Highly sensitive detection for stimulants using an aptamer functionalised gold nanoparticles

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In this paper, a simple, highly sensitive, and cost-effective method was described for cocaine detection as an example in the stimulants using an aptamer-functionalised gold nanoparticles (Au NPs). Cocaine-specific aptamer is single-strand DNA sequence that can specifically bind to cocaine. Carboxyfluorescein (FAM)-labelled aptamer was adsorbed on the surface of Au NPs. Then FAM was quenched owing to the close proximity between aptamer and Au NPs. Cocaine can be detected after the fluorescence of FAM was restored by detaching from the surface of Au NPs. The limit of detection (LOD) was 3 pM. The results show this sensitive method is promising for the detection of stimulants.

Keywords: Cocaine detection, Aptamer, Gold nanoparticles, Highly sensitive detection, Stimulants

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Introduction

Doping abuse is an increasingly outstanding problem in modern sports games. According to the World Anti-Doping Agency (WADA) doping rules, stimulants are prohibited substances for athletes.1 Some methods for stimulants analyses have been developed in recent years involving colourimetry,2 electrochemistry,3 mass spectrometry,4 fluorescence assays,5 surface plasmon resonance,6 capillary electrophoresis,7 and high-performance liquid chromatography (HPLC).8 However, some methods are complicated, high cost or the sensitivity needs to be further improved. Therefore, it is of great importance to develop a simple, low-cost and high sensitivity method to detect stimulants in sport games.

Cocaine, or benzoylmethylecgonine, is one of stimulants with adverse effects on human health and has an instantaneous effects on the central nervous system.9 Aptamers are single-strand nucleic acid sequences that are easily synthesised, easily modified with chemical groups, have high affinity to the target.10 In recent years, the methods of cocaine detection using fluorescence resonance energy transfer (FRET)-based aptamer sensors since 2000 have been particularly attractive because of the inherent sensitivity.11–14 Au NPs have excellent chemical and physical properties, such as facile surface chemistry, unique optical properties, and appropriate size scale.15 In this paper, we used Au NPs as a fluorescence quencher for the Carboxyfluorescein (FAM)-labelled aptamer and optimised experiment conditions for the detection of cocaine as an example in the stimulants and developed a highly sensitive detection method for stimulants.

Materials and methods

Materials and chemicals

The aptamer for cocaine was purchased from Sangon (China) and used as received. The sequence of aptamer modified with FAM was used based on the discovery of Stojanovic et al.16 in this study is as follows: 5'-FAM-5'-CCATAGGGAGACAAGGATAAATCCTTGAA GTGGGTCTCCC-3'. Au NPs (13 nm diameters), cocaine, and other chemicals were purchased from Sigma (Shanghai, China). Ultrapure water (18.2 MΩ cm) was used in the experiments.

Sensor preparation and sensitivity of the assay

The solution containing 15 nM FAM-modified aptamer was obtained by using PBS buffer to dilute the stock solution. The 1 mL solution containing 45 nM aptamer was added to 2 mL of the solution containing 4.73 nM AuNP. Then different concentrations of cocaine were added and incubated for 5 min for the detection of sensitivity.

Selectivity of the assay

Cocaine and others (immunoglobulin G (IgG), lysozyme, and Adenosine) were added into the solution containing 15 nM aptamer with 3.15 nM AuNPs. Each concentration was 0.3 nM. They were incubated for 5 min at room temperature for further measurements.

Fluorescence spectroscopy analysis

The fluorescence measurements were carried out using a Cary Eclipse spectrophotometer. The optical path length of a quartz fluorescence cell was 1.0 cm. The emission spectra were recorded in the wavelength of...
Results and discussion

Design strategy for cocaine detection

The principle of the detection for cocaine using the Au NP-aptamer based sensor was shown (Fig. 1). FAM-modified aptamer was adsorbed on the surface of Au NPs. In the absence of cocaine, the fluorescence of FAM was efficiently quenched by Au NPs via FRET. In the presence of cocaine, cocaine caused the conformational change of aptamer and the desorption of aptamer from the surface of Au NPs. Then the fluorescence of FAM turned on. As shown in the Supplementary Material, Fig. S1 (www.maneyonline.com/doi/suppl/10.1179/17535557B15Y.000000003), the fluorescence intensity showed strong in the presence of FAM-modified aptamer or aptamer-cocaine (Fig. S1, curve a and b). The fluorescence signal decreased after Au NPs were added into the FAM-modified aptamer solution (Fig. S1, curve c). This also showed Au NPs can quench FAM with high efficiency. After the cocaine was added into the mixture solution, FAM-modified aptamer subsequently showed significant fluorescence restoration (Fig. S1, curve d).

Kinetic behaviour of the aptamer functionalised Au NPs

The kinetic behaviour of the aptamer functionalised Au NPs for the detection of cocaine was further studied. In the presence of Au NPs, Fig. S2, showed the fluorescence quenching of FAM-modified aptamer. The fluorescence was quenched by Au NPs and the release of the cocaine–aptamer complex from Au NPs can reach an equilibrium in 5 min.

Effect of Au NPs concentration for the detection

As shown in Fig. S3, the fluorescence intensities of FAM-modified aptamer changed with different concentrations of Au NPs. The fluorescence intensities of FAM-modified aptamer decreased with the increase of Au NPs concentration.

To achieve a best quenching efficiency to improve the sensitivity of detection for cocaine, it was essential to optimise the concentration of aptamer-Au NPs. As shown in Fig. 2a, the fluorescence intensity of solution decreased with the addition of Au NPs. After the addition of cocaine, the fluorescence intensity showed the corresponding restoration. Figure 2b further showed the changes between the values of black column bars and the values of corresponding of grey column bars. Error bars were obtained from three experiments.

Sensitivity of the detection for cocaine

Figure 3 illustrated the fluorescence intensity further increased with the addition of cocaine. As illustrated in Fig. 4a, the fluorescence intensity increased with the increasing concentrations of cocaine from 0 to 10 nM. Figure 4b showed the calibration curve of fluorescence intensity with increasing the concentration of cocaine. The calibration curve of fluorescence intensity and a linear correlation ($R^2 = 0.78487$) between the value of
The concentration of cocaine over the range 0.003–10 nM were showed in Fig. 4b. The limit of detection (LOD) was 3 pM. The detection limit was higher than that obtained from those of previously reported assays, such as a visual bioassay based on AuNPs and engineered aptamers (2 μM), an aptamer biosensor on Au NPs and progressive dilution (1 nM), electrochemical aptasensor (1.3 nM), an optical aptamer sensor (0.2 μM), a microfluidic aptamer-based biosensor (10 pM), an aptasensor based on silver nanoparticles measured (150 pM), fluorescence aptameric sensor (2 nM).

Selectivity of the detection for cocaine

High sensitivity is important, high selectivity is also crucial for sensing. To investigate the selectivity of aptamer functionalised Au NPs-based sensors, we preformed the detection of other small molecule and proteins for evaluation under the same experimental conditions as cocaine. As shown in Fig. S4, the aptamer functionalised Au NPs-based sensor could effectively differentiate between cocaine and other targets. This demonstrated that aptamer functionalised Au NPs-based sensor responded selectively towards cocaine over others.

Conclusions

Authors have developed a method for the detection of cocaine using an aptamer-functionalised Au NPs. This method can detect cocaine as low as 3 pM. Furthermore, that aptamer functionalised Au NPs-based sensor responded selectively towards cocaine over others. These results show this method can readily be highly sensitive detection for cocaine. Therefore, it is a promising technique for the detection of cocaine and may inspire application in modern sport games.

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3 The fluorescence intensity of FAM-modified aptamer (15 nM)-Au NPs (3.15 nM) in the presence of different concentrations of cocaine (0, 0.003, 0.006, 0.1, 0.2, 0.3, 10 nM)

4 a The calibration curve of fluorescence intensity with increasing the concentration of cocaine. b The inset shows the values of \( F/F_0 \)' for assay with the concentration of cocaine. \( F_0 \) and \( F \) were the values of fluorescence intensities without and with cocaine. The values shown in Fig. 3b represented the average of three experiments.
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