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Highly sensitive and selective dopamine detection by an amperometric biosensor based on tyrosinase/MWNT/GCE

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Abstract−Dopamine (3,4-dihydroxylphenyl ethylamine) is the most significant neurotransmitter in the human nervous system. Abnormal dopamine levels cause fatal neurological disorders, and thus measuring dopamine level in actual samples is important. Although electrochemical methods have been developed for detecting dopamine with high accuracy, certain substances (e.g., ascorbic acid) in actual samples often interfere with electrochemical dopamine detection. We developed tyrosinase-based dopamine biosensor with high sensitivity and selectivity. An electrochemically pretreated tyrosinase/multi-walled carbon nanotube-modified glassy carbon electrode (tyrosinase/MWNT/GCE) was prepared as an amperometric biosensor for selective dopamine detection. For optimizing the biosensor performance, pH, temperature, and scan rate were investigated. The electrochemically pretreated tyrosinase/MWNT/GCE exhibited not only the highest sensitivity (1,323 $mAM^{-1}cm^{-2}$) compared to previously reported tyrosinase-based dopamine sensors, but also good long-term stability, retaining 90% of initial activity after 30 days. Additionally, ascorbic acid, a major interfering substances, was not oxidized at the potential used to detect dopamine oxidation, and the interfering effect of 4 mM ascorbic acid was negligible when monitoring 1 mM dopamine. Consequently, the electrochemically pretreated tyrosinase/MWNT/GCE is applicable for highly selective and sensitive dopamine detection in actual samples including interfering substances, thereby extending the practical use to monitor and diagnose neurological disorders.

Keywords: Tyrosinase, Dopamine, Biosensor, Multi-walled Carbon Nanotube

INTRODUCTION

Tyrosinase (E.C. 1.14.18.1) is a widely distributed oxidoreductase in nature for catalyzing hydroxylation of monophenols to *ortho*-diphenols by cresolase and oxidation of *ortho*-diphenols to *ortho*-quinones by catecholase activity using molecular oxygen as an electron acceptor [1,2]. In addition, tyrosinase utilizes various phenolic compounds as the substrate, so it is used for detecting phenolic substances in food [3], the environment [4], and for medical diagnosis [5].

Dopamine, known as 3,4-dihydroxyphenyl ethylamine, is the most important catecholamine neurotransmitter in the mammalian nervous system. As abnormal dopamine levels often cause neurological disorders such as Parkinson's disease, schizophrenia, and epilepsy [6], dopamine detection is significant to diagnose and monitor neurological disorders [7]. Given that dopamine is an electroactive chemical, various electrochemical sensors have been attempted to measure dopamine level in actual samples. Electrochemical dopamine sensors are usually highly accurate and easy to operate with a fast response time, but the sensitivity often decreases sharply in the co-existence of interfering substances (e.g., ascorbic acid) in actual samples [8]. Accordingly, a tyrosinase-based amperometric biosensor might be a promising alternative to improve sensitivity for dopamine detection in actual samples; dopamine is one of the primary substrates for catecholase activity of tyrosinase, whereas ascorbic acid, main interfering substance existing in actual samples, is not the substrate of tyrosinase [9].

Recent advances in nanotechnology enable carbon-based nanomaterials to be paid special attention as emerging electrode materials for fabricating biosensors due to their chemical inertness, large surface area for high loading density, and outstanding electrical conductivity [1,2,5,10,11]. Among various carbon nanomaterials, multi-walled carbon nanotubes (MWNT) were of interest, because enzyme-immobilized MWNT electrodes often not only minimize surface fouling from analytes or interfering substances [12] but also operate at a lower working potential [13].

As shown in Scheme 1, our aim was to develop a modified glassy carbon electrode with tyrosinase-immobilized MWNT (tyrosinase/MWNT/GCE) as an amperometric biosensor for selective as well as sensitive dopamine detection in the co-existence of ascorbic acid. Performance of the biosensor was evaluated in terms of sensitivity

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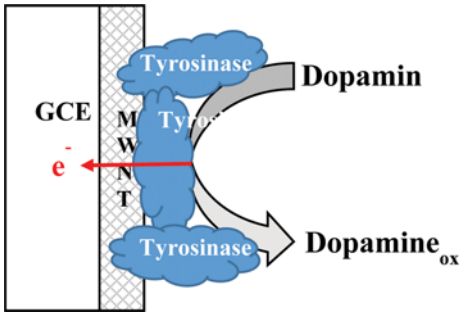

Scheme 1. Schematic illustration of tyrosinase/MWNT/GCE for dopamine detection.

and detection limit, and then compared with tyrosinase-based dopamine sensors previously reported. Additionally, the interfering effect of ascorbic acid on dopamine detection was examined to evaluate sensitivity.

MATERIALS AND METHODS

1. Materials

All chemicals including MWNT (>90%) and tyrosinase from mushroom were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the highest grade available and used without further purification. Platinum wire as counter electrode was bought from Dongsan Science (Ansan, Korea). Glassy carbon electrode (GCE) and the Ag/AgCl electrode were purchased from WonA Tech. (Seoul, Korea).

2. Preparation of Tyrosinase/MWNT/GCE as the Amperometric Dopamine Biosensor

The bare GCE was polished with aluminum slurry and ultrasonically washed with distilled water to remove the adsorbed impurities [10]. For introducing carboxyl groups, MWNT (100 mg) was treated with 10 mL of a nitric acid and sulfuric acid mixture (3 : 1 v/v %) for four hours, washed with distilled water until pH reached at 7, and then dried at ambient temperature [14]. For covalent immobilization, 2 mg of acid treated MWNT was dispersed in 400 μL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (200 mM) including 4 μL of tyrosinase (500 unit) for 1 hours [11]. The mixed solution (8 μL) was dropped on the cleaned GCE and dried at 4 °C, and 4 μL of Nafion® was used to cover the surface. Then, the modified GCE was transferred into an electrochemical cell containing phosphate buffer (50 mM, pH 7.0) and electrochemically pretreated at 2.0 V for 300 sec [15,16]. The electrochemically pretreated tyrosinase/MWNT/GCE was used for further experiments and stored in dry condition at 4 °C before use.

3. Morphological Characterization

The surface morphology of tyrosinase/MWNT/GCE before and after the acid treatment was analyzed by scanning electron microscopy (SEM, Hitachi-S4700, Tokyo, Japan) at 10 kV. A specimen coated with tyrosinase/MWNT was prepared on separate GCE and the upper portion was analyzed after cutting and fixing with an adhesive tape holder [17].

4. Electrochemical Measurement

Cyclic voltammetry and amperometric measurement were performed using an AUTOLAB potentiostat (PGSTAT302N, Metrohm, Netherlands) with commercial NOVA software. The electrochemically pretreated tyrosinase/MWNT/GCE, coiled Pt wire, and Ag/AgCl electrode were used as the working, counter, and reference electrodes, respectively. All measurements were carried out in a one-compartment reactor (50 mL) at 25 °C.

RESULTS AND DISCUSSION

1. Surface Morphology of Tyrosinase/MWNT/GCE

Fig. 1(A) and (B) show the SEM images of the tyrosinase/MWNT composite before and after acid treatment. As a result, the acid treatment did not destroy the long cylindrical structure of the MWNTs and additionally the MWNTs were well-dispersed. The well-dispersed MWNTs shown in Fig. 1(B) might contribute to a much larger surface area than the apparent geometric area.

2. Electrochemical Behavior of Tyrosinase/MWNT/GCE as the Amperometric Dopamine Biosensor

As shown in Fig. 2(a), the cyclic voltammogram of dopamine was investigated to amperometrically detect tyrosinase-catalyzing dopamine oxidation. As a result, bare GCE (× in Fig. 2(a)) slightly oxidized dopamine at 251 mV (vs. Ag/AgCl). Given that carbon nanomaterials often have dopamine detection activity [18], we also examined the cyclic voltammogram of dopamine using the MWNT/GCE as the working electrode (○ in Fig. 2(a)), but a significant peak was not observed at all. The electrochemically pretreated

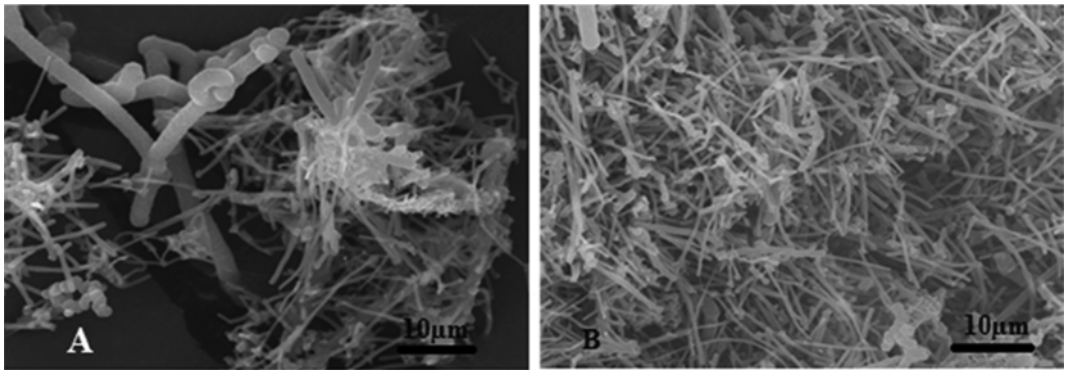

Fig. 1. SEM image of the tyrosinase/MWNT composite (A) before and (B) after acidic treatment.

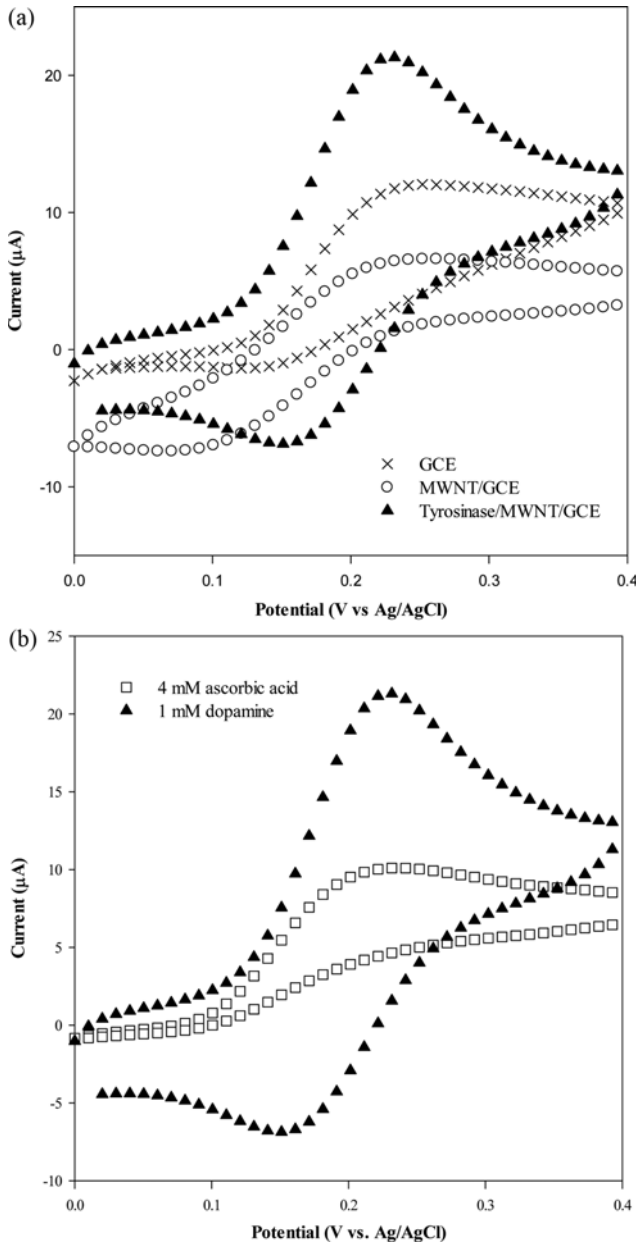

Fig. 2. Cyclic voltammogram of (a) 1 mM of dopamine in 50 mM phosphate buffer (pH 7.0) with bare GCE (×), MWNT/GCE (○), tyrosinase/MWNT/GCE (▲) and (b) 4 mM of ascorbic acid (□) and 1 mM of dopamine (▲) in 50 mM phosphate buffer (pH 7.0). Tyrosinase/MWNT/GCE, coiled Pt wire and Ag/AgCl were used as the working, counter and reference electrodes, respectively. Scan rate: 50 mVs^{-1}.

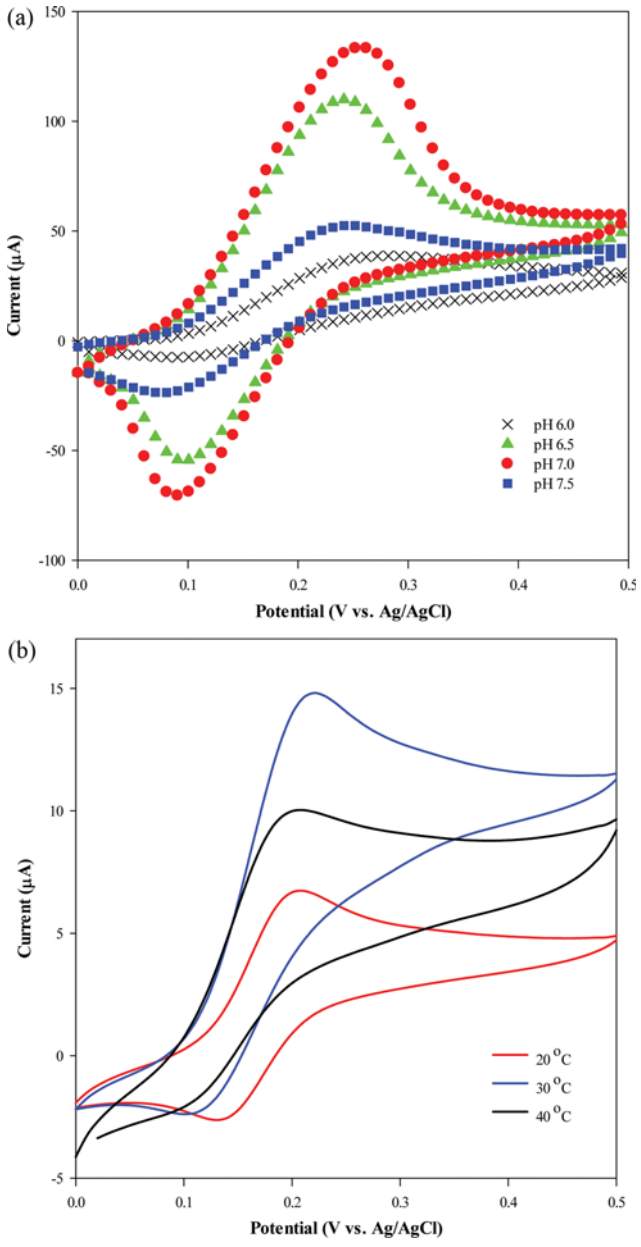

Fig. 3. Effect of (a) pH (100 mM of phosphate buffer) at 30 °C and (b) temperature at pH 7.0 on the anodic peak current of 1 mM of dopamine using the electrochemically pretreated tyrosinase/MWNT/GCE as the working electrode. Coiled Pt wire and Ag/AgCl were used as the counter and reference electrodes, respectively. Scan rate: 50 mVs^{-1}.

tyrosinase/MWNT/GCE oxidized dopamine at 231 mV, and then a reduction peak was detected at 150 mV (vs. Ag/AgCl), thereby ΔE_p of 80 mV (▲ in Fig. 2(a)). The tyrosinase/MWNT/GCE was more sensitive for dopamine oxidation compared to that of bare GCE, probably due to the tyrosinase specificity for dopamine. In addition, the small ΔE_p value represents that the electron obtained from tyrosinase-catalyzed dopamine oxidation was quickly transferred to the working electrode. For investigating whether ascorbic acid, a major inferring compound for amperometric dopamine detection in actual samples, was oxidized at the oxidation potential of dopamine, cyclic voltammogram of ascorbic acid was performed with tyrosinase/MWNT/GCE as the working electrode. As shown in Fig. 2(b), no oxidation peak was observed, even if ascorbic acid concentration was 4-fold higher than the dopamine concentration. Consequently, tyrosinase/MWNT/GCE was useful for selective dopamine detection in actual samples, including ascorbic acid.

3. Effect of pH and Temperature on Current Response

For optimizing the biosensor performance, the effect of pH and

temperature on the anodic peak response was examined with a cyclic voltammogram to optimize performance of the electrochemically pretreated tyrosinase/MWNT/GCE for dopamine detection. The pH effect was investigated at pH 6.0-7.5, resulting in optimum pH of 7.0 as shown in Fig. 3(a). The optimum pH for tyrosinase is usually known as pH 7.0 [19], and thus we concluded that the optimum pH for the biosensor was definitely dependent on the tyrosinase activity. In addition to pH, temperature influences biosensor performance. Fig. 3(b) shows that temperature did not nearly affect the reduction peak current, while anodic peak current was maximized at 30 °C. Considering that optimum temperature of tyrosinase was of 25-35 °C [20-22], the optimum temperature of the biosensor was also dependent on the tyrosinase activity. All further experiments in this study were performed at pH 7.0 (50 mM phosphate buffer) and 30 °C.

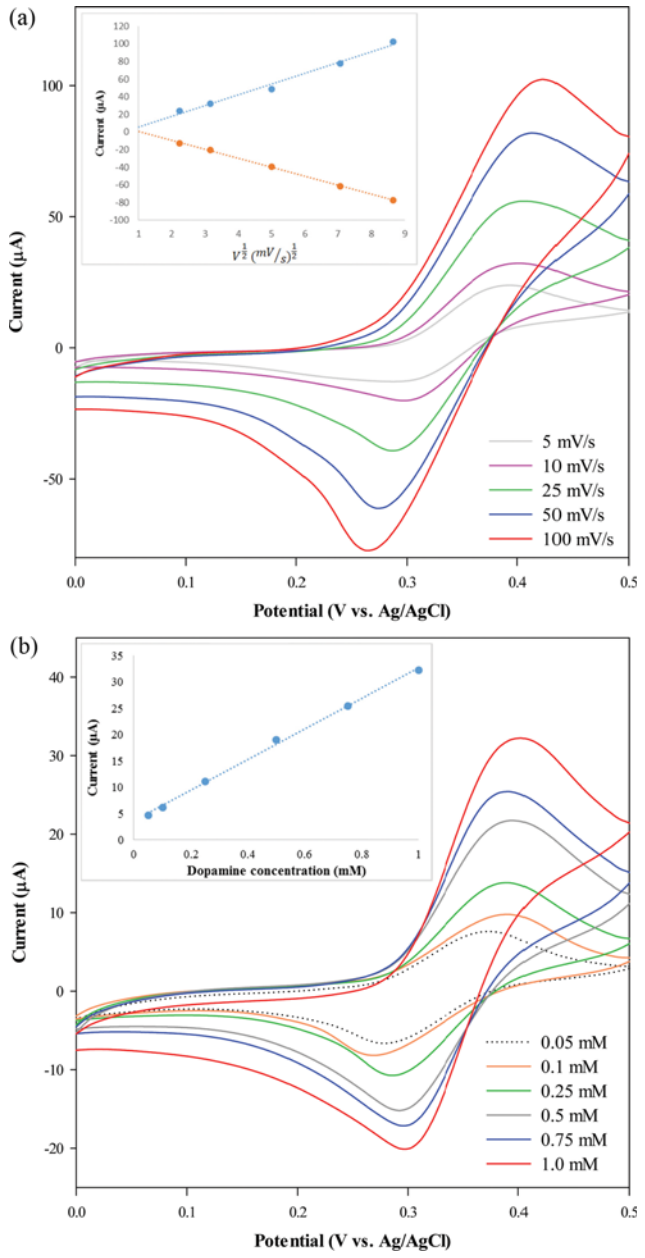

Fig. 4. Amperometric response of the electrochemically pretreated tyrosinase/MWNT/GCE to (a) scan rate with 1 mM of dopamine (Inset: Relationship between anodic [🔵] and cathodic current response [🔴] and scan rate) and (b) dopamine concentration (scan rate: 50 mVs^{-1}) (Inset: Linearity between dopamine concentration and anodic current response). Coiled Pt wire and Ag/AgCl were used as the counter and reference electrodes, respectively.

4. Performance of the Electrochemically Pretreated Tyrosinase/MWNT/GCE as an Amperometric Dopamine Sensor

We conducted amperometric detection of dopamine by cyclic voltammograms using the electrochemically pretreated tyrosinase/MWNT/GCE as a working electrode. As shown in Fig. 4(a), the anodic and cathodic peak currents were linearly proportional to scan rate and the regression equations for the oxidation (I_{pa}) and reduction peak (I_{pc}) were the following.

$$I_{pa}\ (\mu A)=12.15V^{1/2}-6.52\ (R^2=0.987)$$
$$I_{pc}\ (\mu A)=10.18V^{1/2}-11.12\ (R^2=0.999)$$

The linearity indicates that dopamine oxidation by the electrochemically pretreated tyrosinase/MWNT/GCE was a surface control process.

Additionally, a cyclic voltammogram was performed to validate the relationship between the oxidation peak current and dopamine concentration for evaluating biosensor performance such as sensitivity, linear detection range, and detection limit. As a result, the oxidation and reduction peak of dopamine was linearly dependent on dopamine concentration in the range of 0.05-1.0 mM (Fig. 4(b)). The inset of Fig. 4(b) represents the regression equation for dopamine oxidation.

$$I_{pa}\ (\mu A)=29.04V\mathrm{Conc.}_{dopamine}\ (\mathrm{mM})+3.61\ (R^2=0.998)$$

Accordingly, sensitivity and the linear detection range of the electrochemically pretreated tyrosinase/MWNT/GCE were of 1.3 $AM^{-1}cm^{-2}$ and 50-1,000 μM, respectively. The detection limit was estimated to be 50 μM (S/M=3), where S and M indicate the standard deviation of the peak currents and the slope of the calibration curve, respectively. We compared the performance of the electrochemically pretreated tyrosinase/MWNT/GCE as an amperometric dopamine biosensor with previously reported tyrosinase-based dopamine sensors (Table 1). Similar to our system, Tsai et al. prepared an amperometric dopamine sensor using tyrosinase and MWNT. Their MWNT-Nafion-tyrosinase modified GCE showed a much lower detection limit (0.52 μM), but sensitivity (12 $mAM^{-1}cm^{-2}$) was 108-fold lower than our system under a narrow linear range (5-23 μM) [1]. Zhou et al. fabricated an amperometric biosensor based on tyrosinase immobilized on a boron-doped diamond electrode (tyrosinase-BOD). The tyrosinase-BOD exhibited not only the lowest detection limit (0.1-0.5 μM) ever reported but also good sensitivity (12 $mAM^{-1}cm^{-2}$). Nevertheless, the tyrosinase-BOD was critically limited by a narrow linear range of 0.5-20 μM [8]. We previously fabricated an activated carbon/tyrosinase/Nafion-modified GCE as an amperometric dopamine sensor that functioned under a wider linear range (50-1,000 μM), but sensitivity (103 $mAM^{-1}cm^{-2}$) was not remarkable (data not shown). So, instead of activated carbon, we used MWNT to amplify the current response and then achieved outstanding sensitivity (1,323 $mAM^{-1}cm^{-2}$) under

Table 1. Performances of tyrosinase-based amperometric dopamine biosensors

Type	Sensitivity ($mAM^{-1}cm^{-2}$)	Linear range (μM)	Detection limit (μM)	Stability	Interfering compounds	Reference
MWNT-Nafion-tyrosinase composite	12	5-23	0.52	Decay of 80% after 5 min		[1]
Tyrosinase-immobilized eggshell membrane	10.6	50-250	25	Shelf life>6 month at 4 °C		[7]
Tyrosinase-immobilized on boron-doped diamond electrode	68.6	5-120	1.3	Retaining 90% of initial activity after 1month at 4 °C	Ascorbic acid	[8]
Tyrosinase entrapped in polyacrylamide gel	1.66	120-360	39.6	Useful lifetime: 27 days		[23]
Tyrosinase/NiO/ITO electrode	60.2	2-100	1.038			[12]
Poly(indole-5-carboxylic acid)/tyrosinase electrode	1300	0.5-20	0.1-0.5		Ascorbic acid Uric acid	[24]
Electrochemically pretreated tyrosinase/MWNT/GCE	1323	50-1000	50	Retaining 90% of initial activity after 1 month	Ascorbic acid	This study

a wide linear range (50-1,000 μM) as shown in Table 1.

5. Stability and Interference Study

For evaluating the stability of the electrochemically pretreated tyrosinase/MWNT/GCE as the dopamine biosensor, the oxidation peak current at cyclic voltammogram of dopamine (1 mM) was time-dependently monitored. As a result, the electrochemically pretreated tyrosinase/MWNT/GCE retained 90% of the initial anodic current response after 30 days, thereby showing good long-term stability (Table 1).

Ascorbic acid, which is electroactive and usually 50-70 μM in human blood [17], is well-known as the most common interfering compound for dopamine detection in actual samples (Table 1). As shown in Fig. 5, 4 mM of ascorbic acid, which is much higher than physiological level, increased the current response, but the current response was rapidly stabilized within 1 min. Consequently, the interference effect of ascorbic acid was negligible to monitor 1 mM of dopamine, and thus the electrochemically pretreated tyrosinase/MWNT/GCE is applicable for highly selective and sensitive dopamine detection.

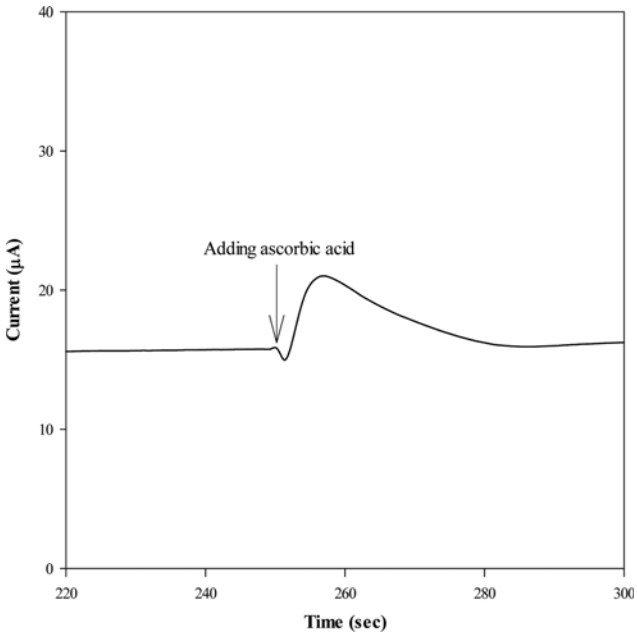

Fig. 5. Interference study using 4 mM of ascorbic acid to monitor 1 mM of dopamine by exposing the biosensor (tyrosinase/MWNT/GCE).

CONCLUSION

An electrochemically pretreated tyrosinase/MWNT/GCE was developed as an amperometric biosensor to selectively and sensitively detect dopamine in actual samples, including ascorbic acid that is a major interfering substance for electrochemical dopamine detection. Compared with previously reported tyrosinase-based dopamine sensors, the electrochemically pretreated tyrosinase/MWNT/GCE exhibited the highest sensitivity (1,323 $mAM^{-1}cm^{-2}$) and a wide linear range (50-1,000 μM). Cyclic voltammogram of ascorbic acid revealed that ascorbic acid was not oxidized at the potential for dopamine oxidation. Additionally, 4 mM of ascorbic acid had a negligible interfering effect when monitoring 1 mM of dopamine. Consequently, the results indicated that this electrochemically pretreated tyrosinase/MWNT/GCE is applicable for dopamine detection in actual samples with high selectivity and sensitivity, thereby extending its feasibility to monitor and diagnose neurological disorders.

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