ABSTRACT

We report fabrication and preliminary testing results of an electrochemical sensor that utilizes a differently modified cyclodextrin-based micro electrode array to simultaneously detect catecholamine neurotransmitters. Individual concentrations of multiple neurotransmitters are known to have critical effects on various human behaviors and diseases; however, their simultaneous detection has been never achieved due to the limited resolutions in previous enzyme-based sensors. We employ various types of cyclodextrins, known to recognize diverse targets through size-matching, into a micro electrode array to precisely detect such subtle differences in, particularly, catecholamine neurotransmitters. We have successfully fabricated a α-, β-, and γ-cyclodextrin-based electrode array. Also, the fabricated electrode array has successfully identified the neurotransmitter, L-tyrosine, L-dopa, and dopamine in 10mM solutions.

KEYWORDS: Cyclodextrin, Neurotransmitter, Electrochemical Sensor

INTRODUCTION

Numerous medical literature has reported that Parkinson’s disease has high correlation with the lack of dopamine (DA) through the damage of dopaminergic neurons in the nigrostriatal pathway of the brain [1-2]. DA is a part of catecholamine neurotransmitter and its biosynthetic pathway is well-known: DA is converted from dihydroxyphenylalanine (L-dopa) by an enzyme of dopa decarboxylase, and L-dopa is converted from L-tyrosine by another enzyme of tyrosine hydroxylase. Thus, simultaneous measurements of the concentrations of these individual neurotransmitters could provide an meaningful insight on the failure mechanisms of individual enzymes, their causes, and thus some health risks exposed. Many efforts have been made to develop a few analytical methods for monitoring catecholamine neurotransmitters [3-4]. However, the simultaneous detection of these neurotransmitters have not been successful because the conventional enzyme-based electrochemical sensors, based on the redox reaction of catechol groups with tyrosinase (only counting the number of electron transfers), could not distinguish which neurotransmitters have contributed how much percentage of the measured results. Thus, there have been no such successful methods of detecting multiple neurotransmitters at the same time.

Our approach, to overcome such issues, is the use of cyclodextrins (CDs) modification to separately induce each neurotransmitter into each designated electrode such that the outputs of each electrode would indicate individual concentrations of the targets. The different affinity of each neurotransmitter to each CD’s are reported in some previous articles [3-5]. Here we employ different diameters of the α-, β-, and γ-CDs, which have six, seven, and eight D-glucopyranose units to respectively match with individual sizes of catecholamine neurotransmitters, such as L-tyrosine, L-dopa, and DA. In this paper, we report the development of a miniaturized electrochemical sensor by utilizing using a α-, β-, and γ-CD modified micro electrode array and by developing a new fabrication method for individual surface modification on the same device substrate, thus to achieve the simultaneously monitoring of multiple catecholamine neurotransmitters.

STRUCTURE AND OPERATION PRINCIPLE
Figure 1 illustrates the final fabricated device consisting of differently functionalized electrodes on the silicon substrate with attaching reservoir. A conventional three electrode cell was applied, with saturated Ag/AgCl electrode as the reference electrode, an untreated thin film Au electrode as the counter electrode and each α-, β-, and γ-CD modified Au electrodes as the working electrodes within a footprint of 21×13mm².

Au electrodes for cyclodextrin-modification were formed on SiO₂ layer, as shown in Fig. 2 (a). The integrated micro-electrode array was fabricated using the following steps: (1) growth of the SiO₂ layer (300nm) by thermal oxidation, (2) deposition of TiW/Au (30/600nm) on an insulated silicon wafer by sputter, (3) coating with photoresist by spin coating, (4) prebaking 80 °C for 20 min, (5) patterning by aligner, (6) immersion in drying solution for 20s, and (7) etching at the outside of micropatterned electrodes by KI and diluted H₂O₂ solution. After cleaning micropatterned electrodes on SiO₂ with H₂O for 5 min, the electrodes were annealed in thermal chamber.

The fabricated micro-electrode array for functionalizing amino-terminated monolayer was treated in a 1mM cysteamine for 12 h, which was prepared in ethanol. Cysteamine (95%), α-, β-, and γ- CD for functionalizing Au surface were purchased from Sigma-aldrich, USA. The modified electrodes were immersed in a 30mM of chlorobutyric acid for 2h, which are mixed with N-hydroxysuccinimide (NHS) and ethyl (dimethylaminopropyl) carbodiimide (EDC) for amide binding between chlorobutyric acid and the amino group on electrodes. Small droplets of the each mixedt of 1mM α-, β-, and γ-CD, 20ml of aqueous KOH (4M) were deposited manually on the each arrayed electrode surface at 65°C for 24h, as shown Fig 2 (b).

RESULTS AND DISCUSSION

FT-IR spectra were used to investigate the CDs modification on Au surface, as shown in Fig. 3. This FT-IR measurements were used over a wavelength range of 500 – 4000nm with a Perkin-Elmer Spectrum. In the IR spectra of cystamine treated Au (a), the characteristic peaks of -NH₂ bending vibration appeared at 1545cm⁻¹ and the symmetrical stretching vibrations were served, corresponding to the C-O-C stretching vibration and amide binding between amine and carboxylic group, respectively. Additionally, in comparison  with the cystamine treated Au spectrum, two new peaks at 1018cm⁻¹ and 1732 cm⁻¹ in spectrum of chlorine-terminated Au (b) after treating 4-chlorobutyric acid were observed, corresponding to the C-O-C stretching vibration and amide binding between amine and carboxylic group, respectively. After immobilizing α-CDs on Au surface (c), the new peaks of typical CDs also were observed at 847 cm⁻¹ (C-H deformation in α-pyranose compounds) and 953 cm⁻¹ (asymmetric pyranose ring stretch). These evidences indicated CDs had immobilized on Au surface.

In figure 4, the survey scans on functionalized substrates primarily used to observe C 1s, N 1s, O 1s and Cl 2p peaks according to the corresponding process; (a) preparation of bare Au electrode, (b) cysteamine treatment for amino termination, (c) chlorobutyric acid treatment for chlorine termination and (d) α-cyclodextrin attachment for 24 hours. These results were measured by X-ray Photoelectron Spectroscopy (Kratos AxisUltraDLDL). From this figure (b), we can attribute the cysteamine attached on Au surface because the carbon (281.6 eV) and nitrogen (397.1 eV) peaks after treatment of cysteamine are observed. Additionally, it is observed the chlorine peak (192.6 eV) on Au surface after treating chlorobutyric acid although it is difficult to do quantitative analysis of the chlorine due to weak signal. However, the O 1s spectrum observed a peak at 529.4 eV is due to the increased hydrophilicity on the functionalized Au surface and thus the surface easily absorbs water molecules. Because chlorine peak is not observed after immobilization of α-cyclodextrin, the existence of the α-cyclodextrin on the Au electrode was confirmed by the presence of the Cl 2p peak in Fig. 4(d). Therefore, the results show that the α-cyclodextrin was successfully immobilized on the Au surface.

Figure 5 shows quasireversible voltamogram in 10mM (a) DA, (b) L-DOPA, and (c) L-tyrosine solutions at pH 7 at 100mV/s scan rate on β-CDs-modified electrode for measuring selectivity. Electrochemical measurements were performed on CHI-400D electrochemical Workstation (CH Instrument, Inc). Also, standards of the tested compounds, dopamine (DA), L-tyrosine, and 3,4-dihydroxyl-L-phenylalanine (L-dopa), were obtained from Sigma. In the DA solution,
The ΔE_p was found to be 380mV and the I_p/I_c was 0.3 on β-CDs-modified electrode. Also, in both of L-dopa and L-tyrosine, the ΔE_p were obtained 280mV and 200mV, and I_p/I_c were 0.44 and 0.67, respectively. This results indicated that the CD-modified electrode include quasireversible characteristics and selectivity in neurotransmitters although it is shown that a pair of redox on β-CDs-modified electrode is obtained with strong increase in both anodic and cathodic peak current than both of L-DOPA and L-tyrosine solutions. So, the cyclic voltammograms obtained on the β-CDs-modified electrode show good electrocatalytic properties and well-defined selectivity against tested neurotransmitters.

The sensitivity was tested by applying 10mM dopamine solution to each electrode. Figure 6 shows the graph of anodic peak current (I_p) vs. square root of scan rate (v^1/2) in 10mM DA solution obtained linear relationship on the α-, β-, γ-CDs-modified electrodes, respectively. The results show that the anodic currents in dopamine grow gradually on all of modified electrode when scan rate increases from 5 to 100mVs^{-1}. The correlation co-efficient were found to be 0.9973, 9763, and 9721, respectively. According to the Randle-sevcik equation, i_p=(2.687x10^{5})n^{3/2}v^{1/2}D^{1/2}AC, the ratio of slopes also shows that the active site of dopamine oxidation on α- and γ-CDs-modified electrodes is approximately 4 times higher sensitivi-ty than the β-CDs-modified electrodes in 10mM dopamine solution.

CONCLUSION
In this paper, we successfully fabricated the a α-, β-, and γ-CDs-modified electrode array on silicon substrate for detection of neurotransmitter by developing a isolation-based fabrication technologies, and the fabricated electrode array has successfully identified L-tyrosine, L-dopa, and dopamine allowing the simultaneous detection. The cyclic voltammogram on the β-CDs-modified electrode show well-definded selectivity in these solution. Also, the α - and γ-CDs-modified electrodes is approximately 4 times higher sensitivity than the β-CDs-modified electrodes in 10mM dopamine solution.

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REFERENCES

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