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Rapid determination of dopamine in human plasma using a gold nanoparticle-based dual-mode sensing system

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ABSTRACT

Dopamine plays a very important role in biological systems and has a direct relationship with the ability of learning and cognition, human desires, feelings and mental state, as well as motor functions. Traditional methods for the detection of dopamine are complicated and time-consuming, therefore it is necessary to explore rapid and accurate detection of dopamine with high sensitivity and specificity. Herein we report a dual-mode system of colorimetric and fluorometric analyses based on gold nanoparticles (AuNPs) and aptamers specifically targeting dopamine. Aptamers modified with the fluorophore were used as dopamine specific recognition probe and the sensing mechanism is based on the color change of AuNPs and the fluorescence recovery of fluorophore conjugated on the aptamers in the presence of dopamine. The addition of aptamers into AuNPs colloid solution would prevent the AuNPs from aggregation in the high-salt solution. The close distance between AuNPs and fluorophore conjugated on the aptamers would lead to the quenching of fluorescence signal. In the presence of dopamine, the conformation of the aptamers and the inter-particle distance would be changed, leading to the aggregation of AuNPs, which subsequently results in color change from red to blue and fluorescence signal recovery. The dual-mode sensing system demonstrated high specificity towards dopamine with the detection limit as low as 78.7 nM. The sensing system reflects on its simplicity as no surface functionalization is required for the nanoparticles, leading to less laborious and more cost-effective synthesis. The reaction time is only 6 min, demonstrating a simple approach for rapid analysis of dopamine. More importantly, the sensing system allows the detection of dopamine in both aqueous solution and complicated biological sample with sensitive response, illustrating the feasibility and reliability for the potential applications in clinical and biomedical analysis in the future.

1. Introduction

Dopamine, one of the small molecules in the monoaminergic neuroreceptor family [1], has been widely studied since it was discovered [2]. Dopamine plays key roles in central nervous systems and serves many functions in dopaminergic neuron process. The disorder levels of dopamine may lead to many neurological diseases such as sleeping and eating disorders, Parkinson's disease, addictive behaviors combined with drug abuse, schizophrenia, and Alzheimer's disease [3–5]. Meanwhile, it is unlawful to use dopamine as a new alternative of "Clenbuterol" as the residues of dopamine in livestock are harmful to human, which presents a challenge for food safety monitoring. Therefore, methods for rapid and sensitive detection of dopamine in biological system are very important for neurological disorder diagnosis [6] and food safety monitoring. Conventional methods for determining dopamine include high performance liquid chromatography (HPLC) [7], mass spectroscopy [8], electrochemical analysis [9,10], chemiluminescence [11], enzyme linked immunosorbent assay [12], surface plasmonic detection [13] and so on. However, most of these methods are time-consuming and require sophisticated pre-treatment process, expensive instruments and expertise for operation. Therefore, it is necessary to develop a new sensing system for rapid detection of dopamine with high sensitivity and specificity. Due to its simplicity and practicability with good sensitivity, electrochemical and optical techniques have been extensively explored for developing dopamine sensors. Electrochemical sensors are the most straightforward, rapid, and cost-effective way for dopamine detection. In order to improve their performance, many new materials, for example conducting polymers, ionic liquids, and nanomaterials have been used for sensor construction [9,10,14,15]. Especially, nanomaterials have been demonstrated as excellent sensing components due to their unique physical and chemical properties, which have been widely applied in analytical chemistry and biomedical diagnosis [14–17]. To circumvent the overvoltage and slow kinetics of the electrode process, metal nanoparticles (Au, Ag, Pt, Pd, Cu) are commonly used as active materials to modify the working electrodes for enhancing the sensitivity and selectivity of

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electrochemical dopamine sensors [14]. For example, the electrode modified with Au nanoparticles (AuNPs) which have been decorated with polypyrrole/reduced graphene oxide hybrid sheets showed the lowest detection limit of 18.29 pM and also demonstrated the good analytical performance in real samples such as human serum and urine [9]. However, determination of dopamine at the nanomolar level characteristic of living system is still challenging for electrochemical sensors in the presence of dominant interferents [10].

Optical sensing incorporated with nanoparticles as signal generators has been proven as the most convenient and comprehensible method. Several colorimetric methods based on the distance-dependent optical properties of AuNPs have been developed for dopamine detection. The color variation of AuNPs associated with the reversible process from dispersion to aggregation induced by the intermolecular interactions between dopamine and surface modifiers on the AuNPs can be observed by naked eyes and quantified by UV–vis spectra [18–22]. In addition, several fluorometric assays have been demonstrated for the detection of dopamine with high sensitivity using fluorophores such as calcein blue–Fe^{2+} complex [23], carbon dots [24], silicon nanoparticles [25], molecule capped QDs [26–29], and gold nanoclusters (AuNCs) [30,31]. To the best of our knowledge, only one dual-mode assay has been reported for dopamine detection, where BSA-stabilized AuNCs were employed as sensing components [32]. However, the instability of AuNCs hinders the wide application of the assay.

In this report, a dual-mode sensing system with both colorimetric and fluorometric readouts for rapid detection of dopamine has been developed. Our proposed dual-mode sensing system consists of AuNPs and dopamine-binding aptamers. In the colorimetric mode, dopamine could specifically bind to the aptamers thus changing the stability of the AuNPs in solution, resulting in the color change of AuNPs solution. In the fluorometric mode, a fluorescence resonance energy transfer system (FRET) is developed using the fluorophore labeled aptamers as donors and AuNPs as acceptors [33–37]. The dual-mode sensing system has high specificity towards dopamine with the detection limit of as low as 78.7 nM. Compared with conventional methods, the advantages of the dual-mode sensing system include its simplicity, rapid analysis, comprehensible qualitative and quantitative results and robust in completed biological sample. As no surface functionalization is required for the nanoparticles, the simplicity of the probe preparation leads to less laborious and more cost-effective synthesis. The reaction time is only 6 min and the results could be visualized by naked eyes and quantified by spectrophotometer, which demonstrates the sensing system as a robust and simple approach for rapid analysis of dopamine. More importantly, the sensing system allows the detection of dopamine in both aqueous solution and complicated biological sample with sensitive response, which could be potentially applied in clinical diagnosis and food safety monitoring in the future.

2. Materials & methods

2.1. Chemicals and materials

Hydrogen tetrachloroaurate (III) tetrahydrate, trisodium citrate, dopamine, norepinephrine, hydroquinone, ascorbic acid, homocysteine, L-tryptophan, L-phenylalanine, 5-hydroxytryptamine, 3,4-dihydroxyphenylalanine, glucose, and uric acid were all of analytical reagent grade and purchased from J&K (Beijing, China). Two 58-mer oligonucleotides strands were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China): one strand of oligonucleotides with the base sequences of 5′-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3′6-FAM [18] was designed specifically for capture of dopamine and the other strand of oligonucleotides was used as control with the sequences of 5′-ACG GTT GCA AGT GGG ACT CTG GTA GGC TGG GTT AAT TTG G-3′. The single stranded DNA was dissolved in 100 mM phosphate-buffered saline (PBS, pH = 7.0) before use. Other solutions were prepared by ultra-pure water (Milli-Q plus, Millipore Inc., Bedford, MA).

2.2. Apparatus

Morphology of the synthesized nanoparticles was characterized by transmission electron microscope (TEM, JEOL JEM 1400). UV–vis spectra of the nanoparticles were obtained by a Beckman DU370 UV–vis spectrometer and fluorescence spectra were obtained on a Fluoromax-4P spectrometer (Horiba).

2.3. Synthesis of citrate-protected AuNPs

Aqueous dispersions of citrate-stabilized AuNPs were prepared according to the published protocol [18]: 120 mL of 1 mM $HAuCl_4$ solution was heated to boiling with vigorous stirring in the round-bottom flask. 12 mL of 38.8 mM trisodium citrate solution was rapidly added into the solution and the reaction continued for another 15–20 min. After that, the solution was cooled down to room temperature and filtered by a 0.45 μm cellulose acetate (CA) membrane. The prepared AuNPs were stored at 4 °C in the refrigerator for further use.

2.4. Detection of dopamine using the dual-mode sensing system

200 μL of dopamine with different concentrations was mixed with 10 μL of 10 μM aptamers in 1.5 mL plastic tubes, respectively. After incubation for 5 min, 200 μL of 11.3 nM AuNPs was added and reacted for another 5 min in solution. Then 75 μL of 0.25 M NaCl was rapidly added into the vial and incubated for another 6 min. 30 mM PBS buffer was used to keep the total volume of 600 μL before spectrum measurement. For colorimetric readout, 130 μL of the mixture was transferred to a 1 cm path length quartz cuvette for UV–vis measurement. For fluorometric readout, 300 μL of the mixture was transferred to cuvette for recording emission spectra.

2.5. Interference experiments

To evaluate the selectivity of the dual-mode system, 30 μL of 100 μM of different kinds of interferents including hydroquinone (HQ), glucose (Glu), ascorbic acid (AA), L-phenylalanine (L-Phe), L-tryptophan (L-Try), norepinephrine (NE), serotonin hydrochloride (5-HT), and 3,4-dihydroxyphenylalanin (DOPA) was mixed firstly with 10 μL of 10 μM dopamine-binding aptamers respectively and incubated for 5 min. Then 200 μL of 11.3 nM AuNPs was added and reacted for 5 min, followed by the rapidaddition of 75 μL of 0.25 M NaCl into the vial and incubated for another 6 min. 300 μL of the mixture was transferred for fluorescence measurement and 130 μL of the mixture was transferred for colorimetric measurement. 30 mM PBS buffer was used to keep the total volume of 600 μL before spectrum measurement.

2.6. Detection of dopamine in human plasma

The detection of dopamine was carried out in deproteinized human plasma. The plasma proteins were precipitated using acetonitrile (two thirds of the reconstitution volume) by centrifugation at 4000 rpm for 30 min [38]. The supernatant was collected and 1000 times diluted with ultra-pure water before use. Different concentrations of dopamine were spiked into the deproteinized blank human plasma and quantified by the present sensing system with dual-mode readouts. The recovery rate was calculated accordingly and compared with HPLC results.