Selective Oxidation of Dopamine and Serotonin with an Alkanethiol-Modified Electrode

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A surface of gold electrode was modified with a self-assembled monolayer in order to protect the oxidation of ascorbic acid, which exists in biological fluid. The electrode was prepared by the modification with aqueous solution of 5-carboxypentanethiol (5C), 7-carboxyheptanethiol (7C) and 10-carboxydecanethiol (10C). At pH 7.0, alkanethiol modified electrodes did not show any anodic response to ascorbic acid but anodic peak to dopamine and serotonin. Dopamine and serotonin were most selectively oxidized with the 7C-modified electrode prepared in 0.15mg/ml 7C. The anodic current to dopamine was 500 times higher than that of ascorbic acid. The selective oxidation of dopamine and serotonin was performed by the ionization of alkanethiol.

Key Words: Dopamine, Serotonin, Ascorbic Acid, Alkanethiol Monolayer

1 Introduction

A self-assembled monolayer (SAM) is highly packed and oriented layer, and easily prepared on a solid surface.1 The characteristics of SAMs on the surface have been investigated intensively.2–4 It was reported that SAMs are formed spontaneously by the immersion of an appropriate substrate such as metallic electrode or glass plate into a solution of an active surfactant in an organic solvent.3) SAM is effective to introduce functional groups on the surface of the electrode. From the standpoint of electrochemical reaction, SAM of alkanethiols on metal electrodes has been investigated.5–7

Dopamine and serotonin are known as neurotransmitter, easily oxidized and their concentrations are determined electrochemically. However, in biological fluid or tissues, electro active species such as ascorbic acid co exist with neurotransmitters and interfere their electrochemical determination.

To eliminate ascorbic acid from the surface of the electrode, Nafion® has been used widely. Nafion® is hydrophilic polymer retaining sulfate group and negatively charged, so that ascorbic acid is excluded by electrostatic repulsion.8) However, the reported diffusion coefficient of the neurotransmitter inside the film was relatively low (D[ascorbic acid] = 2.3 × 10⁻⁷ cm² s⁻¹).9) Another approach to eliminate ascorbic acid is the utilization of ascorbic acid oxidase prior to the electrochemical reaction of neurotransmitter.9, 10) In order to achieve rapid and selective determination of dopamine, the SAMs modified electrodes have been investigated and the effect of negatively charged groups of SAM was reported by Mandeler11) and Savinnell et al.12) They applied negatively charged SAM to the selective oxidation of dopamine and succeeded in its selective determination against ascorbic acid. Ohsaka and Raj reported simultaneous detection of ascorbic acid and dopamine utilizing cystamine SAM modified electrode.13) Shinohara et al. prepared the SAM of mercapto undecanoic acid (MUA) and applied it to a glucose sensor.14) Glucose oxidase was immobilized through polyarylimine, which is electrostatically adsorbed on carboxyl groups of MUA. In these studies SAMs were prepared from ethanol solution at the concentration of 1–50 mM, and the preparation took 20 to 24 hrs. These studies focused on differentiation of the detecting substrate from ascorbic acid. Off course there are other antioxidant other than ascorbic acid, however, ascorbic acid is considered to be most important one. Because most of the other antioxidants including nonionic ones are not water soluble, electrochemical reaction is not easily observed in aqueous solution.

In this study, we propose an easy and quick procedure to prepare SAMs from aqueous solution. The negatively charged SAMs prepared in this study were served to eliminate ascorbic acid and selective measurement of dopamine and serotonin.

Malem and Mandeler11) prepared the SAMs of alkanethiols (HS(CH3)nCOOH) with different length (n=2,5,10) to differentiate dopamine to ascorbic acid. They concluded the optimum differentiation was found for n=5 and it was attributed to a compromise between a well-organized SAM and a reasonable electron transfer, which was observed with shorter alkanethiols.

In our system, we examined the effects of chain length of alkanethiols (5-carboxypentanethiol, 7-carboxyheptanethiol, 10-carboxydecanethiol) and hydrophilic group

Fig. 1 Scheme of alkanethiol modified Au electrodes. (a) Carboxy alkanethiol (5C, 7C or 10C) SAM modified electrode, (b) Amino alkanethiol (8A) SAM modified electrode.
of alkanethiol with amino group (8-amino octanethiol) as shown in Fig. 1.

In this work all the negatively charged groups remains and effectively used to exclude negatively charged ascorbic acid.

2 Experiment

5-carboxypentanethiol (5C), 7-carboxyheptanethiol (7C), 10-carboxydecanethiol (10C) and 8-aminoocanethiol (8A) were purchased from DOJINDO, Kyushu Japan. Dopamine and serotonin was laboratory grade of Sigma Japan. Other reagents including ascorbic acid were laboratory grade and used without further purification. Water used in this study was purified with MQ SP system (Millipore) to the resistance of 18.3 MΩ.

Modification of the surface of a gold electrode was performed as follows. The gold electrode of 1.6mm in diameter obtained from Bioanalytical System (BAS) was polished with aluminum powder (0.05 μm), and washed in purified water with sonication, for 15 min. It was cleaned in vacuum by sputtering for 1 min. Each alkanethiol was dissolved in deoxygenated water at the concentration of 0.05 – 0.20 mg/ml. The cleaned electrode was immersed in the solution of alkanethiol at room temperature for 1 hr. After the immersion the electrode was rinsed with water and sonicated for 5min to remove excess alkanethiol.

In order to confirm the modification, cyclic voltammetry of ferrocyanide (1mM) was carried out in phosphate buffer (0.1 M) pH7.0.

In the electrochemical measurement, the gold electrode, platinum wire (auxiliary electrode) and an Ag/AgCl reference electrode obtained from BAS were used. Electrochemical measurements were performed with use of a voltammetry analyzer (CV-50W; BAS). All the measurements were carried out in a vial filled with 10 ml of deoxygenated phosphate buffer (0.1M, pH 7.0). Cyclic voltammetry was performed at the scan speed of 100 mV/sec according to the previously reported procedure.  

Prior to the electrochemical experiment, the buffer in the vial was deoxygenated for 30 min. and purged with dry nitrogen gas for 30 min in order to avoid the chemical oxidation of electro- active reactants such as ascorbic acid and dopamine. The oxidations of ascorbic acid and dopamine were examined at the concentration of 0.3mM.

3 Results and Discussion

The electrode modified with 7C, 0.10mg/ml was served to electrochemical oxidation of ascorbic acid and dopamine. Cyclic voltammograms of those were shown with a bare gold electrode. Both of them were oxidized with the bare gold electrode certainly (Fig. 2). Electrochemical oxidation of ascorbic acid and dopamine has been studied on carbon electrodes and gold electrodes, and the reactions of ascorbic acid (eq.1) and dopamine (eq.2) correspond to two-electron, two-proton process at neutral pH. The result shown in Fig. 2 agreed with those reported one.

![Image](image_url)

Fig. 2 CVs for the oxidation of (a) ascorbic acid (0.3mM) and (b) dopamine (0.3mM) at 7C modified and bare Au electrode in 0.1M phosphate buffer pH 7.0.

With the 7C-modified electrode, ascorbic acid did not show any anodic peak at the range of -100 to 700 mV. This is consistent with the carboxy alkanethiol-SAM modified electrode and acidic end group SAM modified electrode, which were prepared in ethanol solution of alkanethiols.

As for the cyclic voltammogram of dopamine, anodic peak appeared at almost the same potential with the 7C-modified electrode as bare gold electrode, and its peak current was almost 80% of the bare electrode (Fig. 2 b). As dopamine was not blocked by 7C SAM, it was selectively oxidized with the 7C SAM modified electrode against ascorbic acid. As mentioned above, 5C and 10C modified electrode showed similar electrochemical property, which was effected by the charge of the monolayer as well as the length of the monolayer chain.
Elimination of anodic peak of ascorbic acid was compared with use of 5C, 10C and 8A modified electrode. Each electrode was modified with each alkanethiol at 0.10 mg/mL. As shown in Fig. 3, anodic peak was not observed with use of carboxy alkanethiol modified electrode, at the examined potential range. However, the currents observed with those electrodes decreased as to the length of the modified alkanethiol. The tendency was apparent at 700mV. The order of the current was 5C, 7C and 10C modified electrode. Among them, the current at 700mV of 5C-modified electrode was almost same as the peak current of the bare electrode. 5C SAM is less densely packed than 7C or 10C. Lower density of SAM means lower surface coverage. The extent of the surface coverage depends on the length of alkyl chain, so that the difference of current of 5C, 7C and 10C is attributed to the difference in their surface coverage.

To investigate the effect of the surface charge of alkanethiol SAM, 8A modified electrode was compared. Ascorbic acid showed anodic peak with the 8A-modified electrode and the current was 70% of the naked one, although the chain length of 7C and 8A is almost same. Therefore carboxyl group or negatively charged group of alkanethiol is necessary to eliminate the oxidation of ascorbic acid.

Malem and Madler\(^{11}\) prepared 5C and 10C modified electrode at the concentration of 50 mM of ethanol solution of the thiols for 24hs and obtained almost same results with this study. In this study the modification of the electrode was performed in aqueous solution of alkanethiols and the concentration of 0.57mM (0.1mg/ml), which is lower than 1/50 of 50nm. The modification time (1hr) was enough to obtain the result as reported in our previous work\(^{10}\) and much shorter than previously reported studies.\(^{11} - 14\) Thus alkanethiols formed a self-assembled monolayer easier in water than in ethanol. Hydrophobic tails of alkanethiols should interact more effectively in water because of the stronger polarity. In our previous study the electrode modified with N-(5-Amino-1-carboxypentylimidodiacetic acid) (AB-NTA) was prepared. AB-NTA has three carboxyl groups in one molecule and aminopentyl group in its tail and it does not assemble by steric hindrance of head groups. The AB-NTA modified electrode did not show the blocking effect to ascorbic acid nevertheless of its negatively charged groups.\(^{15}\) Therefore the self-assembling of hydrophobic tail is quite effective to block ascorbic acid as well as acidic end group.

To optimize the modification concentration of alkanethiols, 0.05, 0.10, 0.15 and 0.20 mg/ml were examined. The ratios of anodic peak current of dopamine to the current of ascorbic acid at the peak of dopamine were compared with those modified electrode. The ratio of 8A-modified electrode at any concentration of alkanethiols was almost same as that of the bare electrode, and there was no selectivity to dopamine observed, but carboxy alkanethiol-modified electrodes showed much higher ratio than the bare electrode. Among them, the highest ratio was observed at the concentration of 7C, 0.15mg/ml (Fig. 4) and it was higher than 500. The concentration 0.15mg/ml (0.85mM) is still lower than previously reported concentrations (50mM).

With the 7C-modified electrode, the anodic peak current of dopamine showed linear relationship to the concentration at the range of 50 – 300 µM. The observed current at 50 µM was 156nA. With widely used electrochemical analyzer, nA level of current can be measured and nanomolar level of dopamine will be measured with this electrode.

![Fig. 3](image1)

**Fig. 3** CVs for the oxidation of ascorbic acid at 5C, 7C, 10C and 8A modified Au electrode in 0.1M phosphate buffer pH7.0.

![Fig. 4](image2)

**Fig. 4** Alkanethiols (5C, 7C, 10C and 8A) and their concentrations in modification of the electrode and the oxidation current ratio of dopamine to ascorbic acid of each electrode.

AB-NTA modified electrode did not show the blocking effect to ascorbic acid nevertheless of its negatively charged groups.\(^{15}\) Therefore the self-assembling of hydrophobic tail is quite effective to block ascorbic acid as well as acidic end group.
space necessary to charge transfer.

From these results, carboxyl group of alkanethiol is quite important to get rid of ascorbic acid. However, amino group of 8A did not show any effect to the ratio. Amino group of 8A does not ionized at pH 7.0 and electrostatic effect might not be observed. To make clear this phenomenon, at pH 4.0, at which ionization of amino group is considered to occur, oxidation of dopamine and ascorbic acid were examined. At the pH anodic peak of dopamine was not observed with use of 8A-modified electrode. On the other hand, ascorbic acid showed smaller current and the higher potential than those at pH 7.0. The ionization of the SAM is effective to realize the selective determination of electroactive substance.

Serotonin was also oxidized with carboxy alkanethiol SAM modified electrodes. The electrochemical oxidation of serotonin is known as follows.\(^{20}\)

![Diagram of serotonin oxidation](image)

**Fig. 5** CVs for the oxidation of serotonin (0.3mM) at 7C modified electrode, which was modified in the solution of 7C 0.15mg/ml.

Figure 5 shows cyclic voltammograms of serotonin. The anodic peak potential did not changed by the modification with 7C but the peak current decreased 60% of naked electrode. The selectivity was investigated with use of 5C, 7C and 10C. The ratios of anodic peak current of serotonin to ascorbic acid were shown in Fig. 6. Among carboxy alkanethiol modified electrodes, the highest selectivity was obtained with 7C-modified electrode.

10C modified electrode prepared in 0.15mg/ml solution, serotonin was oxidized but the peak was not observed obviously and the current was lower than 7C-modified electrode. The highest selectivity was obtained with use of 7C-modified electrode prepared in 0.15mg/ml, and the peak current ratio of serotonin to ascorbic acid was 430. In case of serotonin, other preparation conditions (5C or 10C at 0.15mg/ml) did not give reproducible result.

In conclusion, preparation of carboxy alkanethiol SAM modified electrodes was investigated and the procedure in aqueous solution of alkanethiol (0.15mg/ml) for only

![Graph of alkanethiol oxidation current ratio](image)

**Fig. 6** Alkanethiols (5C, 7C, 10C) modified electrode and the oxidation current ratio of serotonin to ascorbic acid. Concentration of each alkanethiols was 0.2mg/ml.

Ihr was demonstrated. With the prepared electrode, selective determination of dopamine and serotonin to ascorbic acid was performed with use of carboxy alkanethiol SAM modified electrode, and it was controlled by chain length and ionization of alkanethiol.

**References**