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A cyclodextrin-based approach for selective detection of catecholamine hormone mixtures

Jung-Hoon Yang, Hyun Tae Kim and Hanseup Kim*

Abstract

This paper presents an electrochemical sensing approach that enables quantitative detection of three major catecholamine hormones from a mixture by specifically employing a chemically-modified microelectrode array with α-, β- and γ-cyclodextrin (CD) 'catchers' holding unique physical matching (size and shape) as well as chemical enticing (stereochemistry and surface charge) properties. The developed neurotransmitter sensor has selectively identified L-tyrosine, dihydroxyphenylalanine (L-DOPA) and dopamine in the absence of ascorbic acid. It exhibited the relatively linear sensitivities to each neurotransmitter with logarithmically increasing concentrations range of 5μM-10mM, while demonstrating stability up to 6 hours from the fabrication and the average accuracy of 91.2%.

Keywords: Cyclodextrins; Catecholamine hormone; Neurotransmitter; Micro electrochemical sensor

Introduction

Neurodegenerative diseases present an increasing burden for the world health care sector. Currently, 6.5 million Americans suffer from the neurodegenerative diseases such as Alzheimer's disease (5.4 million), Parkinson's disease (1 million), amyotrophic lateral sclerosis (30,000), and Huntington's disease (15,000) [1-3]. Since the neurodegenerative diseases are primarily an age-based disease with the increasing incidence after age 65 years [4,5] and the U.S. population continue to age [6], it is estimated that, by 2040, more than 13.1 million Americans will be diagnosed with neurodegenerative diseases. Such increase of the neurodegenerative disease population will cause the corresponding growth of social costs of treating neurodegenerative diseases; thus, developing effective treatments for the neurodegenerative diseases is an urgent and critical issue.

For diagnosing and treating the neurodegenerative diseases, monitoring the concentrations of co-existing catecholamine neurotransmitters could be critical because concentrations represent the effectiveness of the derangement of neurotransmitters transferring through a neuron terminal and was shown to be associated with many neurodegenerative diseases. For example, Parkinson's disease has high correlation with the lack of dopamine (DA) due to the loss of dopaminergic neurons in the substantia nigrapars compacta and degeneration of the nigrostriatal pathway [7-9]. Although DA concentration is the direct cause of such diseases, since the biosynthetic pathway of major catecholamine neurotransmitters has been well-understood (Figure 1), the concentration measurements of these individual neurotransmitters (L-Tyrosine, L-DOPA and DA) could provide meaningful insights on the failure mechanisms along the pathway. L-tyrosine is converted into L-DOPA by Tyrosine hydroxylase enzyme, while L-DOPA, in turn, is transformed into DA through Dopa decarboxylase enzyme. Thus, the extra-ordinarily low concentration of DA while normal concentrations of L-tyrosine and L-DOPA would indicate the damage of Dopa decarboxylase enzymes or its neuronal pathway. Such insights, thus, could be an excellent indicator of the status of some neurodegenerative diseases. Therefore, the monitoring of DA as well as the closest pre-derivatives of dopamine, such as L-tyrosine and L-DOPA, would be the main focus of this study.

The detection of neurotransmitters has been performed largely in four ways: electrochemical detection (ECD) [10-17], fluorescence detection (FD) [18-21], chemiluminescence detection (CLD) [22-24], and mass spectrometric detection (MSD) [25]. However, none of those approaches have been successful in selectively detecting the co-existing individual catecholamine neurotransmitters without using additional separation techniques such as liquid chromatography or capillary electrophoresis that require complex

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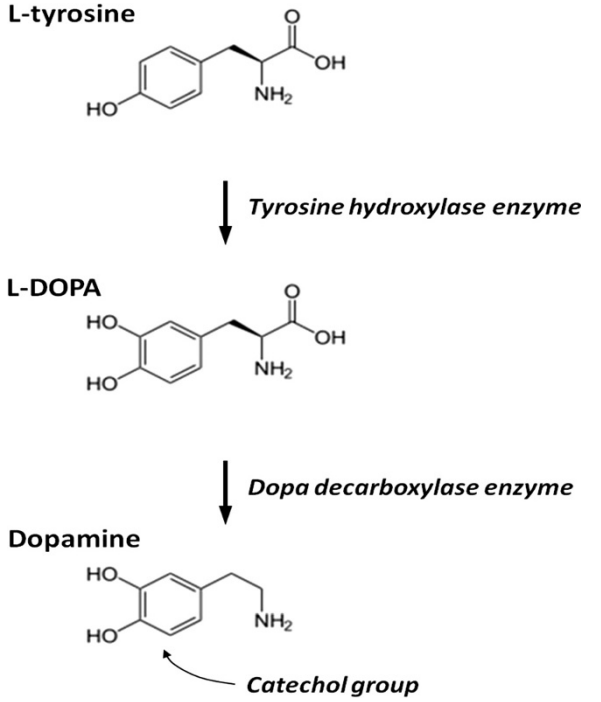

Figure 1 Biosynthetic pathway of catecholamine neurotransmitters.

system, long analysis time, and high power consumption. Particularly, the ECD has been most frequently utilized for the neurotransmitter detection due to its advantages of high sensitivity, real time analysis, low cost, and easiness of system miniaturization [17,26]; however, only the detection of a single catecholamine neurotransmitter has been successfully achieved because the conventional enzyme-based electrochemical sensors, based on the redox reaction of catechol groups with tyrosinase, was incapable of distinguishing the neurotransmitters because each DA and L-DOPA has identical catechol groups (two OH groups attached to one benzene ring) that similarly react with the enzyme attached to electrodes and generate redox reaction current.

To overcome such limitation we developed an electrochemical sensor using a differently-chemically-modified microelectrode array with various CycloDextrin (CD) 'catchers' that are capable of engaging differently-sized target molecules respectively due to physically unique molecular structures. Note that previously it was known that a CD could trap certain molecules better due to its "basket"-type physical shapes, also known as the host-guest recognition [12-14,27-30]; however, it has not been applied for the selective identification of multiple catecholamine neurotransmitters. Here we focus on the use of the CDs' size differences to statistically produce unique affinities to each of co-existing catecholamine neurotransmitters. Especially, we employed three different α-, β-, and γ-CD catchers, which have respectively six, seven, and eight D-glucopyranose units based on their physically 'matching' sizes (Figure 2), to identify three targets of L-tyrosine, L-DOPA and DA.

Recently the use of cyclodextrins has been proven to detect dopamine (DA) despite the interference of ascorbic acid (AA) in a mixture [31-33]. Ascorbic acid has been known to interfere the detection of dopamine due to the overlap of the oxidation potentials and much higher (>100 times) concentrations. Based on these previous results, it was reasonably hypothesized that the proposed approach would properly function even in the presence of ascorbic acids. Thus, in this paper we report the simultaneous and quantitative detection approach to distinguish L-tyrosine, L-DOPA and Dopamine in the absence of ascorbic acids as the proof-of-concept. Particularly, we report the structure, fabrication and characterization of a micro neurotransmitter sensor as the proof-of-concept device, with particular foci on detecting DA as well as its closest pre-derivatives. The details will be reported of (1) microelectrode array fabrication, (2) electrode functionalization, (3) sensing sensitivity and selectivity and (4) simultaneous identification of the mixture ratios among L-tyrosine, L-DOPA and Dopamine.

Findings

Structure and operation principle

The developed micro neurotransmitter sensor consists of a microelectrode array and an electrochemical chamber on a silicon substrate (Figure 3-right bottom). The microelectrode array has four working electrodes (2 mm diameter) at which electrochemical reaction takes place and one counter electrode at the center among the four working electrodes. Three of the four working electrodes are differently and chemically functionalized by immobilizing α-, β-, and γ-CDs respectively on its surface, while the fourth electrode is not coated as a reference electrode. The counter electrode works as a ground in potential measurement. The electrochemical chamber contains the target solution with neurotransmitters and exposes it to all five electrodes for analysis. The total footprint of the sensor was 13×21 mm^2.

The developed neurotransmitter sensor produces three output signals through the host-guest recognition from each of the three CD-coated electrodes, and the combination of those three signals enables the identification of each concentration of the co-existing three target neurotransmitters, such as L-tyrosine, L-DOPA and dopamine. Since each electrode is immobilized with different CDs with unique size, shape, stereochemistry and surface charge, its surface demonstrates variable affinities to each catecholamine neurotransmitter. For example, the total output voltage, measured at the α-CD electrode, is the combined results from the respective responses to L-tyrosine, L-DOPA and dopamine. Thus, each output signal at each electrode is the combined contribution of each CD to all three target neurotransmitters. By combining such a relationship from all

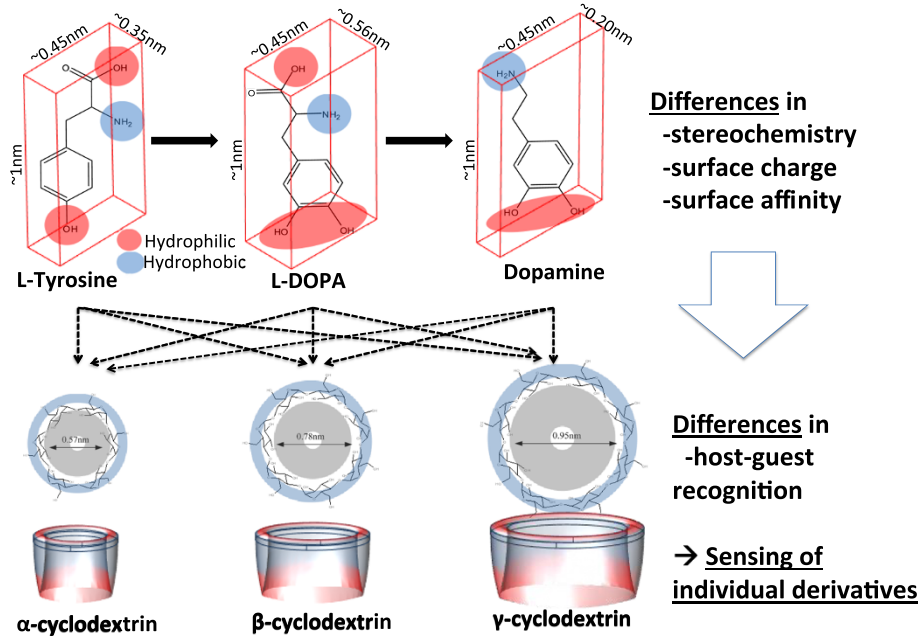

Figure 2 Illustration of the sensing principle: differences in stereochemistry, surface charge and surface affinity of the three catecholamine neurotransmitter derivatives cause different levels of host-guest recognition to α-, β- and γ-cyclodextrins, respectively, enabling the sensing of individual derivative ratios from a mixture.

three electrodes coated with three α-, β-, and γ-CDs, the relationship of the output signals can be expanded into three equations:

$$A = \alpha_1 \cdot LT + \alpha_2 \cdot LD + \alpha_3 \cdot DA + \alpha_4$$
$$B = \beta_1 \cdot LT + \beta_2 \cdot LD + \beta_3 \cdot DA + \beta_4$$
$$C = \gamma_1 \cdot LT + \gamma_2 \cdot LD + \gamma_3 \cdot DA + \gamma_4$$

where A, B and C are the measured output signals at each electrode with α-, β- and γ-cyclodextrins (CDs) coating, respectively. α_1, α_2, and α_3 are the response coefficients of the α-CD electrode, while α_4 are the sum of the offset values to each target neurotransmitter. The same relationship exists for β_1, β_2, β_3, β_4, γ_1, γ_2, γ_3 and γ_4. LT, LD and DA indicate the concentrations of L-tyrosine, L-DOPA and DA, respectively. Under the hypothesis that this matrix is consistent (i.e. all the coefficients are independent), this set of three equations could be solved to determine the concentrations of LT, LD, and DA simultaneously, therefore enabling the identification of the co-existing three neurotransmitters of interests.

$$\begin{bmatrix} LT \\ LD \\ DA \end{bmatrix} = \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \beta_1 & \beta_2 & \beta_3 \\ \gamma_1 & \gamma_2 & \gamma_3 \end{bmatrix}^{-1} \cdot \begin{bmatrix} A-\alpha_4 \\ B-\beta_4 \\ C-\gamma_4 \end{bmatrix}$$

Note that in order to prove the selectivity of the method, one exemplary ascorbic acid (vitamin C) was mixed into a solution for in-vivo-like consideration while measuring the three neurotransmitters.

Fabrication

Micro electrode array fabrication

The microelectrode array was fabricated on a silicon substrate (Figure 3-left). First, a SiO_2 layer (200 nm) was grown by wet oxidation, which served as an insulating layer. Second, a TiW/Au layer (30 nm/600 nm) was sputtered on top of the SiO_2 layer, where the TiW layer enhanced the adhesion between the Au and SiO_2. Third, the deposited metal layers were patterned using photolithography. KI and diluted H_2O_2 solution were used as metal etchant and Shipley 1813 positive photoresist as an etch mask. Finally, the patterned substrate was cleaned by Acetone, IPA, and DI water. The footprint of each electrode was 7.065 mm^2.

Electrode functionalization

The fabricated microelectrode array was functionalized by immobilizing CDs on its surface (Figure 3-right top). First, the fabricated device was soft-baked on a hot plate at 80°C for 10 min to remove residual water molecules on the electrode surface because water molecules can prevent uniform coating of CDs molecules. Second, it was treated in a 1 mM cysteamine for 12 hr, which was prepared in ethanol, for functionalizing amino-terminated monolayer. Third, the modified electrode array was immersed in a 30 mM of chlorobutyric acid (CA) for 2 hr, which was mixed with 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M ethyl (dimethylaminopropyl) carbodiimide (EDC). This procedure enabled to form amide binding between the chlorobutyric acid and amino group, which changed the functionalized

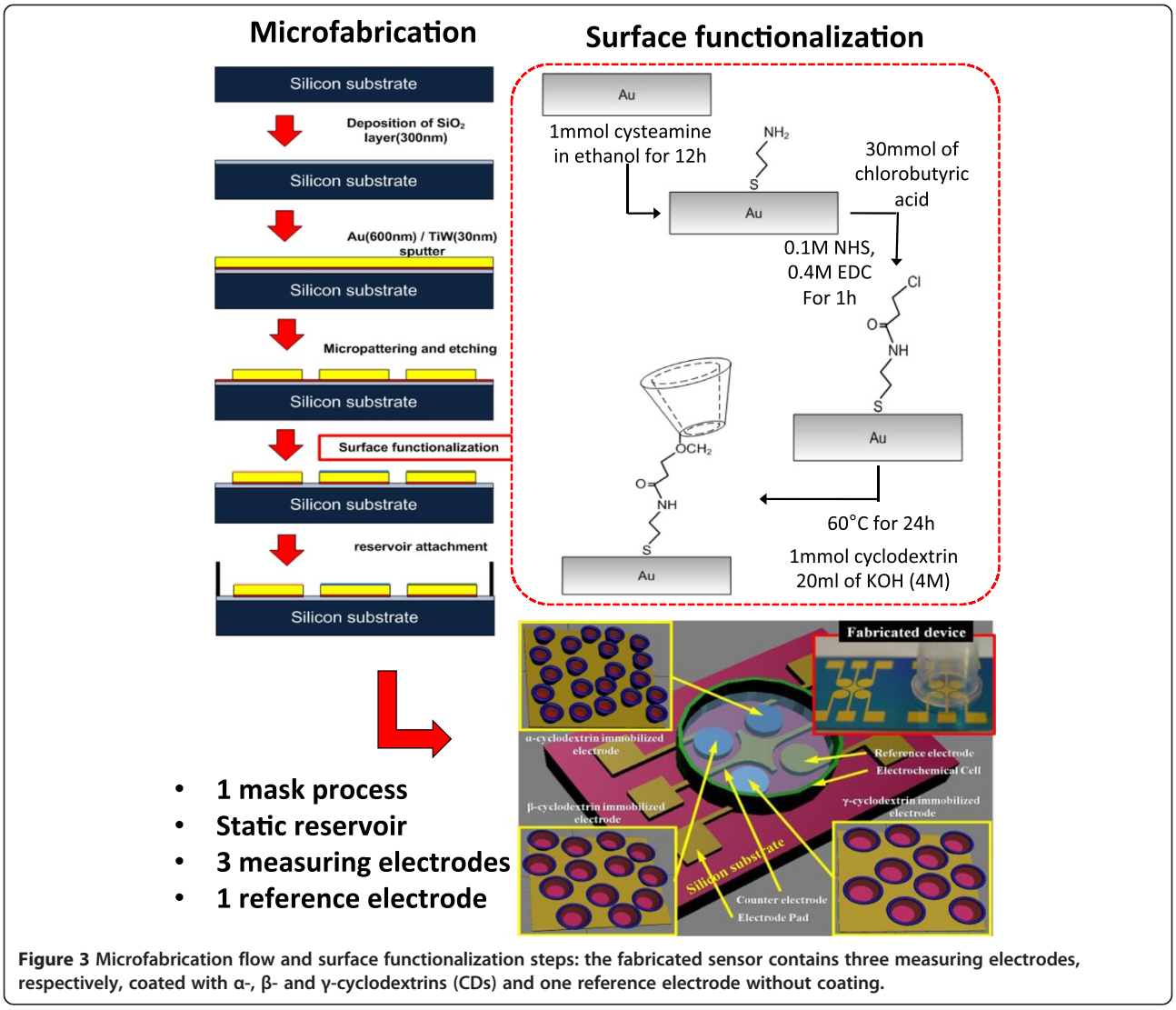

Figure 3 Microfabrication flow and surface functionalization steps: the fabricated sensor contains three measuring electrodes, respectively, coated with α-, β- and γ-cyclodextrins (CDs) and one reference electrode without coating.

electrode to be hydrophilic. Finally, small droplets of the each mixture of 1 mM α-, β-, and γ-CDs and 20 ml of aqueous 4 M KOH were selectively deposited on top of each arrayed electrode at 65°C for 24 hr. The α-, β-, and γ-CDs and all the chemicals that used for CD immobilization such as Cysteamine (95%), CA, NHS, and EDC were purchased from Sigma-Aldrich Co., USA.

Packaging

The functionalized microelectrode array was connected to an electrochemical measurement system using copper wire. The room temperature curable conductive paste (Silver conductive adhesive paste, Alfa Aesar) was used to bond the wire such that the immobilized CDs could avoid the thermal damage from conventional solder bonding. While curing the conductive paste, the microelectrode array was covered with a water-absorbed filter paper (1002–185, Whatman) to keep the CDs being sufficiently moisturized. After the conductive paste cured, an electrochemical chamber was built on top of the fabricated sensor chip by bonding a polycarbonate tube (inner diameter 10 mm, length 15 mm) with epoxy (Quick Set™, Loctite).

Methodology

Analysis of the functionalized electrode

The functionalized microelectrode array was analyzed utilizing Fourier transform infrared spectrometry (FT-IR) and X-ray photoelectron spectroscopy (XPS) to confirm the immobilization of α-, β-, and γ-CDs. The FT-IR results identify existence of chemical bindings after each step of electrode functionalization over a wavelength range of 500-4000 nm (Perkin-Elmer Spectrum 100), while the XPS measurements identify the existing atoms on the electrode over electron beam power of 0-1000 eV (Kratos analytical Axis Ultra DLD).

Electrochemical measurement

The fabricated neurotransmitter sensor was connected to a potentiostat (Reference 600, Gamry Instruments) and test data was monitored using a labview-based data recording system (VFP600). The electrochemical cell was filled with target catecholamine hormone solution, and the fabricated sensor was tested in random mixtures of catecholamine hormone targets (L-tyrosine, L-DOPA, and DA), concentrations (5 μM-10 mM), and scan rate (1-100 mV/s). First, I-V curves of each electrode were measured in a specific neurotransmitter concentration to measure the individual responses of each CDs immobilized electrode. Next, by varying the neurotransmitter concentrations between 5 μM-10 mM at every decade, the response curves were constructed for each CD electrode and neurotransmitter. The slope of the curve provides the sensitivity of each electrode to each neurotransmitter. Then, from the collected sensitivity coefficients, the matrix of quantification was constructed to identify the individual concentrations from a mixture. Finally, the individual concentrations were extracted from random mixtures of L-tyrosine, L-DOPA and dopamine and compared to the original values, in order to validate the developed sensor and method. Measurement was repeated at least five times, and all the catecholamine hormones were purchased from Sigma-Aldrich Co., USA.

Results and discussion

Electrode functionalization

FT-IR spectra measurements at each stage of the surface coating indicated that the CDs were successfully immobilized on the Au surface of each electrode, as shown in Figure 4-top. First, the characteristic peaks of -NH_2 bending vibration and the symmetrical stretching vibration of C-N appeared at 1545 cm^{-1} and at 1153 cm^{-1}, respectively after the first step of cystamine treatment on Au electrodes. This indicates the formation of first amine functional layer for the subsequent hydrogen bonding. Second, two new peaks were measured at 1018 cm^{-1} and 1732 cm^{-1} after the treatment of 4-chlorobutyric acid on the Au electrode. These peaks correspond to the C-O-C stretching vibration and amide binding between amine and carboxylic group, respectively, indicating that the outermost coating now becomes chlorobutyric acid available for CD immobilization. Finally, the additional new peaks were also observed at 847 cm^{-1} (C-H deformation in α-pyranose compounds) and 953 cm^{-1} (asymmetric pyranose ring stretch). These peaks are conventional signatures for typical CDs, evidencing that CDs had been immobilized on the Au surface as the outmost chemical acceptor.

XPS survey scan measurements, complementing the FT-IR spectra results, indicated that the key elements, such as nitrogen and chlorine, appeared and disappeared as the surface coating progresses. Figure 4-bottom displays the baseline binding energy of each element at the bare Au surface. In comparison, the peak of nitrogen (N 1 s) was observed at the binding energy of 397.1 eV after cysteamine treatment that creates amino termination. The peak for carbon (C 1 s) was also measured throughout the coating steps at the binding energy of 281.6 eV. Then, an additional peak of chlorine (Cl 2p) was measured at the binding energy at 192.6 eV, after chlorobutyric acid treatment that forms chlorine termination. Note that the oxygen (O 1 s) spectrum was observed as a distinctive peak at 529.4 eV, indicating the increased hydrophilicity on the functionalized Au surface. This enables the surface to easily interact with water molecules thus efficiently capture CDs. The peak of chlorine was not measured once the cholorobutyric acid replaced chlorine element with α-CDs. This confirms the existence of the α-CDs on the Au electrode.

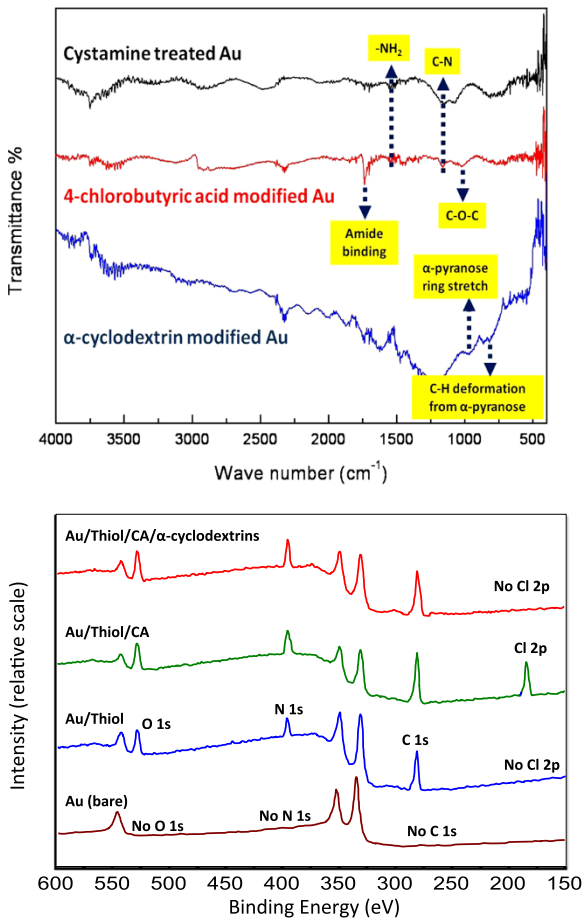

Figure 4 Surface analysis results of (top) FT-IR spectra and (bottom) XPS measurement at each step of the electrode functionalization: bare Au, cysteamine (thiol) coating, chlorobutyric acid (CA) treatment, and α-cyclodextrins (CDs) immobilization. Signature binding as well as key elements such as O 1 s, N 1 s, C 1 s and Cl 2p, distinguishes the surface coating at each functionalization step.

Identification of each catecholamine neurotransmitter from a mixture

Cyclic voltammetry measurements demonstrated that each electrode responses to respective neurotransmitter with different order of preferences and degrees (Figure 5). The γ-electrode produced the highest output under the same concentration of L-tyrosine, while the β-electrode showed the maximum signal outputs from both L-DOPA and dopamine. Although the α-electrode did not demonstrate the highest output to any of the neurotransmitters, it responded the second highest output to L-tyrosine and dopamine. Figure 5 shows the cyclic voltammograms respectively obtained from 1 mM of L-tyrosine, L-DOPA and DA in PBS from each electrode with the footprint of 7.065 mm^2, resulting in different output responses under the same neurotransmitter. In response to 1 mM of L-tyrosine at the L-tyrosine oxidation potential, the γ-electrode produced the highest output current of 1.765 μA and then the β- and α-electrodes produced 1.176 and 0.515 μA, respectively (Figure 5-top). This corresponds to the current densities of 0.250, 0.167 and 0.073 μA/mm^2. The electrodes with chlorobutyric acid or cysteamine coating without cyclodextrins did not produce measurable output currents. The oxidation potential of each neurotransmitter was separately obtained utilizing conventional platinum electrodes. In response to L-DOPA, the generated output signals ranged at 12.123, 7.397 and 6.37 μA with coatings of β-, γ-, α-cyclodextrins, chlorobutyric acid and cystemine (Figure 5-Middle), with corresponding current densities of 1.716, 1.047 and 0.902 μA/mm^2, respectively. In the 1 mM dopamine solution, the β-, α- and γ-electrodes produced current outputs of 11.811, 9.213 and 7.323 μA and densities of 1.672, 1.304 and 1.037 μA/mm^2, respectively (Figure 5-bottom). These results indicate that each cyclodextrins, thus each electrode, holds different affinity in catecholamine hormones. Note that the minimum detectable amount was 5 μM, limited by the resolution of measurement. Also note that the testing was performed up to 10 mM of concentrations to fully cover the maximum range feasible in-vivo environment. Thus, the overall concentration ranges tested were limited between 5 μM and 10 mM in this paper.

Amperometric measurement results demonstrated that the output current produced from each electrode with α-, β-, and γ-immobilization increased linearly with increasing concentrations between 0.005 and 10 mM in logarithmic scale (Figure 6). The linear relationship between each electrode and neurotransmitter produced substantially different slopes, which confirms selective discrimination of each electrode by the terminated CDs. In response to L-tyrosine, α-, β- and γ-electrodes respectively exhibited linear slopes of 0.2035, 0.1055 and 0.2897, which forms a multiplication factor matrix as:

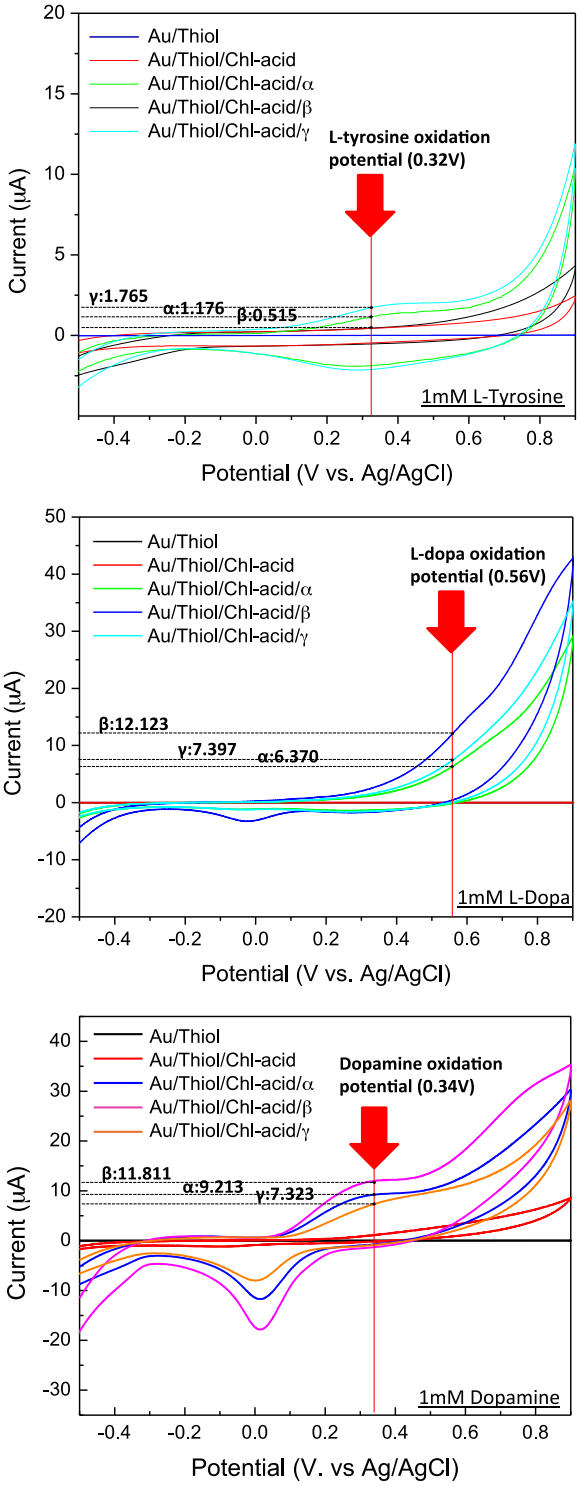

Figure 5 Cyclic voltammograms of each electrode with the α-, β-, and γ-CDs immobilization, respectively, under the 1 mM solutions of (top) L-tyrosine, (Middle) L-dopa and (bottom) dopamine. The scan rate was 100 mV/s. The current amounts at the given concentration (1 mM) were identified to produce the quantification matrix at each oxidation potential point that was measured without CD immobilization. The footprint of each electrode was 7.065 mm^2.

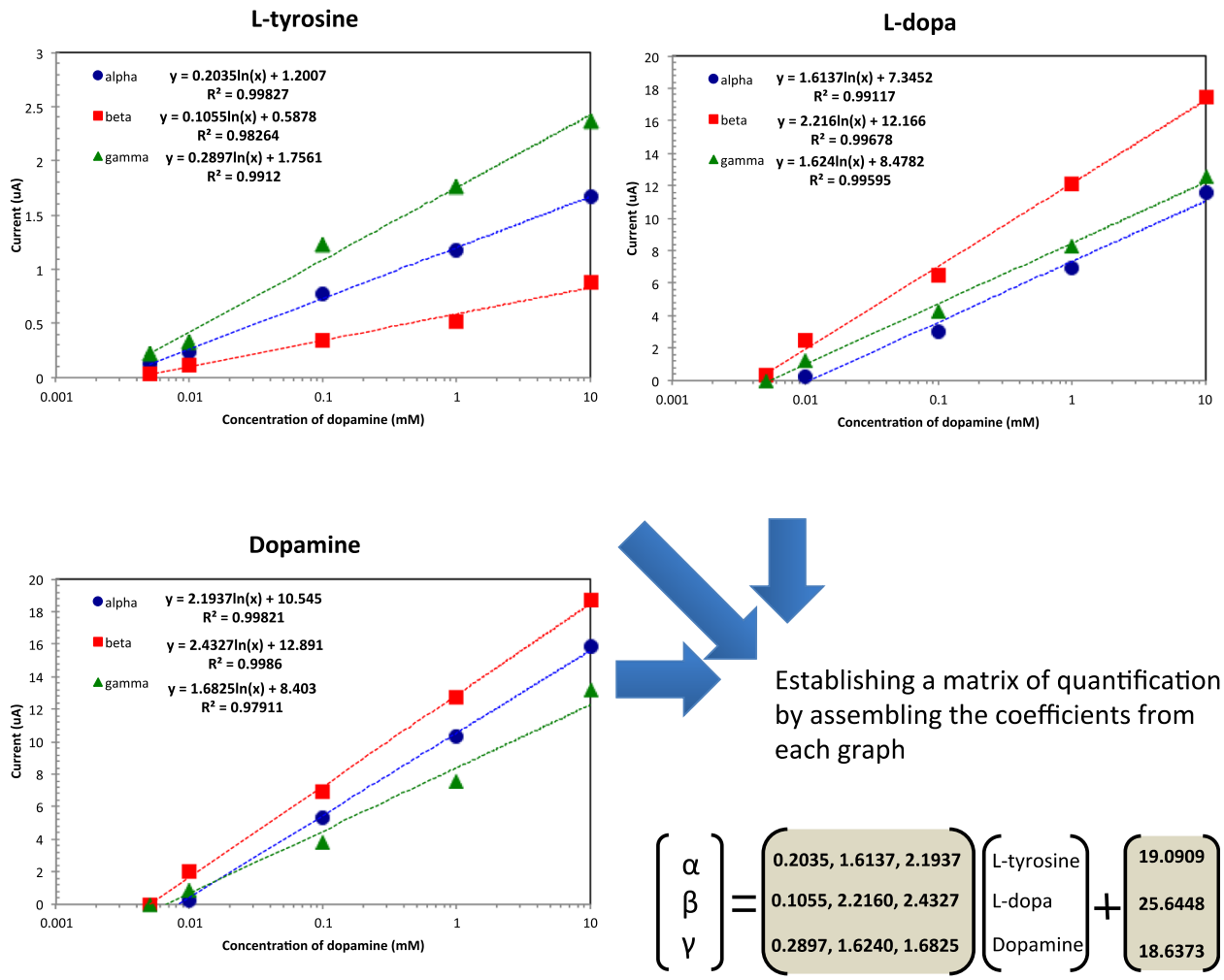

Figure 6 Amperometric responses of each electrode with the α-, β-, and γ-CDs immobilization, respectively, under various concentrations between 10^{-2} and 10^{1} mM at each solution of L-tyrosine, L-dopa and dopamine. The output current amounts were measured as to have linear relationships under logarithmic concentration ranges. The slope and offset data for each curve are extracted to form a matrix of quantification. The footprint of each electrode was 7.065 mm^2.

$$\textit{Multiplication matrix for L–tyrosine} = [0.2035 \quad 0.1055 \quad 0.2897]$$

By expanding the matrix to L-DOPA and dopamine, the complete output current can be expressed as:

$$\begin{bmatrix} I(\alpha) \\ I(\beta) \\ I(\gamma) \end{bmatrix} = \begin{bmatrix} 0.2039, & 0.1055, & 0.2897 \\ 1.6137, & 2.2160, & 1.6240 \\ 2.1937, & 2.4327, & 1.6825 \end{bmatrix} \begin{bmatrix} LT \\ LD \\ DA \end{bmatrix} + \begin{bmatrix} 19.0909 \\ 25.6448 \\ 18.6373 \end{bmatrix}$$

where I(α), I(β) and I(γ) are the output currents at each electrode and LT, LD and DA represent the concentrations in mM. This matrix enables the detection of individual amounts of each neurotransmitters from a mixture.

Random mixture measurement results, without vitamin C (an ascorbic acid), demonstrated that individual concentrations of L-tyrosine, L-DOPA and dopamine can be identified utilizing the CD-based electrode as listed in Table 1. A total of three different mixture cases was evaluated at least five repeated experiments without ascorbic acids as explained earlier. The Mixture 1 included minute amounts of 0.035, 0.010 and 0.005 mM; the mixture 2 contained 0.39, 1.11, and 0.68 mM; and the Mixture 3 consisted of 1.10, 3.24 and 2.37 mM, respectively of L-tyrosine, L-DOPA and dopamine. Each mixture without ascorbic acids produced the output currents of 10.531, 8.921, and 11.531 μA; 18.722, 18.724 and 18.758 μA; and 20.73, 21.11, and 20.56 μA respectively from α-, β- and γ-electrodes. By supplying the output currents into the constructed matrix of quantification, the supplied concentrations were estimated as 0.035, 0.010 and

Table 1 Experimental measurement-based quantification results of three neurotransmitter mixture cases with and without ascorbic acid (1 mM of vitamin C): (Case I) 0.035, 0.010 and 0.005 mM; (Case II) 0.39, 1.11, and 0.68 mM; and (Case III) 1.10, 3.24, and 2.37 mM of L-tyrosine, L-dopa and dopamine, respectively

Mixture I						
Input (No ascorbic acid)		**Output**				**Accuracy output/input (error)**
		Measurement		**Estimated from measurement**		
Type	mM	Electrode	μA	Type	mM	
L-tyrosine	0.035	α-CD	10.531	L-tyrosine	0.0372	**106.30% (+6.30%)**
L-dopa	0.010	β-CD	8.921	L-dopa	0.0093	**92.83% (−7.17%)**
Dopamine	0.005	γ-CD	11.531	Dopamine	0.0052	**105.05% (+5.05%)**
Mixture II						
Input (No ascorbic acid)		**Output**				**Accuracy output/input (error)**
		Measurement		**Estimated from measurement**		
Type	mM	Electrode	μA	Type	mM	
L-tyrosine	0.39	α-CD	18.722	L-tyrosine	0.3915	**100.37% (+0.37%)**
L-dopa	1.11	β-CD	18.724	L-dopa	1.1045	**99.51% (−0.49%)**
Dopamine	0.68	γ-CD	18.758	Dopamine	0.6678	**98.19% (−1.81%)**
Mixture III						
Input (No ascorbic acid)		**Output**				**Accuracy output/input (error)**
		Measurement		**Estimated from measurement**		
Type	mM	Electrode	μA	Type	mM	
L-tyrosine	1.10	α-CD	20.73	L-tyrosine	1.1690	**106.28% (+6.27%)**
L-dopa	3.24	β-CD	21.11	L-dopa	3.3399	**103.08% (+3.08%)**
Dopamine	2.37	γ-CD	20.56	Dopamine	2.2724	**95.88% (−4.12%)**

Experimental data indicated above are the average of at least five experiments. The testing was performed within 10 minutes of fabrication.

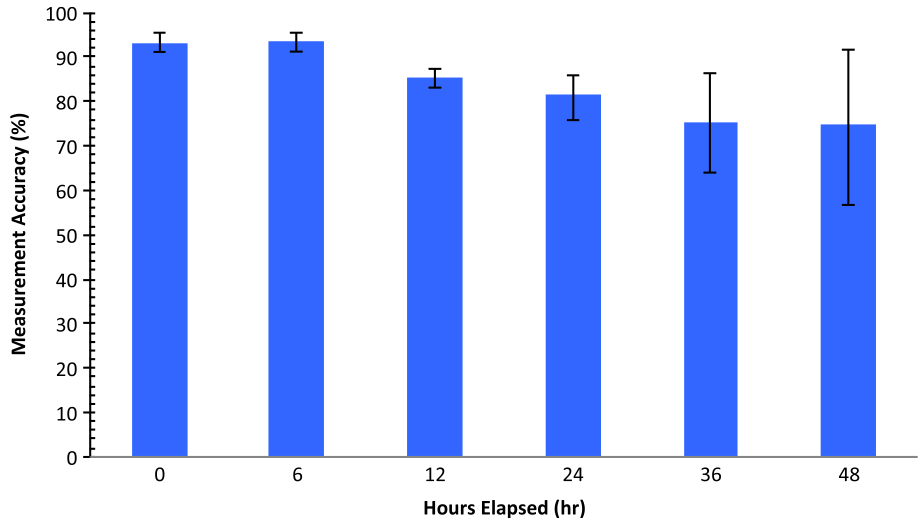

Figure 7 Composition identification accuracy over time utilizing α-, β-, and γ-electrodes under 100uM ascorbic acid (vitamin C). Error bars indicate the deviation over five experiments. The results indicate that the neurotransmitter sensor provides stable outputs up to 6 hours.

0.005 mM; 0.39, 1.11 and 0.68 mM; and 1.10, 3.24 and 2.37 mM respectively for the Mixtures 1, 2 and 3. These estimated results ranged within 92.41% and 106.30% of the supplied values for Mixtures 1, 2 and 3, verifying the concept of CD-based quantitative identification of mixed neurotransmitters. Note that all the Mixtures were evaluated within 6 hours of the device fabrication to minimize the performance drift over time.

The measurement results indicated that the average accuracy of the neurotransmitter sensor degraded over time from 93.2 (used immediately after fabrication) and 93.4 (within 6 hours) to 85.2, 81.4, 75.2 and 74.9%, respectively for within 12, 24, 36 and 48 hours (Figure 7). The measurement also showed that the deviations of the accuracy increased over time from 2.1 to 18.2%, indicating the reliable measurement periods after fabrication up to 6 hours.

Conclusion

A new neurotransmitter sensing technique has been examined to enables quantitative detection of three major catecholamine hormones from a mixture. Specifically, the sensing technique utilized three chemically-modified microelectrodes, respectively, with α-, β- and γ-cyclodextrin (CD) 'catchers'. The sensing technique relied on different physical matching (size and shape) as well as chemical enticing (stereochemistry and surface charge) properties of α-, β-, and γ-CDs in order to produce statistically different affinities to each target catecholamine neurotransmitters of L-tyrosine, dihydroxyphenylalanine (L-DOPA) and dopamine. The developed neurotransmitter sensing technique has successfully identified three individual catecholamine hormones, without the existence of ascorbic acid, with respective CD 'catchers': α-CDs better responded to L-tyrosine and dopamine; β-CDs to L-DOPA and dopamine; and γ-CDs to L-tyrosine, respectively. It also demonstrated the linear sensitivities to each neurotransmitter with logarithmically increasing concentrations range of 5 μM-10 mM. The measurement accuracy changed over time, indicating the stability up to 6 hours from the fabrication. The demonstrated accuracy, over >5 tests near 1 mM of target concentrations, was within 91.2%, and the total device footprint was 13×21 mm^2.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JY performed the fabrication and testing of the developed electrochemical sensors. HTK performed a variety of electrochemical measurements. JY, HTK and HK analyzed the measurement data and drafted the manuscript. All authors read and approved the final manuscript.

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