotide (NADH) in aqueous solution is increasingly important in order to develop amperometric biosensors for determining substrates which react with NAD' to produce NADH under enzymatic conditions. One of problems in the electrooxidation of NADH is large overpotentials such as 1.1 V at carbon and 1.3 V at platinum electrodes. Therefore, much effort has been expended to circumvent the problem so far. Degrand and Miller reported the electrocatalytic oxidation of NADH using an electrode modified with polymer-bound dopamine redox-mediator. Although this electrode showed an electrocatalytic effect for NADH oxidation, the density of an electroactive quinone functional group was extremely low and the durability of the electrode was also unsatisfactory.

Considering this, we have developed self-assembled thio-monolayer modified electrodes to which dopamine is covalently bound.

2 EXPERIMENTAL
2.1 Preparation of a modified electrode
The electrode modification process was as follows. A gold electrode (1×1 cm²) was immersed overnight in a 0.1 mmol dm⁻³ cysteamine/ethanol solution followed by dipping in a 5 % aqueous glutaraldehyde solution for 2 h. The electrode was again immersed in a 10 mmol aqueous dopamine solution for 2 h and then treated with 1 % NaBH₄ solution for 1 min. The electrode thus modified was sonicated in distilled water for 30 sec to remove physically bound cysteamine and dopamine on the electrode. Those procedures were done at room temperature.

2.2 Electrochemical measurements
The electrochemical measurements were carried out using a three-electrode cell at room temperature. The modified electrode was placed in 0.1 mol dm⁻³ NaClO₄/MeCN solution or 0.1 mol dm⁻³ aqueous Na₂SO₄ solution and the solutions were deaerated by passing nitrogen gas through them before experiments were begun. A platinum wire (diameter: 0.2 mm, length: 6 cm) and saturated calomel electrode (SCE) were used as a counter and reference electrodes, respectively.

3 RESULTS AND DISCUSSION
Previously, we prepared a self-assembled D,L-α-lipoic acid monolayer modified gold electrode having immobilized dopamine as shown in Fig. 1. Although this modified electrode showed a symmetrical reversible redox
wave (E_{pox}^\text{ox} = +0.30 \text{ V} \text{ and } E_{p\text{red}}^\text{red} = +0.15 \text{ V at 100 mV sec}^{-1} \text{ in acetonitrile}) in the cyclic voltammogram, the redox peaks were diminished significantly after potential scanning several times.

In this study, we employed another modification process. Dopamine was chemically bound to self-assembled cysteamine monolayer on a gold electrode with use of glutaraldehyde as shown in Fig. 2.

As shown in Fig. 3, this modified electrode shows a symmetrical reversible redox wave in a cyclic voltammogram. The redox potential was +0.62 V vs. SCE. This electrode was found to be quite stable after potential scanning many times.

The peak separation was 50 mV and a linear relationship was observed between the sweep rate and the anodic and cathodic peak currents (I_p^{ox}, I_p^{red}), respectively as shown in Fig. 4. Furthermore, the amount of immobilized dopamine as determined by peak area measurement of the cyclic voltammogram was ca. 3.2x10^{-11} \text{ mol cm}^{-2}. This value is one order lower than those estimated in the cases of self-assembled monolayer anthraquinone and violagen modified electrodes. These facts indicate that dopamine was immobilized on the electrode, probably as a monolayer.

Next, electrocatalytic oxidation of dihydronicotinamide adenine dinucleotide (NADH) was examined in an aqueous solution using this modified electrode. The voltammogram observed in the presence of NADH exhibited a greatly enhanced oxidation current as shown in Fig. 5 and the enhanced anodic peak potential is less positive compared to the potential observed at a bare gold electrode (+0.88 V at 100 mV sec^{-1}).
Fig. 5 Cyclic Voltammograms of a dopamine modified electrode in 0.1 mol dm$^{-3}$ Na$_2$SO$_4$ in the absence (dashed line) and presence (solid line) of 1 mmol dm$^{-3}$ NADH. Scan rate: 100 mV sec$^{-1}$.

Fig. 6 Relationship between anodic peak current ($I_{pa}$) and NADH concentration (C).

Since this electrode was found to have electrocatalytic activity, the anodic peak current was measured by changing the concentration of NADH in order to obtain a calibration curve of the electrode for amperometric detection of NADH. As shown in Fig. 6, relatively good relationship between the anodic peak current ($I_{pa}$) and the NADH concentration (C) was obtained only at lower concentrations of NADH. Deviations at higher concentrations may arise through the limited electrocatalysis of the electrode.

Although the redox potential of this modified electrode is more positive compared to that of the electrode modified with polymer-bound dopamine, the catalytic current, namely, the sensitivity of NADH was much greater than the latter electrode$^6$. In addition, this electrode has rather high durability and shows resistance against surface fouling. Therefore, the proposed modified electrode has a potentiality of use for NADH analysis although further studies must be done to reduce the redox potential of this electrode.

In summary, we have developed a self-assembled dopamine modified electrode and examined its electrocatalysis for NADH oxidation. The present system can be used both for fundamental studies of functional modified electrodes and for biosensors.

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REFERENCES


