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Facile and sensitive detection of dopamine based on *in situ* formation of fluorescent polydopamine nanoparticles catalyzed by peroxidase-like ficin

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ABSTRACT

Polydopamine nanoparticles (pDA NPs), a kind of popular polymer material, have been broadly applied to various areas owing to its excellent physicochemical properties. In this work, we employed ficin as a peroxidase-mimic to *in situ* synthesize intrinsic fluorescent pDA NPs and developed a fluorescence turn-on method for rapid dopamine (DA) sensing which is based on monitoring the intrinsic fluorescence of pDA NPs. Ficin possesses intrinsic peroxidase-like activity that could catalyze the oxidization of dopamine to its quinone derivative and autopolymerize into fluorescent pDA NPs by H_2O_2. Thus, the fluorescence intensity of pDA NPs gradually enhanced with increasing concentration of DA. Under the optimum conditions, the strategy showed a good linear relationship from 10 nM to 5.0 μM (R^2 = 0.996) and provided an exciting detection limit of 5.5 nM. More importantly, the presented sensing approach has high sensitivity, good selectivity and has been successfully applied to DA sensing in human serum samples. With its good analytical figures of merit, our proposed analytical method is promising for the detection of DA-related diseases.

1. Introduction

Dopamine (3,4-dihydoxyphenethylamine, DA), a catecholamine neurotransmitter, mainly exists in the central nervous system of human and other mammals, which plays an important role in the nervous activity [1,2]. It can regulate many biological processes in the cardiovascular system and hormone secretion system as well as manage a variety of cognitive functions of central nervous system, such as memory, behavior, learning and emotion [3–7]. It has been reported that the abnormal concentration of DA in biological fluids is regarded as one of the indicators used in the diagnosis of several neurological diseases [8], including Schizophrenia [9], Parkinson's diseases [10], restless leg syndrome [11], and Alzheimer's diseases [12]. Besides, these diseases attract considerable attentions due to the physiological and pathophysiological effect of DA levels. Therefore, it is essential to develop a sensitive and selective method that can efficiently detect DA. In the past few years, a variety of available analytical techniques have been developed and devoted to DA detection, such as electrochemical analysis [13], capillary electrophoresis [14], chromatography coupled with spectroscopy [15], colorimetric method [16] and fluorescent probes [17]. Dismayingly, most of the above methods generally suffer from time-consuming, tedious or lengthy procedures, and relatively high cost. Accordingly, a facile and sensitive method for the determination of DA is highly desirable for researching human physiological functions, which was propitious to achieve clinical diagnosing of neurological diseases.

DA, as generally known, is susceptible to be oxidized to its quinone derivative and then self-polymerizes to form pDA NPs under oxidative stress or alkaline environment [18,19]. With the merits of excellent physicochemical properties, pDA NPs have attracted a great deal of application to various areas [20–24]. Very recently, it has been reported that pDA has fluorescent properties under certain conditions. For instance, Zhang et al. reported pDA NPs that obtained by treating DA with concentrated H_2O_2 possess fluorescence, which can be applied to cellular imaging [25]. Up to now, however, the development of the intrinsically fluorescent pDA NPs for biosensing application was rarely explored. As far as we know, only Bayindirz et al. reported the formation of fluorescent pDA NPs under alkaline conditions, which is further used for the sensing of DA [26] and Chu et al. reported the fluorescent pDA NPs are catalytically synthetized by Fe_3O_4 nanozyme and its applica-

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tion in detection of Zn^{2+} [27]. Therefore, it is quite indispensable to further explore pDA NPs with intrinsic fluorescence for biosensing application.

Ficin (EC 3.4.22.3), a cysteine proteolytic enzyme, mainly presents in the latex of ficus trees, playing pivotal roles in many plant physiological processes and applying to a variety of industries such as food and pharmaceutical [28–30]. Recently, our group has found that ficin exhibited intrinsic peroxidase-like activity, which can catalyze H_2O_2 decomposition into •OH radicals that are a powerful oxidizing agent [31]. On this basis, we developed a new method for DA sensing based on the *in situ* formation of fluorescent pDA NPs by catalyzing the oxidation of DA in the presence of H_2O_2 with the aid of the peroxidase-like activity of ficin. The conceptual framework of this method for DA sensing was shown in Scheme 1. In the presence of ficin, H_2O_2 can be decomposed into •OH radicals that are able to rapidly oxidize dopamine to its quinone derivative and then auto polymerizes into fluorescent pDA NPs. Meanwhile, within a certain concentration range the fluorescence intensity of pDA NPs gradually enhanced with increasing concentration of DA. The sensing approach was verified to be effective for the detection of DA in human serum samples, which provides a broad prospect in practical applications. Furthermore, this study gives a new insight on the application of intrinsic fluorescent pDA NPs to develop a facile and sensitive biosensing system.

2. Experimental section

2.1. Reagents and materials

Hydrogen peroxide (H_2O_2, 30 wt%), acetic acid, sodium acetate, glucose and sucrose were purchased from Chongqing Pharmaceutical Co., Ltd. (Chongqing, China). Dopamine hydrochloride (DA), ascorbic acid (AA), uric acid (UA), gallic acid (GA), urea, glutamic acid (Glu), glycine (Gly), aspartic acid (Asp), lysine (Lys), alanine (Ala) and phenylalanine (Phe) were obtained from Aladdin (Shanghai, China), premium grade ficin (F4165, $\geq$0.1 U mg^{-1}) was purchased from Sigma-Aldrich (USA). All other chemicals were of analytical reagent grade and used without further purification. Ultrapure water purified with a Milli-Q system (18.2 MΩ, Millipore) was used in all runs.

2.2. Measurement

The fluorescence measurements were conducted on an F-4500 spectrophotometer (Hitachi, Japan). The excitation wavelength was set at 400 nm, the Ex and Em slits were set at 10.0 and 10.0 nm, respectively, with a 700 V PMT voltage. The absorption spectra were recorded by a UV–2450 spectrophotometer (Shimadzu, Japan). Transmission electron microscopy (TEM) was performed on a JEM-2100 instrument (JEOL Ltd., Japan). All ESR measurements were carried out using a JES-FA200 Spectrometer (JEOL, Japan) at an ambient temperature.

2.3. Detection of DA

0.20 mL of freshly prepared DA aqueous solution with concentrations from 0 to 50 μM, 0.20 mL of 4.0 mg mL^{-1} ficin, 0.20 mL of 0.40 mM H_2O_2, 1.0 mL of 0.20 M HAc-NaAc buffer (pH 4.0), and 0.40 mL H_2O were introduced into 2.0 mL eppendorf tube, and incubated for 150 min at 30 °C to form fluorescent pDA NPs. And then, the fluorescence spectra of the reaction solution were recorded.

2.4. Detection of DA in human blood samples

The human blood sample permitted and obtained from healthy volunteers was collected in a heparin anticoagulated tube. Furthermore, the volunteers' consent and approval from the Institutional Research Ethics Committee of Southwest University hospital were obtained for research purposes. And then, the blood samples were treated according to previous literatures [32]. Briefly, the collected samples were first treated by centrifugal ultrafiltration using a filter (cutoff is 1 kDa) at 3000 rpm for 15 min at room temperature to remove haemocytes. The detection of DA in human samples was carried out by well-established spiked method. Firstly, the aliquot (0.20 mL) of the filtrates were spiked with 0.20 mL of different concentrations standard DA solution. Then, 0.20 mL of 4.0 mg mL^{-1} ficin, 0.20 mL of 0.40 mM H_2O_2, and 0.2 mL H_2O were added into above reaction solution containing 1.0 mL of 0.20 M HAc-NaAc buffer (pH 4.0), and incubated for 150 min at 30 °C. The subsequent operations and fluorescence measurements were performed in the same as described above.

3. Results and discussion

3.1. Feasibility and mechanism of the system for DA sensing

The oxidation of DA by H_2O_2 using DNAzymes [33] or nanozymes [34] was reported by some groups. However, only a few papers mentioned the fluorescent properties of the oxidation products. Therefore, in this work, we constructed a novel fluorescence sensing platform for detecting DA based on the *in situ* formation of fluorescent pDA NPs *via* catalyzing the oxidation of DA in the presence of H_2O_2 by the peroxidase-like activity of ficin. To demonstrate the feasibility of the proposed strategy for sensing DA, fluorescence spectra were recorded. As shown in Fig. 1a, a remarkable fluorescence signal was observed when DA was mixed with both ficin and H_2O_2 (curve 4), while barely any fluorescence signal was generated in the presence of free DA, DA with ficin alone and DA mixed with H_2O_2 alone (curve 1–3). It is noteworthy that we used rather low concentration of H_2O_2 during the synthesis compared to the previously reported method [35]. All the above results indicated that ficin can successfully catalyze the oxidation of DA by H_2O_2 into quinone and initiate the polymerization reaction to form fluorescent pDA NPs that showed an excitation wavelength of 400 nm and the maximum emission peak at 476 nm. To prove the catalytic mechanism of ficin, a series of electron spin resonance (ESR) experiments with 5, 5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trap was used to confirm the generation of the •OH radicals. From Fig. 1b, one can see that there are no apparent •OH radical signals in the systems of DMPO and DMPO—H_2O_2. In contrast, the ESR spectrum in the system of DMPO—H_2O_2-ficin displayed a characteristic peak of the typical DMPO/•OH spin adduct signal, demonstrating that ficin converts H_2O_2 to •OH radical [36]. However, after the addition of DA to this solution, the typical DMPO/•OH spin adduct signal intensity is obviously weakened, clearly indicating that the generated •OH radicals are consumed owing to rapidly oxidize DA into fluorescent pDA NPs.

Additionally, as displayed in Fig. S1a, we also found that the emission intensity initially enhanced with increasing excitation wavelength from 360 nm to 400 nm and then began to decrease gradually from 410 nm to 430 nm. As shown in Fig. S1b, with the increasing excitation wavelength, the emission peak shifted accordingly to the longer wavelength, illustrating its excitation-dependent fluorescent property, which is similar to the fluorescence spectra of dopamine oligomers synthesized in an acidic condition *via* a hydrothermal method [37].