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# An electrochemical aptasensor for sensitive and selective detection of dopamine based on signal amplification of electrochemical-chemical redox cycling

Lin Liu *, Ning Xia, Jing-Jing Meng, Bin-Bin Zhou, Su-Juan Li

*Key Laboratory of New Optoelectronic Functional Materials (Henan Province), College of Chemistry and Chemical Engineering, Anyang Normal University, Anyang, Henan 455000, People's Republic of China*

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ABSTRACT

This paper presented an electrochemical aptasensor for dopamine detection with high sensitivity and selectivity. The electrochemical signal was amplified by electrochemical-chemical redox cycling with tris(2-carboxyethyl)phosphine (TCEP) as the reducing reagent. Specifically, dopamine captured by the aptamer-covered gold nanoparticles-modified carbon glass electrode was cycled by TCEP after its electrochemical oxidization, enabling an increase in the anodic current. Because of the high specificity and strong binding affinity of aptamer to dopamine, the sensor is fairly selective in not responding to common interferences. The results of chronoamperometry indicated that this aptasensor allowed for the detection of dopamine in a linear concentration range of 5 nM to 0.5 μM. The detection limit was estimated to be 1.8 nM. Additionally, the aptasensor was successfully applied to determine dopamine spiked in the serum sample and gave recoveries ranging from 94.6 to 107.2%. This work would also be valuable for development of new types of electrochemical sensors for determination of other electroactive substances by marrying specific receptors and effective reducing or oxidizing reagents.



## 1. Introduction

Dopamine (DA), one of the most important neurotransmitters, is widely distributed in the central nervous system brain tissues and body fluids of mammals. It plays pivotal roles in the function of central nervous, renal, hormonal and cardiovascular system. Concentration change of DA has been associated with various diseases and disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, epilepsy, pheochromocytoma and neuroblastoma [1,2]. For example, the concentration of DA ranges between a basal level of 1 nM in human serum/blood plasma and is extremely low ($<10^{-10}$ M) in the extracellular fluid of Parkinson's disease patients [3,4]. Thus, it is of paramount importance to develop strategies for the precise detection of DA in diagnose, monitoring, prevention and treatment of the DA-related diseases.

Because of the inherent redox activity of DA and the excellent characteristics of electrochemical techniques, fabrication of electrochemical sensors for determination of DA in human fluids has received considerable interest in recent years. To overcome the interference from other electroactive compounds such as ascorbic acid and uric acid, present in biological samples with the concentration of 100–1000 times higher than that of DA, a wide range of materials have been used as the active materials for chemically modifying the electrodes, such as carbon nanotubes, graphene, metal nanoparticles, organic redox mediators and polymers [5–10]. These modified electrodes have distinguished the overlap peaks to some extent and achieved the selective or simultaneous determination of DA, ascorbic acid and uric acid. However, their analytical performances (e.g. detection limit, stability and selectivity) are not entirely satisfactory for the practical application in biological samples. For example, the large background current of modified electrode may overlap with the oxidation current of low concentration of DA, thus leading to an unsatisfactory detection limit. Moreover, the fouling of the electrode surface by the oxidation products of DA results in poor stability of the modified electrode, and the overlapping electrochemical signals resulting from other neurotransmitter (NT) metabolites cause poor selectivity of the electrochemical DA sensors. Therefore, a simple, sensitive and selective electrochemical method is still desirable for the quantification of DA in the coexistence of other biological species.

Aptamers are single strand DNA or RNA molecules that can specially and strongly bind to the molecular targets from small molecules to proteins, even to whole cells [11–13]. They have been widely employed as recognition elements for biosensors because of their distinctive properties, such as simple synthesis, easy labeling, good stability as well as excellent recognition and binding ability with target. Aptamer-based

* Corresponding author.
*E-mail address:* liulin@aynu.edu.cn (L. Liu).

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detection techniques can resolve problems of limited selectivity. High specificity of the aptamer-ligand biorecognition has facilitated the fabrication of aptasensors for selective analysis of small molecules including cocaine, ATP, adenosine and theophylline [14–18]. It has been reported that the DA-specific RNA and DNA aptamer can bind to DA with a dissociation constant of 1.6 μM and 0.7 μM, respectively [4,19–21]. Upon binding to DA, the aptamer formed a stable framework like a DA pocket with two stems and two loops. Based on the specific and strong DA-aptamer interaction, several electrochemical aptasensors have been reported recently for the quantification of DA [3,22–29]. For example, Ferapontova's group first demonstrated the detection of DA by direct electrochemical oxidation of DA on a DA RNA aptamer/cysteamine-covered gold electrode [22]. This method allowed for the amperometric detection of DA at the concentration down to 100 nM. Compared to the RNA aptamer, the DNA aptamer has good stability and improved affinity to DA [4,21]. Huang's group reported an electrochemical aptasensor for DA determination based on the immobilization of the DNA aptamer on the graphene-polyaniline (GR-PANI) nanocomposites film [24]. Luo and co-workers presented an electrochemical DA aptasensor using a gold electrode modified with carbon nanoparticles (CNPs) coupled to thionine-labeled gold nanoparticles (AuNPs) acting as signal amplifiers [29]. These aptasensors are selective and feasible for DA detection, but their practical application is still limited due to the poor sensitivity and/or time-consuming and costly preparation of labeled nanomaterials. Thus, there still remains significant room to develop a sensitive and theoretically and technically simple electrochemical aptasensor for DA detection.

It is well known that signal amplification for electrochemical sensors can be obtained by redox cycling, which is related to the regeneration of electroactive species after their electrochemical oxidation or reduction [30–34]. The redox cycling reactions, which are usually performed electrochemically, enzymatically or chemically, provide an enhanced electrochemical signal. A classical example of electrochemical sensors based on the amplification of redox cycling is the oxidoreductase-catalyzed electrochemical reaction. This technique has been applied to sensitive detection in enzyme-linked immunoassays and DNA hybridization assays [35–37]. Also, the hemin/G-quadruplex DNAzyme exhibits useful bioelectrocatalytic functions toward the electrocatalyzed reduction of $H_2O_2$ [38]. In a general electrochemical-chemical redox cycling system, an excess of nonelectroactive species that act as reducing or oxidizing reagents is required to regenerate a starting electroactive species. Recently, we found that tris(2-carboxyethyl)phosphine (TCEP), a stable reducing regent, shows low background current on self-assembled monolayers (SAMs)-covered gold surface [39–41]. More importantly, TCEP exhibits a fast chemical reaction rate with quinone derivates. Inspired by these results, herein, we developed a simple and sensitive electrochemical aptasensor for DA detection by the signal amplification of redox cycling with TCEP as the reducing reagent. After the captured DA was electrochemically oxidized into dopaminequinone (DAQ), it was immediately regenerated by TCEP, leading to the enhancement in the electrochemical signal.

## 2. Experimental

### 2.1. Materials and chemicals

Tris(carboxyethyl)phosphine hydrochloride, dopamine hydrochloride, 3-mercaptopropionic acid (MPA), 3.4-dihydroxyphenylacetic acid, norepinephrine, epinephrine, uric acid, ascorbic acid, L-3.4-dihydroxyphenylalanine, serotonin, $K_2HPO_4$ and $KH_2PO_4$ were obtained from Sigma–Aldrich (Shanghai, China). Thiolated aptamer with a sequence of (5′-SH-$(CH_2)_6$-GTC TCT GTG TGC GCC AGA GAC ACT GGG GCA GAT ATG GGC CAG CAC AGA ATG AGG CCC-3′) was ordered from Sangon Biotech. Co., Ltd. (Shanghai, China). The 1 mM DA stock solution was freshly prepared with water and then diluted with phosphate buffer solution (PBS, pH 7.4) to the desired concentrations. To avoid the auto-oxidation of DA in air, TCEP was added into the PBS solution to a final concentration of 0.5 mM in the dilution step. The supporting electrolyte was 50 mM PBS (pH 7.4). All aqueous solutions were prepared with $N_2$-saturated deionized water treated with a Millipore system (Simplicity Plus, Millipore Corp., Billerica, MA). The serum sample from one yellow staff (male, 52 years old) was obtained from the health center of Anyang Normal University (Anyang, China) and stored at −20 °C for use.

### 2.2. Instruments

Electrochemical determination was performed on a CHI660E electrochemical workstation (CH Instruments, Shanghai, China). Carbon glass electrode (GCE) with a diameter of 3 mm was used as the working electrode. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively.

### 2.3. Preparation of MPA/aptamer-covered gold nanoparticles-modified electrode

The GCE was first polished with 0.3 and 0.05 μm alumina slurries and washed ultrasonically in water and ethanol for a few minutes. The cleaned GCE was then electrodeposited in a 1 mL of 1% $HAuCl_4$ solution at a constant potential of −0.2 V for 30 s to obtain the gold nanoparticles (nano-Au) modified GCE electrode (nano-Au/GCE). The morphology of nano-Au/GCE was characterized by Atomic force microscope (AFM) (Dimension Edge microscope, Bruker Nano Inc., Santa Barbara, CA), as shown in the inset of Fig. 2A. The aptamer-covered electrode (aptamer/nano-Au/GCE) was prepared by immersing the nano-Au/GCE in a solution composed of 10 μM thiolated aptamer and 50 μM TCEP in the darkness for 12 h. This step was followed by washing the electrode thoroughly with deionzied water and soaking it in a MPA solution (1 mM) for 20 min to block the unreacted gold surface. Again, the MPA/aptamer/nano-Au/GCE was rinsed with ethanol/water to rid any non-specifically adsorbed substances. The modified electrode was characterized by cyclic voltammetry and electrochemical impedance analysis. Cyclic voltammogram (CV) was collected in 1 mM $[Fe(CN)_6]^{3-}$ solution containing 0.1 M $KNO_3$. Electrochemical impedance spectroscopy (EIS) was obtained in 1 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) solution containing 0.1 M $KNO_3$.

### 2.4. Detection procedure

The MPA/aptamer/nano-Au/GCE was incubated with 50 μL of different concentration of DA solution for 10 min. After the electrode had been rinsed with water, voltammetric or amperometric measurement was performed in the PBS solution containing 200 μM TCEP. To test the stability, the MPA/aptamer/nano-Au/GCE was stored in PBS at 4 °C with a sealed container. This allowed long-term stability testing for up to ten days. To examine the reusability of the aptasensor, the used electrode was immersed in 7.0 M urea for 15 min, and then thoroughly rinsed with PBS [26]. The regenerated electrode was then used for DA determination as the aforementioned procedure.

For the assay of DA in the serum sample, 50 μL of serum was diluted with 50 μL of PBS solution containing a given concentration of DA. Then, the sensing electrode was incubated with the diluted serum sample for 10 min, which was followed by the amperometric measurement in the TCEP-containing PBS solution.

## 3. Results and discussion

### 3.1. Principle of our detection strategy

The principle of the aptamer-based electrochemical strategy for DA detection is illustrated in Fig. 1. Thiolated DA aptamer was immobilized onto the nano-Au/GCE surface through the Au–S interaction. After blocking with MPA, the MPA/aptamer-covered sensing electrode was