

Voltammetric Behaviors of Dopamine and Ascorbic Acid at a Glassy Carbon Electrode Anodized in 1, ω -Alkanediol

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The voltammetric behaviors of dopamine (DA) and ascorbic acid (AA) at a glassy carbon (GC) electrode anodized in 1, ω -alkanediol were examined. On cyclic voltammetry (CV) at a 1,5-pentanediol-modified GC electrode, the electrode process of AA was totally depressed, while DA still showed an anodic peak. Although similar phenomena were observed at a GC electrode anodized in 1,3-propanediol or 1,4-butanediol, the 1,5-pentanediol-modified electrode suppressed the anodic peak of AA most strongly. The voltammetric response of DA at the modified electrode was enhanced by the presence of AA, and the extent was controllable by a proper selection of the sweep rate. Thus, the electrochemical detection of DA with high sensitivity was achieved through CV with 0.1 V s⁻¹ at the modified electrode by adding excess AA, while electrochemical discrimination of DA from excess AA was realized at 20 V s⁻¹.

Keywords Dopamine, voltammetric detection, modified electrode, ascorbic acid

Recently, we have found that the anodic oxidative treatment of a glassy carbon (GC) electrode in a simple alkanol containing a small amount of H₂SO₄ allows alkanol molecules to be fixed on the electrode surface *via* an ether-linkage.^{1,2} The method is simple and highly reproducible, providing a useful tool to obtain a covalently modified GC electrode as a counterpart of a gold electrode covered with a self-assembled monolayer of an alkanethiol. However, it has been suggested that the resulting membrane on a GC electrode anodized in an alkanol is not as densely packed as that on a gold electrode modified by the self-assembly technique.² Accordingly, an alkanol-modified GC electrode, though not useful as model systems to study the interfacial chemistry of organic surfaces, seems to be applicable to electrochemical sensors, because pinhole defects in the membrane permit an electro-active analyte to reach the electrode surface and to show its electrochemical response under certain conditions.² Thus, our attention has turned to develop an electrochemical sensing system by employing an alkanol-modified GC electrode, taking the fact into consideration that various substituted alkanols are easily available in comparison with alkanethiols. Since terminal functional groups of alkanethiols have been demonstrated to play important roles in determining interfacial characters of self-assembled monolayers³⁻⁶, we have undertaken to investigate the electrochemical performance of a GC electrode anodized in ω -substituted-1-alkanols. Such attempts will shed light on the scope of our modification

method as a general access to a covalently modified GC electrode, and should help to design a chemically modified electrode as a novel chemical sensor. According to the observation that the introduction of oxygen-containing functional groups, such as carboxylic acids, ketones, and alcohols, onto carbon electrode surfaces through electrochemical pretreatment seems to affect the electrode reactions of dopamine (DA) and ascorbic acid (AA)⁷⁻¹⁰, 1, ω -alkanediols were first chosen as substituted alkanols to modify a GC electrode anodically in expectation of attaining an electrode surface covered with hydroxy alkyl groups, and the voltammetric behaviors of DA and AA at the modified GC electrode were investigated. In this paper we describe that a GC electrode anodized in 1,5-pentanediol is utilized for the voltammetric detection of DA in the presence of AA; the latter is known as an interfering factor.

Experimental

Reagents

Deionized water was distilled and used throughout the present study. All other reagents were of reagent grade and were used without further purification. Phosphate buffer (0.1 M, pH 7.0; Na₂HPO₄+NaH₂PO₄) was used for all measurements.

Apparatus

A GC electrode was polished with a Maruto (Tokyo, Japan) polishing system, Model ML-150P. An electro-

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chemical modification of a GC electrode was performed by a Hokuto Denko (Tokyo, Japan) potentiostat/galvanostat (Model HA 301) connected to a Hokuto Denko Coulomb/amperehour meter (Model HF 201). Cyclic voltammograms were obtained with a Huso (Tokyo, Japan) potentiostat (Model 315A) equipped with a Riken Denshi (Tokyo, Japan) X-Y recorder (Model F-5C) or with a computer-controlled electroanalysis system (Model CS-1090, Cypress Systems, Inc., Lawrence, KS). A three-electrode configuration was employed: a glassy carbon-disk (7.07 mm^2) electrode with or without a modification as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. GC rods (GC 30, $3 \text{ mm}\phi$) were obtained from Tokai Carbon (Tokyo, Japan). The fabrication of a GC electrode has been described previously.¹¹

Electrode modification and measurements

Before the anodic modification of a GC electrode with $1,\omega$ -alkanediol, the electrode was mechanically polished using a Maruto system with polishing paper (#1200), followed by alumina powder ($0.05 \mu\text{m}$) on a polishing cloth, sonicated in deionized water for 5 min, washed with water, MeOH, and CHCl_3 , and dried by a stream of nitrogen. The modification of a GC electrode was performed as follows: (1) a GC electrode was anodized in $1,\omega$ -alkanediol containing $0.1 \text{ M H}_2\text{SO}_4$ by controlled-potential electrolysis at $+2.0 \text{ V vs. Ag wire}$, where 2 mC of electricity was allowed to be consumed, and washed with MeOH and water; (2) the anodized electrode was electrochemically treated by five repetitive potential sweeps (sweep rate, 0.1 V s^{-1}) between 0 and -0.5 V vs. SCE ¹² in a phosphate buffer solution (0.1 M , $\text{pH } 7.0$), washed with water and MeOH, and dried by a stream of nitrogen; and the procedure (1) and (2) were repeated three times, unless otherwise mentioned. All voltammetric measurements at the bare and the modified electrodes were carried out at room temperature between -0.2 and 0.8 V vs. SCE for a daily prepared buffer solution containing DA and/or AA. The reported data at a $1,\omega$ -alkanediol-modified electrode are the average values of more than three measurements obtained with newly prepared electrodes.

Results and Discussion

Voltammetric behaviors of DA and AA at a GC electrode anodized in a $1,\omega$ -alkanediol

The voltammetric behaviors of DA and AA at a $1,5$ -pentanediol-modified GC electrode were first examined. The results of cyclic voltammetry (CV) obtained for a phosphate buffer solution ($\text{pH } 7.0$) of DA (1 mM) or AA (1 mM) alone at the modified GC electrode at a sweep rate of 0.1 V s^{-1} are compared in Fig. 1 with those at a bare GC electrode. The anodization of a GC electrode in the alkanediol had small effects on the electrode reaction of DA, while the response of AA was remark-

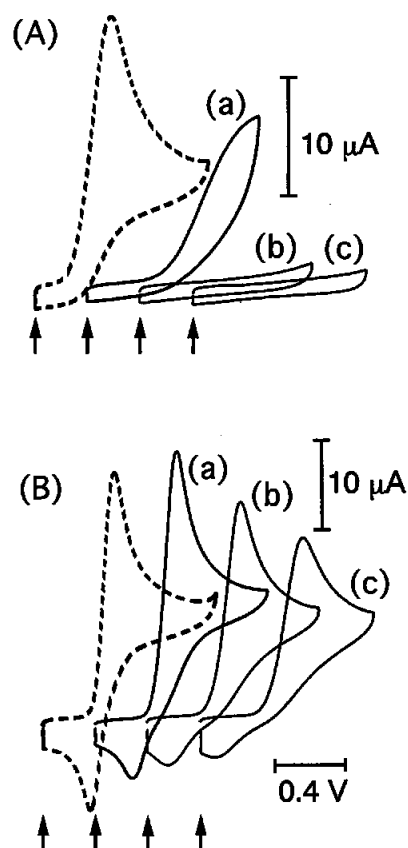


Fig. 1 Cyclic voltammograms for phosphate buffer solutions ($\text{pH } 7.0$) of (A) AA (1 mM) and (B) DA (1 mM) at a bare GC electrode (broken line) and at a GC electrode subjected to the modification procedure using $1,5$ -pentanediol (see Experimental) a) once, b) twice, and c) three times (solid line): sweep rate, 0.1 V s^{-1} ; the potential indicated by an arrow is -0.2 V .

ably suppressed. The retardation in the electrode process of AA became successively enhanced as the modification sequence of (1) and (2) (see Experimental) was repeated, and the voltammograms of AA at the GC electrode obtained by triplicate repetition of the modification procedure was indistinguishable from that in the absence of AA (Fig. 1A-c). At the same modified GC electrode, the response of DA was not influenced as much as that of AA, and the anodic wave was still noted (Fig. 1B-c), of which the peak potential was positively shifted by 210 mV and the peak current was about 60% of that at a bare electrode. No further change was recognized for the responses of AA and DA at a GC electrode treated by the procedure for more than three times. Instead of repeating the modification sequence three times, passing more than 2 mC of electricity, e.g. 6 mC , during process (1) all at once followed by process (2) gave a modified GC electrode with similar voltammetric characteristics. However, the electrode-to-electrode reproducibility on the responses of DA and AA within $\pm 5\%$ was attained when a $1,5$ -pentanediol-

Table 1 Voltammetric results of ascorbic acid (AA) and dopamine (DA) at a glassy carbon electrode anodized in 1, ω -alkanediol^a

1, ω -Alkanediol	AA (1 mM)		DA (1 mM)	
	E_p / V vs. SCE	I_p / μ A	E_p / V vs. SCE	I_p / μ A
— ^b	0.24	22.8	0.19	33.6
Ethylene	0.33	21.8	0.26	26.8
1,3-Propanediol	— ^c	12.6 ^d	0.25	30.6
1,4-Butanediol	— ^c	6.7 ^d	0.29	30.0
1,5-Pentanediol	— ^c	0.8 ^d	0.40	20.5
1-Pentanol	0.4	14.8	0.24	34.2

a. Sweep rate, 0.1 V s⁻¹. b. At a bare electrode. c. No peak was observed at all. d. Anodic current observed at 0.8 V vs. SCE.

modified GC electrode was obtained by the repeating method, rather than by the latter method.

The voltammetric results of AA (1 mM) and DA (1 mM) at a GC electrode anodized in various alkanediols, that is, HO(CH₂)_nOH ($n=2, 3, 4,$ or 5) are summarized in Table 1. At a GC electrode anodized in ethylene glycol, both AA and DA still exhibited their anodic peaks, the potentials of which were slightly shifted toward the positive directions. When alkanediols with alkyl chains longer than that of ethylene glycol were used as a modifier, the anodic oxidation of AA at the GC electrode anodized in each modifier was remarkably inhibited, in contrast to the response of DA at the same electrode. Although the electrochemical performance of GC electrodes anodized in alkanediols longer than 1,5-pentanediol were attractive, they were not investigated. This is because such alkanediols are solid and the anodic modification must be performed in an organic solvent containing an alkanediol as a modifier, for which no satisfactory procedure has yet been established. Among the alkanediols examined here, 1,5-pentanediol has proved to be the modifier of choice for a voltammetric discrimination between AA and DA, suggesting that an alkanediol-modified GC electrode can be applicable to an *in vivo* electrochemical analysis of DA, which is one of the major concerns in the field of electrochemical analysis, provided that a corresponding microelectrode is accessible (as discussed below).

In order to determine the origin of the voltammetric behaviors of AA and DA at a GC electrode anodized in 1,5-pentanediol, the CV of AA or DA was performed at a GC electrode anodized in 1-pentanol; the results are included in Table 1. At a 1-pentanol-modified GC electrode, the anodic response of AA or DA was still recognized as a peak, though at a potential more positive than that at a bare electrode; a slightly stronger distortion was observed in the response of AA compared with that of DA. The results indicate that anodization of a GC electrode in a particular alkanediol is indispensable for the observed repression in the voltammetric response of AA. Namely, the presence of another hydroxy func-

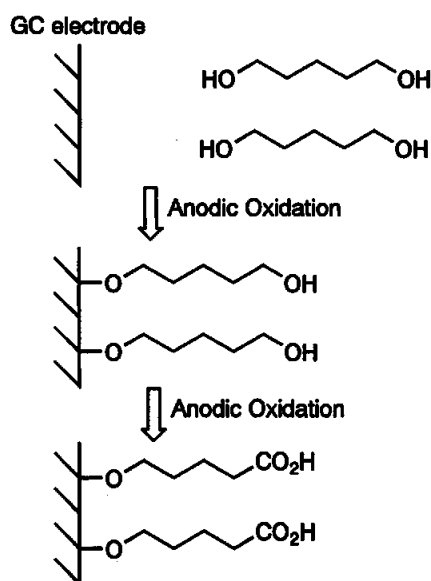


Fig. 2 Schematic representation of the change in the surface structure of a GC electrode during the anodic treatment in 1,5-pentanediol.

tion, in addition to the hydroxyl group available for the anodic fixation of the modifier on the electrode surface, seems to play an important role in realizing a specific retardation in the anodic oxidation of AA. It has been reported that an electrochemical discrimination between AA and DA is achieved at a Nafion-coated carbon electrode¹³⁻¹⁵, a stearate modified carbon-paste electrode^{16,17}, or a gold electrode covered with a self-assembled monolayer of an ω -mercapto carboxylic acid¹⁸, which is attributed to an electrostatic repulsion between AA and a particular functionality in each electrode modifier, excluding negatively charged AA from the electrode surface. Judging from the similarity of the electrochemical performance between a 1,5-pentanediol-modified GC electrode and those modified electrodes, the anodic treatment of a GC electrode in the alkanediol seems to introduce anionic groups on the electrode surface. This assumption is also supported by the fact that on CV at a bare GC electrode, 3,4-dihydroxyphenylacetic acid (DOPAC) (1 mM), which is negatively charged at pH 7.0, as is AA, exhibited an anodic wave with a peak potential and current of 0.33 V and 18 μ A, respectively, while no peak was observed at a GC electrode anodized in 1,5-pentanediol; the anodic response was 4.5 μ A, even at 0.8 V (0.5 μ A at 0.33 V). A plausible functional group inducing the observed voltammetric behavior is believed to be a carboxylate, which would be formed during the modification procedure by an anodic oxidation of the terminal hydroxy group of the alkanediol fixed anodically to the surface *via* an ether-linkage, as schematically depicted in Fig. 2. Thus, it appears that the anodic treatment of a GC electrode in a 1, ω -alkanediol provides easy access to an electrode modified with alkyl carboxylate molecules, although

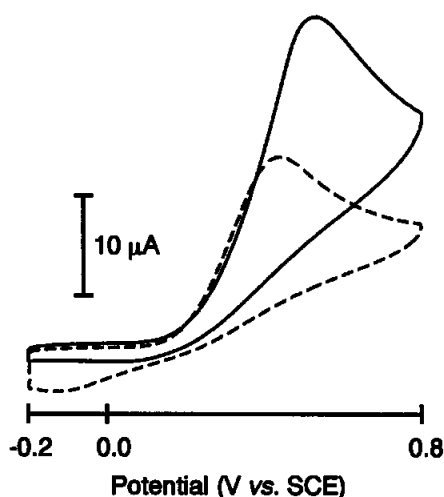


Fig. 3 Cyclic voltammograms for phosphate buffer solutions (pH 7.0) of DA (1 mM) in the absence (broken line) and the presence (solid line) of AA (1 mM) at a GC electrode anodized in 1,5-pentanediol at a sweep rate of 0.1 V s^{-1} .

further study is needed to confirm the proposed surface structure.

Voltammetric behavior of DA in the presence of AA at a GC electrode anodized in 1,5-pentanediol

Figure 3 shows the results of CV obtained for DA (1 mM) in the presence of AA (1 mM) as well as for DA alone at a GC electrode anodized in 1,5-pentanediol. Despite the fact that a significant inhibition of AA oxidation was observed at the modified electrode, the response of DA in the presence of AA increased by about 1.7 times. This observation demonstrates that the oxidation of DA in the presence of AA at a 1,5-pentanediol-modified GC electrode results in a homogeneous catalytic oxidation of AA. Based on the proposed process (Fig. 2) for the present anodic modification, a GC electrode anodized in 1,5-pentanediol must resemble a gold electrode¹⁸ modified with 6-mercaptohexanoic acid very closely in surface structure. However, no change was observed between the voltammograms for DA in the absence and presence of AA at the modified gold electrode.¹⁸ The difference in electrochemical performance between the 1,5-pentanediol-modified GC electrode and the modified gold electrode seems to reside in the size of the electrodes: the diameters of ours and the latter electrode are 3 mm and 10 μm , respectively. Namely, the catalytic oxidation of AA at a 1,5-pentanediol-modified GC electrode can be explained by the remarkably large radius of the electrode, compared with the thickness of the diffusion layer, which will induce a high probability for DA, regenerated through the oxidation of AA by the oxidized form of DA, to return to the electrode. The same argument has been applied to rationalize the observation that the contribution of the catalytic current to the response of DA in

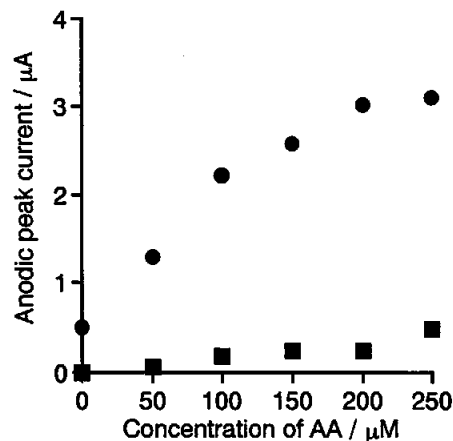


Fig. 4 Effects of the concentration of AA on the anodic peak current in the CV for a mixture of DA (10 μM) and AA (circles) and for AA alone (squares) at a 1,5-pentanediol-modified GC electrode: potential sweep rate, 0.1 V s^{-1} ; the response for AA alone corresponds to the current observed at 0.8 V vs. SCE, since no peak was observed.

the presence of AA at a carbon fiber electrode with a 2.8 μm radius was much smaller than that at a carbon-paste electrode with a 790 μm radius.¹⁹ This rationale is supported by the fact that a similar catalytic current was observed at a stearate-modified carbon-paste electrode of 6.35 mm diameter.¹⁶

In order to evaluate the efficiency of the catalytic reaction at the modified electrode, the effects of the concentration of coexisting AA and the potential sweep rate upon the voltammetric response of DA were examined. As shown in Fig. 4, the anodic peak current observed for a mixture of DA (10 μM) and AA increased with the concentration of AA, and reached an almost constant value in the presence of more than 200 μM of AA, which is about 8 times as large as that for DA alone. It is noteworthy that no peak was observed for AA alone, and that the anodic response at 0.8 V for 200 μM of AA was less than 10% of the peak current observed for the mixture of DA and AA. The effects of the potential sweep rate upon the voltammetric response obtained for a mixture of DA (10 μM) and AA (200 μM) at a 1,5-pentanediol-modified GC electrode are summarized in Table 2. The extent of the catalytic process was evaluated by the ratio between I_0 and I , which stand for the peak currents obtained for DA alone and a mixture of DA and AA, respectively. At a slow sweep rate, the anodic response of DA was substantially enhanced by the presence of AA, while a sweep rate of 20 V s^{-1} rendered the effect of AA almost negligible. Although it was expected that CV at a sweep rate higher than 20 V s^{-1} will more effectively minimize the contribution of the catalytic oxidation of AA to the response of DA, such experiments were not carried out, since under such conditions the anodic peak was no longer observed within $-0.2 - 0.8 \text{ V}$, probably due to the conventional size (3 mm ϕ) of the electrode, causing a large ohmic drop.

Table 2 Effects of potential sweep rate upon the voltammetric response of DA ($10 \mu\text{M}$) in the absence and the presence of AA ($200 \mu\text{M}$)

Sweep rate/ V s^{-1}	Anodic response ^a		Ratio of I/I_0
	DA (I_0)/ μA	DA+AA (I)/ μA	
0.1	0.4	3.2	8.0
1	2.1	5.9	2.8
5	4.1	9.2	2.2
10	6.7	10.4	1.6
20	14.0	16.0	1.1

a. Reported as peak current.

Voltammetric detection of DA in an amplified or highly selective manner

The results described so far allow us to expect that the voltammetric detection of DA in the presence of a large excess of AA will be achieved at a 1,5-pentanediol-modified GC electrode in dual ways, that is, in an amplified or a highly specific manner, the mode of which can be altered simply by proper choice of the voltage sweep rate. In order to examine the expectation, the dependence of the voltammetric response of DA at a 1,5-pentanediol-modified GC electrode on its concentration was investigated. Figure 5 shows the results obtained at a sweep rate of 0.1 V s^{-1} . The peak current observed for a mixture of DA and AA ($200 \mu\text{M}$) at the modified GC electrode bore a linear relationship to the concentration of DA from 2 to $10 \mu\text{M}$ with a slope and correlation coefficient of 0.300 A/M and 0.997 , respectively. The response was reproducible within $\pm 5\%$ over the concentration range. As a control experiment, working curves for the determination of DA were also obtained for DA alone at the modified and bare GC electrodes, in which the slope and correlation coefficient were of 0.031 A/M and 0.970 at the former electrode and of 0.047 A/M and 0.997 at the latter. These results indicate that a highly amplified detection of DA can be achieved by CV with a slow potential sweep rate at a GC electrode anodized in 1,5-pentanediol in the presence of excess AA. Thus, it is suggested that by deliberately adding AA a minute amount of DA can be electrochemically determined, which will be realized in HPLC analysis with the modified electrode and a buffer solution containing AA in a large amount as an electrochemical detector and an eluent, respectively. Although a similar enhanced response of DA induced by excess AA was noted at a carbon-paste electrode modified with stearic acid, and a flow analysis of DA was claimed to be a potent application of the modified electrode¹⁶, the present modified electrode seems to be more stable than the carbon-paste electrode, implying that the former electrode will find more practical applications to the *in vitro* analysis of DA.

Figure 6 compares the anodic peak currents for a mixture of DA and AA ($200 \mu\text{M}$) with that for DA alone at a 1,5-pentanediol modified GC electrode at a sweep

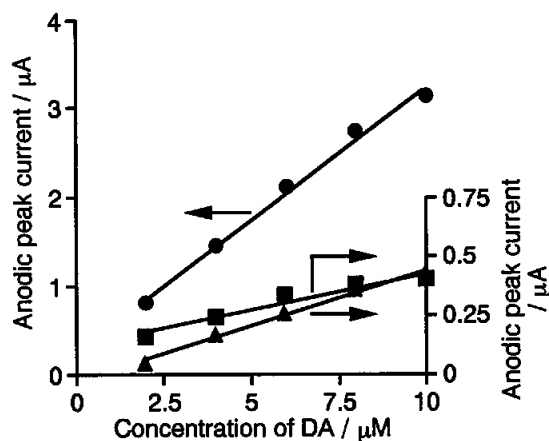


Fig. 5 Relationship of the concentration of DA with the anodic peak currents at a 1,5-pentanediol-modified GC electrode at a sweep rate of 0.1 V s^{-1} for a mixture of DA and AA ($200 \mu\text{M}$) (circles) and for DA alone (squares), and with that at a bare electrode for DA alone (triangles).

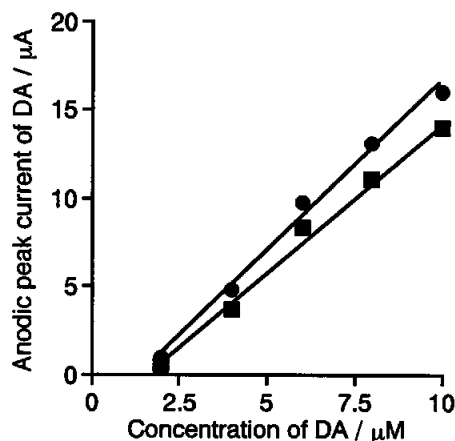


Fig. 6 Relationship of the concentration of DA with the anodic peak currents at a 1,5-pentanediol-modified GC electrode at a sweep rate of 20 V s^{-1} for a mixture of DA and AA ($200 \mu\text{M}$) (circles) and for DA alone (squares).

rate of 20 V s^{-1} , where both peak currents were correlated linearly with a concentration of DA from 2 to $10 \mu\text{M}$ (slope and correlation coefficient, 1.915 A/M and 0.995 for the mixture and 1.710 A/M and 0.995 for DA alone). The close proximity of the correlation lines suggests the possibility of a specific detection of DA in the presence of AA, as mentioned above for the results in Table 2. Since it has been demonstrated that CV at a carbon fiber electrode at a sweep rate of 300 V s^{-1} can be useful for *in vivo* monitoring of DA^{20,21}, the anodic modification of the carbon fiber electrode in 1,5-pentanediol is believed to provide the reported methodology with a higher accuracy. Further studies on the point are under way.

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