Simultaneous Detection of Ascorbic Acid and Dopamine at Gold Electrode Modified with a Self-Assembled Monolayer of Cystamine

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The electrochemical oxidation of ascorbic acid (AA) and dopamine (DA) at a gold electrode modified with a self-assembled monolayer (SAM) of cystamine (CYSA) has been studied. A large decrease in the overpotential for the oxidation of AA was noticed at CYSA-Au electrode. Well-separated square wave voltammetric peaks for AA and DA were observed at this electrode, which can be used for the simultaneous detection of these species.

Key Words : Cystamine, Self-assembled Monolayer, Ascorbic Acid, Dopamine

1 Introduction

The development of voltammetric methods for the simultaneous detection of dopamine (DA) and ascorbic acid (AA) in biological samples such as brain tissue received much interest. The common problem in the oxidation of these molecules is that it requires a large overpotential in case of using bare electrodes. Moreover, the direct oxidation of these species occurs at very similar potential. And very often the electrode suffers from the fouling effect, which results in rather poor selectivity and sensitivity. Since the concentration of AA in the biological samples is very high, both the selectivity and sensitivity are very important in developing the voltammetric procedure for the detection of DA. Different approaches have been attempted, particularly using the ion-exchange membrane coated electrodes such as Nafion-coated electrodes, to solve the problem. However, this approach has some drawbacks, e.g., (i) the non-uniform thickness of the polymer film, (ii) the poor response time, and (iii) the reported diffusion coefficient of DA inside the film was relatively small (typically $10^{-10}$ to $10^{-9}$ cm$^2$ s$^{-1}$). The recent development in electrode modification is formation of a self-assembled monolayer (SAM) of mercaptoalkanes on Au electrode. SAMs can provide a means of controlling the chemical nature of the electrode-solution interface. Mandler et al. attempted the oxidation of dopamine and ascorbic acid at carboxyl terminated SAM. Similarly Savinell et al. utilized different SAMs with negatively charged end groups for the selective detection of DA. However, the simultaneous detection of both AA and DA has not been achieved owing to the homogeneous catalytic oxidation of ascorbic acid by the oxidized dopamine in solution. In the present investigation we have attempted the simultaneous detection of both AA and DA using the cystamine, diethiobisethanamine (CYSA), modified electrode. The potential utilization of SAMs in electroanalytical chemistry is demonstrated.

2 Experimental

Electrochemical studies were performed using a two-compartment three-electrode cell with an Au working electrode (diameter 1.6 mm), a Pt wire auxiliary electrode and a NaCl saturated Ag/AgCl reference electrode. The Au working electrodes were polished with alumina powder (1.0 and 0.06 μm) and sonicated in water for 5-10 min. The polished electrodes were then electrochemically pretreated in 0.05 M H$_2$SO$_4$ before each experiment. The self-assembled monolayer of CYSA was formed by immersing the pretreated electrode in an aqueous solution of 50 mM CYSA. Hereafter the Au electrode modified with the SAM of CYSA will be referred as CYSA-Au. Cyclic voltammetry was carried out using a computer-controlled electrochemical analyzer (BAS 100B/W).

3 Results and Discussion

The cyclic voltammograms (CVs) recorded for AA and DA at bare and CYSA-Au electrodes are shown in Fig. 1. At the bare electrode, the AA oxidation occurs around 0.5 V and the electron transfer kinetics is rather sluggish owing to fouling of the electrode surface due to the deposition of the oxidation product of AA. The oxidation of DA at the bare electrode takes place around 0.2 V. It has been established that the oxidation of AA involves two protons and two electrons at acidic pH. However, at pH higher than the first $pK_a$ (4.17) of AA, the oxidation process involves only the loss of single proton owing to the fact that AA exists as anion. The amino group of DA is positively charged ($pK_a$ 8.9) at neutral pH. The
CYSA monolayer is expected to be positively charged in 0.1 M phosphate buffer (pH 7.2) since the $pK_a$ of CYSA is 8.19. As can be readily seen from Fig. 1, an enormous increase in the oxidation peak current accompanied with a large negative shift ($\sim 450$ mV) in the peak potential was observed for the oxidation of AA at the CYSA-modified Au electrode. The negative shift in the oxidation peak potential at the CYSA-Au is attributed to more favorable interactions between positively charged monolayer and negatively charged AA. On the other hand, the electron transfer kinetics for the oxidation of DA at the CYSA-Au electrode was found to be rather sluggish owing to the electrostatic repulsion.

By considering the nature of the terminal group, the following important factors can be accounted for the oxidation of AA. (i) At an electrode modified with a monolayer of cationic species, the effective electrode potential experienced at the monolayer-solution interface would have been altered by the development of a Donnan potential at the interface. The effective electrode potential, thus, will shift positively at a monolayer of cationic species and hence the electrode potential must be made more negative to effect the electrode reaction of AA. (ii) The change in the concentration of electroactive species at the electrode surface. The ionic terminal groups will affect the distribution of ionic electroactive species in the vicinity of the electrode surface. The positively charged SAM (CYSA-Au) will attract the anionic AA and repel the positively charged DA. Attractive interaction between the positively charged SAM and the anionic AA increases the concentration of AA in the vicinity of electrode surface, resulting in an enhancement in the oxidation current of AA. The opposite effect has happened for the positively charged DA at the SAM.

Figure 2(a) shows the square-wave voltammogram of 0.15 mM of AA and DA at bare electrode. A rather broad oxidation peak was obtained and the peak

![Figure 1](image1.png)

**Fig. 1** CVs for the oxidation of (A) AA (1 mM) and (B) DA (0.5 mM) at (a) CYSA-Au and (b) bare Au electrodes in 0.1 M phosphate buffer (pH 7.2).

![Figure 2](image2.png)

**Fig. 2** Square-wave voltammograms of AA and DA at (a) bare and (b) CYSA-Au electrodes in 0.1 M phosphate buffer containing 0.15 mM AA and 0.15 mM DA. Square wave amplitude: 25 mV; Frequency: 15 Hz; Step potential: 4 mV; Quite time: 2 s.

![Figure 3](image3.png)

**Fig. 3** Square-wave voltammograms of AA (0.1 mM) in the presence of different concentrations of DA, $[DA]$ : (a) 6, (b) 45, (c) 109, (d) 129 $\mu$M. Other experimental conditions are the same as in Fig. 2.
potentials of AA and DA were indistinguishable. It is impossible to determine the concentration of the species from the broad voltammetric peak. As shown in Fig. 2(b), the modification of electrode surface with CYSA resolved the merged voltammetric peak into two well-defined voltammetric peaks at potentials 0.04 and 0.2 V. The homogeneous catalytic oxidation of AA by the oxidized DA has been observed. The oxidized DA, dopamine-oxquinone, is chemically reduced by AA. Thus, one would anticipate that the oxidation of DA would be affected by AA. Very recently, Savinell et al. reported the oxidation of AA and DA at self-assembled monolayers with different negatively charged terminal groups. They observed the homogeneous catalytic oxidation of AA by the oxidized DA and they could not simultaneously detect both AA and DA. In the present investigation, we could successfully determine the DA concentration in the presence of high concentration of AA. Figure 3 shows the square wave voltammograms of AA (0.1 mM) at different concentrations of DA. The homogeneous catalytic oxidation has not been observed and the voltammetric peak of AA was unaltered by the addition of DA. The calibration curves for both AA and DA are linear for a wide range of concentrations with a correlation coefficient 0.997, and this electrode can be used to detect both species at a sub-micromolar level. The detection limits for AA and DA were found to be 0.5 and 1 μM, respectively. The electrode response was very stable and showed excellent anti-fouling properties against the oxidation products of AA, which are notorious for their surface fouling at the bare electrode.

In summary, we have demonstrated the use of an Au electrode modified with the SAM of CYSA for the simultaneous detection of AA and DA. The modified electrode showed excellent sensitivity and selectivity and anti-fouling properties. The use of ionic SAM modified electrodes provides many advantages over polymer-coated electrodes. Since the thickness of monolayer is very thin when compared with the polymer-coated electrode, fast measurements are possible in the SAM modified electrode due to the easy diffusion of electroactive species. The homogeneous catalytic oxidation AA by the oxidized DA has been advantageously eliminated at the CYSA-Au electrode.

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