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Label-free fluorescent aptasensor of Cd^{2+} detection based on the conformational switching of aptamer probe and SYBR green I

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ABSTRACT

A label-free fluorescence aptasensor based on conformational switching of aptamer induced by Cd^{2+} was established for the good selective determination of Cd^{2+}. The aptamer could act as a recognition element for Cd^{2+}. SYBR green I (SG), as a signal reporter, can bind to the small groove of double-stranded DNA (dsDNA) constituted the aptamer and the complementary strand, establishing the dsDNA-SG complex and generating high fluorescence intensity. In the presence of Cd^{2+}, the specific recognition and binging of aptamers preferentially with Cd^{2+} induce free of complementary strands from dsDNA and conformational switching of aptamer from dsDNA to a stem-loop structure, fluorescence intensity of system decrease dramatically. Under the optimum experiment parameters, the aptasensor shows a good linear range from 1.12 μg L^{-1} to 224.82 μg L^{-1} and the excellent detection limit as low as 0.34 μg L^{-1}. The relative standard deviations (RSD) for determination of Cd^{2+} (n = 8) were lower than 4%, and the recoveries were in the range of 98.57% ~ 102.49%. The use of fluorescence aptasensor has good selectivity and does not require any sample treatment or target preconcentration for the detection of Cd^{2+} in real samples.

1. Introduction

Heavy metal pollution is a great concern due to their persistence and high toxicity [1,2]. Cadmium (Cd) is classified as one of heavy metals with biological half-life in the range of 10–30 years and is used widely in different industrial processes such as industrial production, agricultural implementation and public life [3]. Cadmium pollution even at very low concentrations can been accumulated in tissues and organisms, causing potentially adverse health effects such as kidney, liver and immune system damage, as well as respiratory disorders [4–7]. World Health Organization (WHO) and United States Environment Protection Agency (EPA) define the maximum permissible limit of 3 μg L^{-1} and the maximum contamination concentration of 5 μg L^{-1} for cadmium in drinking water, respectively [8,9]. Also, European legislation has set a limit of 0.3–0.5 mg L^{-1} for cadmium discharge in the environment from various industries [10]. Therefore, quantitative determination of cadmium at trace or even ultra-trace concentrations is of great interest in environmental chemistry.

Up until now, a large number of analytical methods have been proposed for cadmium determination. According to the different principles of design, these developed methods can be mainly classified into the following types: inductively couples plasma mass spectrometry (ICP-MS), ICP-optical emission spectrometry (ICP-OES) [11–14], atomic absorption spectroscopy [15–19] and fluorescence spectrometry [20,21]. For example, Beiraghi. A., et al. have reported the cationic micellar precipitation method to preconcentrate simultaneously several heavy metals and apply it to determinate cadmium, cobalt and nickel in various water by the ICP-OES, lowering to the detection limit of 0.008 μg L^{-1} [11]. Martínez, D., et al. have reported the electrothermal atomic absorption spectrometry (ETAAS) coupled with dispersive liquid-liquid micro-extraction for Cd^{2+} determination in different types of wines, and the limit of detection (0.01 μg L^{-1}) was lower than previously reported ETAAS [15]. These above-mentioned methods have great improvement in the sensitivity and selectivity. However, the serial of extraction methods such as liquid-liquid extraction, coprecipitation, microextraction, solid phase extraction and so on, still be required in the sample treatment due to the low levels of cadmium in the samples and the high complexity of sample matrices [11,13–19,22–24]. Also,

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these methods require the skilled operator and expensive instrumentation. Therefore, great efforts improving the cost and efficiency of detection must be paid to develop the alternative techniques for cadmium determination.

In recent years, aptamers are artificially selected single-stranded DNA or RNA sequences through systematic evolution of ligands by an exponential enrichment (SELEX) process, and show the unique advantages in affinity and special binding for their target molecules [25,26]. Because of these characteristics, the aptamers have attracted increasing popularity in establishing sensor for determination of heavy metals, and many aptasensors with high sensitivity and selectivity coupled with colorimetry [25,27,28], electrochemistry [29,30] and fluorescence [31,32] have been applied successfully for determination of heavy metals.

Aptamers of cadmium with twelve nonrepeating G and T-rich sequences have been developed by SELEX [33], Cd-4 aptamer exhibits the highest affinity to bind Cd^{2+}, of which the domain of 30-random sequence folds into a stem-loop structure. The loop may offer the sufficient binding sites for Cd^{2+} through the coordination bonds between the adjacent short fragment rich in T or G and Cd^{2+}. The stem is to maintain the stability of such a stem-loop structure via hydrogen bonds among the base pairing of AT or GC [33]. Although the Cd-4 aptamer has been reported, few methods use this aptamer to determine Cd^{2+}. Lotfi Zadeh Zhad. H.R., et al. reported the "signal-on" electrochemical aptasensor for Cd^{2+} determination and indicated that the sensor had good selectivity, fast analysis time and regenerable merits [34]. Recently, we used a singly labeled multifunctional Cd-specific aptamer to construct sensitive and selective analytical method for Cd^{2+} determination and found the strategy had the lower detection limit than the electrochemical aptasensor [35].

In this work, we supposed that the specific recognition of aptamers for Cd^{2+} induced conformational switching of dsDNA, resulting in the change of fluorescence intensity. Based on this, we used two unlabled oligonucleotides to develop a novel and label-free aptasensor for the sensitive and selective detection of Cd^{2+}, as the typical and most common one of heavy metals. The proposed method would reduce the testing cost and simplify the experiment procedure for the determination of cadmium.

2. Experimental

2.1. Reagents and materials

HPLC-purified aptamer, complementary strand and SYBR green I (10, 000×, SG) were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China.) The working solutions concentrations of oligonucleotides were all 1 μmol L^{-1}, and the sequences were as follow:

Aptamer:5′-GGGAGGGAACTGTTGTGGTATTATTTTTGGTTGTGCAG TAGGGCGGG-3′.

Complementary strand (7 bases): 5′-ACAACAG-3′.

Complementary strand (9 bases): 5′-ACAACAGTT-3′.

Complementary strand (11 bases): 5′-ACAACAGTTCC-3′.

Complementary strand (13 bases): 5′-ACAACAGTTCCCT-3′.

Tris (hydroxymethyl) aminomethane, PBS buffer and Citrate buffer were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). SG was dissolved and diluted to working solution (20×) in ultrapure water. All other chemicals employed are analytical grade without purther purification. All solutions in the whole experiments were prepared with ultrapure water (≥18 MΩ, Milli-Q, Millipore, USA).

2.2. Apparatus

The fluorescence spectra were recorded on a Hitachi F-4500 fluorophotometer (Tokyo, Japan). A pH meter (Sartorius AG, Germany) were used for pH adjustment and a MVS-1 vortex mixer (Beijing, China) were employed to blend mixtures.

2.3. Absorbance spectra measurements

Absorbance spectra from 400 nm to 700 nm were obtained by a Shimadzu UV-2550spectrophotometer (Kyoto, Japan) to declare the conformational change of aptamers.

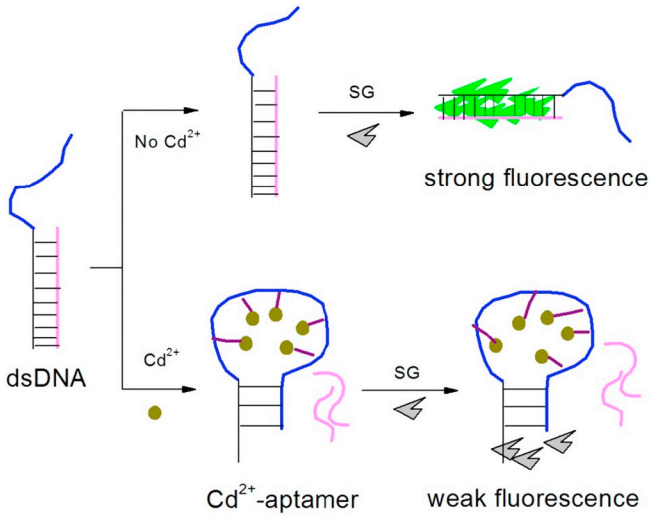
2.4. Detection of Cd^{2+}

Into a 2-mL EP tube, a 20 μL of 1 μmol L^{-1} aptamer and complementary strand were added into 100 μL of Tris-HCl (pH = 7.3), followed by adding different concentrations of Cd^{2+} and the mixture was diluted to a volume of 300 μL in ultrapure water. After incubating 40 min, 200 μL of SG (20×) was added, keeping it for 30 min. Thereafter, the emission spectra were obtained by scanning from 508 to 575 nm at λ_{ex} = 497 nm. The slits width of excitation and emission spectra obtained were set to 5 nm. The change of fluorescence intensity was recorded at λ_{em} = 525 nm, representing as $\Delta F = F_0 - F$, where F_0 and F were the fluorescence intensities of the system without and with Cd^{2+}, respectively.

3. Results and discussion

3.1. Design strategy and sensing mechanism

The design principle for the Cd^{2+} determination is outlined in Scheme 1. In this proposed assay, the thirteen bases of aptamer at the 5′ end are perfectly complementary to the complementary strand, forming the dsDNA. In the absence of Cd^{2+}, SG gradually binds to the small groove of dsDNA, establishing the dsDNA-SG complex and generating remarkable fluorescence intensity [36]. Upon adding Cd^{2+}, we supposed that the specific recognition and binging of aptamers preferentially with Cd^{2+} induced free of complementary strands and conformational switching of aptamer from dsDNA to a stem-loop structure along with the change of fluorescence intensity. Wu Y., et al. have also reported that the dissociation constant of Cd-4 aptamer determined as 34.5 nmol L^{-1} for Cd^{2+}, which shows that the aptamer has the high affinity to Cd^{2+} [33]. As we all know that SG is commonly used dsDNA-specific fluorescent dye and only keep active when it combines with dsDNA [37]. Moreover, SG interacts not only with dsDNA but also with ssDNA, while dsDNA has a higher affinity than ssDNA for the SG [37,38]. When the aptamer binds with the targets, SG also only emits a weak fluorescence because of the relative inaccessibility of the duplex region to SG-I [39]. Therefore, after the introduction of Cd^{2+}, the SG generates a low fluorescence signal between



Scheme 1. the design principle for the Cd^{2+} determination.