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Design and characterization of electrochemical dopamine–aptamer as convenient and integrated sensing platform

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ABSTRACT

Here, an ultrasensitive label-free electrochemical aptasensor was developed for dopamine (DA) detection. Construction of the aptasensor was carried out by electrodeposition of gold–platinum nanoparticles (Au–PtNPs) on glassy carbon (GC) electrode modified with acid-oxidized carbon nanotubes (CNTs–COOH). A designed complementary amine-capped capture probe (ssDNA1) was immobilized at the surface of PtNPs/CNTs–COOH/GC electrode through the covalent amide bonds formed by the carboxyl groups on the nanotubes and the amino groups on the oligonucleotides. DA-specific aptamer was attached onto the electrode surface through hybridization with the ssDNA1. Methylene blue (MB) was used as an electrochemical indicator that was intercalated into the aptamer through the specific interaction with its guanine bases. In the presence of DA, the interaction between aptamer and DA displaced the MB from the electrode surface, rendering a lowered electrochemical signal attributed to a decreased amount of adsorbed MB. This phenomenon can be applied for DA detection. The peak current of probe (MB) linearly decreased over a DA concentration range of 1–30 nM with a detection limit of 0.22 nM.

Aptamers are artificially synthetic single-stranded DNA or RNA that have been selected in vitro from large randomized oligonucleotide libraries by systematic evolution of ligands by exponential enrichment (SELEX) [1,2]. They are similar to antibodies, exhibiting high specificity and affinity for a wide range of target molecules such as protein [3], small molecules [4], amino acids [5], and even cells [6]. Because of the high specificity between aptamers and target molecules, they have attracted particular attention and been widely employed in verity scientific fields such as sensor preparations, drug screenings, and disease diagnoses. In addition, they have been applied for sensor fabrication based on various analytical methods, including optical transduction [7], circular dichroism [8], electrochemical techniques [9], fluorimetry [10], colorimetry [11], atomic force microscopy [12], surface plasmon resonance [13], and quartz crystal microbalance [9]. Electrochemical schemes for the detection of analytes possess some merit over other techniques such as high sensitivity, simple operation, rapid response, and portability. In general, additional electroactive tags, such as ferrocene derivatives [14] and methylene blue [15], have been used to label aptamer to obtain the measurable signal. But labeling aptamer is a complex and time-consuming process, which might affect the affinity of aptamer toward target. Therefore, fabricating and designing the label-free aptamers instead of directly labeling them has attracted a great deal of attention [16].

Dopamine (DA) is one of the most important catecholamine neurotransmitters in the mammalian central nervous system. It plays a crucial role in learning and memory, and it can control a variety of motivated behaviors and biological functions such as emotion, endocrine regulation, motivation, and locomotion [17]. Some diseases, such as Parkinson's disease, epilepsy, senile dementia, and HIV infection, have been found to be associated with either low concentration or abnormal metabolisms of dopamine

Abbreviations used: DA, dopamine; CNT, carbon nanotube; Au–PtNP, gold–platinum alloy nanoparticle; GC, glassy carbon; CNTs–COOH, acid-oxidized carbon nanotubes; MB, methylene blue; $HAuCl_4 \cdot 4H_2O$, hydrogen tetrachloroaurate; $H_2PtCl_6 – 5H_2O$, hexachloroplatinate(IV) hydrate; KCl, potassium chloride; NHS, *N*-hydroxysuccinimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDTA, ethylenediaminetetraacetic acid; PBS, phosphate-buffered solution; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; DPV, differential pulse voltammetry; SEM, scanning electron microscopy; WDX, wavelength-dispersive X-ray; RSD, relative standard deviation; NP, norepinephrine; AA, ascorbic acid; UA, uric acid.

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[18,19]. Therefore, there is significant importance in developing methods for the highly selective and portable determination of DA in biological systems. Determination of DA has been reported using chromatography [20], spectroscopy (optical) [21], electrophoresis [22], fluorescence [23], colorimetric [24], and electrochemical analysis [25]. Nearly all of these methods require sophisticated equipment and/or time-consuming procedures, which bring significant limitations on their application. In addition, most of them suffer from limitations for practical use and cross-sensitivity toward other chemicals.

Because DA, ascorbic acid, and uric acid possess similar oxidation/reduction potentials, electrochemical determination of DA is mainly compromised by the interference of ascorbic acid and uric acid. Aptamer technology can resolve the problems of limited selectivity because of its excellent recognition and binding ability with the target molecules. DA–aptamer has been discovered recently, and some methods based on the DA–aptamer fabrication have been reported [26,27].

During past decades, there has been increasing interest in the use of carbon nanotubes (CNTs) due to large surface area, good physical tubular structure, high surface-to-volume ratio, strong adsorptive ability, and electrocatalytic properties. Extensive studies have proved that CNTs can be used as excellent supporting materials of metal nanoparticles in hybrid materials. The combination of two or three materials (i.e., CNTs and other kinds of nanoparticles) is particularly useful to integrate the properties of these components in hybrid materials. Over past years, many studies have been devoted to fabrication of metal nanoparticles-decorated CNTs for unique electrical, magnetic, and optical properties, and various noble metal nanoparticles such as Pt, Au, Sn, Pd, and Ag have been constructed on the CNTs' sidewalls via different methods [28–30].

In this study, electrodeposited gold–platinum alloy nanoparticles (Au–PtNPs) on glassy carbon (GC) electrode modified with acid-oxidized carbon nanotubes (CNTs–COOH) was designed as a sensing platform for immobilization of aptamer. Our constructed electrochemical biosensor featured a DA–aptamer as the detection probe and its complementary sequence as the capture probe. The fabrication of the aptasensor was performed based on the covalent attachment of amine-terminated capture probe onto the nanocomposite. Immobilization of the DA-specific aptamer at the surface of modified electrode was carried out through its hybridization with the capture probe. Methylene blue (MB) was chosen as the electrochemical indicator because of its specific binding to double-stranded DNA [31,32]. In the presence of DA, the MB current response decreased due to the MB release from the electrode surface. In general, by interaction with the aptamer and target, MB was removed from the electrode surface and thus the peak current of MB decreased linearly with increasing target concentration. The lower the DA concentration in the sample, the lower the number of DA–aptamer complexes formed, leading to higher bound MB molecules and a larger MB redox current.

Materials and methods

Chemicals

Multiwalled carbon nanotubes with 95% purity, 10–20 nm diameter, and 1 μm length were obtained from Nanolab (Brighton, MA, USA). Potassium ferricyanide [$K_3Fe(CN)_6$], potassium ferrocyanide [$K_4Fe(CN)_6 \cdot 4H_2O$], hydrogen tetrachloroaurate ($HAuCl_4 \cdot 4H_2O$), hexachloroplatinate (IV) hydrate (H_2PtCl_6–$5H_2O$), and potassium chloride (KCl) were obtained from Merck (Germany) and Fluka. *N*-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma and used as received without further purification.

The sequences of these oligonucleotides were listed as follows:

Amine-terminated capture probe: 3′-NH_2-$(CH_2)_6$-CAG AGA CAC ACG-5′
DA–aptamer: 5′-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3′.

All other chemicals were of analytical reagent grade and used without further purification. Tris–HCl buffer (pH 7.4) was prepared by using $CaCl_2$, KCl, NaCl, and ethylenediaminetetraacetic acid (EDTA). Phosphate-buffered solution (PBS) with different pH values served as working buffer throughout the experiment and contained 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, and 0.1 M KCl. All experiments were performed at ambient temperature of 25 ± 1 °C. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were recorded to study the fabrication of modified electrode. DPV was recorded with modulation amplitude of 0.05 V, pulse width of 0.05 s, and sample width of 0.0167 s.

Apparatus

All electrochemical measurements were carried out using a μAutolab III potentiostat/galvanostat (Eco Chemie, The Netherlands) equipped with NOVA 1.8 software. A conventional three-electrode cell was employed with an Ag/AgCl electrode (KCl, 3 M) as reference electrode, a Pt wire as counter electrode, and a modified GC electrode as working electrode. The cell had one compartment and an internal volume of 10 ml. The pH was measured with a JENWAY pH meter (model 3345). The size and morphology of materials were investigated by taking scanning electron microscopy (SEM) images with a scanning electron microscope (XL-30, Philips, The Netherlands). Wavelength-dispersive X-ray (WDX) images were also taken on a Vega-Tescan electron microscope.

Electrode treatment and aptamer immobilization

According to literature reported prior to the modification [33,34], GC disk electrode was cleaned by polishing with 0.05-mm alumina slurries on a polishing cloth, followed by successive sonication in 1:20 (v/v) nitric acid and ethanol to obtain a mirror-like surface. Then the GC electrode was cleaned in doubly distilled water in order to remove absorbed particles. Purified CNTs were synthesized by refluxing CNTs in 3 M nitric acid for 12 h at a temperature of 150 °C. After washing the sediments with double-deionized water, the suspension was filtered and dried at a temperature of 60 °C. This process was repeated until the neutral filtrate was obtained. After that, the resultant solid was sonicated in the mixed solution of HNO_3 and H_2SO_4 (1:3, v/v) for 3 h. Then the pH of the CNTs was adjusted to 8.0 using NaOH (15%), and the obtained CNTs were subsequently centrifuged and dried until the proposed CNTs–COOH solution was obtained.

Then 10 μl of DMSO–CNTs–COOH solution (0.4 mg ml^{-1}) was cast at the surface of GC electrode and dried in air to form a CNTs–COOH film at electrode surface. To prepare metal nanostructure film on the surface of electrode, electrodeposition is often used as an easy and controllable method that allows electrodeposited film to tightly attach at the surface of electrode. Therefore, the CNTs–COOH/GC electrode was immersed in 0.2 M Na_2SO_4 solution containing 1 mM $HAuCl_4$ and 1 mM H_2PtCl_6 solution for potentiostatic electrodeposition at a potential of −0.2 V for 400 s. During this process, $PtCl_6^{2-}$ and $AuCl_4^-$ adsorbed on the CNTs–COOH