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Cationic-perylene-G-quadruplex complex based fluorescent biosensor for label-free detection of Pb^{2+}

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ABSTRACT

In this work we use a water-soluble cationic perylene derivative (compound 1) as the G-quadruplex (G4) structure fluorescence indicator to construct a fluorescent biosensor for simple, rapid and label-free detection of Pb^{2+}. In the absence of Pb^{2+}, strong electrostatic interactions between compound 1 and the G-rich DNA probe (PW17) induced the aggregation of compound 1 and resulted in the fluorescence quenching. In the presence of Pb^{2+}, the PW17 formed Pb^{2+}-stabilized G4 structure, which reduced the aggregation of compound 1 and gave rise to high fluorescence. This allowed us to use convenient "mix-and-detect" protocol for quantitative analysis of Pb^{2+}. Since Pb^{2+} can specially induce PW17 to form compact DNA fold, our proposed biosensor displayed high selectivity for Pb^{2+}. It also exhibited a high sensitivity to Pb^{2+}, with a limit of detection of 5.0 nM observed. Furthermore, the biosensor was applied for the detection of Pb^{2+} in urine and paint samples, and both showed satisfactory results.

1. Introduction

It is well-known that Ionic lead is a common environmental and industrial pollutant. It is quite harmful to human body, especially on children, and a very small amount of Pb^{2+} could cause renal malfunction and inhibit brain development [1,2]. Therefore, the development of sensitive and selective methods for the detection of Pb^{2+} is a current research focus in the fields of modern environmental science and life science. A variety of conventional methods have been developed for the detection of Pb^{2+}, including atomic absorption spectrometry [3], inductively coupled plasma-mass spectrometry [4], and inductively coupled plasma-atomic emission spectrometry [5]. However, these techniques are rather expensive, complex, time consuming and not suitable for on-site detection.

In recent years, some functional DNA molecules such as 8–17 DNAzyme and G4 oligonucleotide have been applied for lead ion detection [6–10]. 8–17 DNAzyme is a RNA-cleaving DNAzyme, which displays cleavage activity at the scissile ribo-adenosine position of the substrate strand with Pb^{2+} as the cofactor. Researchers have made use of this unique enzyme-like feature of 8–17 DNAzyme to develop fluorescent, [11,12] colorimetric, [13,14] electrochemical sensors [15,16], which exhibit high sensitivity and selectivity for Pb^{2+} analysis. However, the RNA molecules used in these sensors are unstable and vulnerable in vivo or complex media. G4s are a kind of four-stranded DNA structure, in which G-rich nucleic acid sequences form stacked arrays of G-quartets connected by Hoogsteen-type base pairing, and are stabilized in the presence of coordination cations [17–19]. Recently, some DNA sensors based on Pb^{2+}-induced allosteric G4 structure have been developed for Pb^{2+} detection [20–23]. For example, Liu and co-workers present a new assay for the sensitive detection of Pb^{2+} using a thrombin-binding aptamer (TBA probe) labeled with a fluorophore and a quencher at its 5′ and 3′ termini, respectively [20]. Li and co-workers developed a sensitive and selective electrochemical sensing platform for lead detection based on a Pb^{2+}-induced G-rich DNA conformational switch with crystal violet as the G4-binding indicator [21]. Nevertheless, in these assays, the DNA probes required an additional DNA tagging process or immobilization technique that are technically demanding, time consuming, and expensive. Therefore, it still remains a challenge to develop a simple, label-free and sensitive method for Pb^{2+} detection.

Water-soluble cationic perylene diimide derivative (compound 1, Fig. 1) is attractive because it exhibits high fluorescence quantum yield, good photostability, and excellent chemical inertness [24–27]. Due to the repulsive interactions among the positive charges of the compound 1, it shows strong monomer fluorescence in an aqueous buffer solution. Nevertheless, nucleic acid containing multiple negatively charged phosphate functional groups

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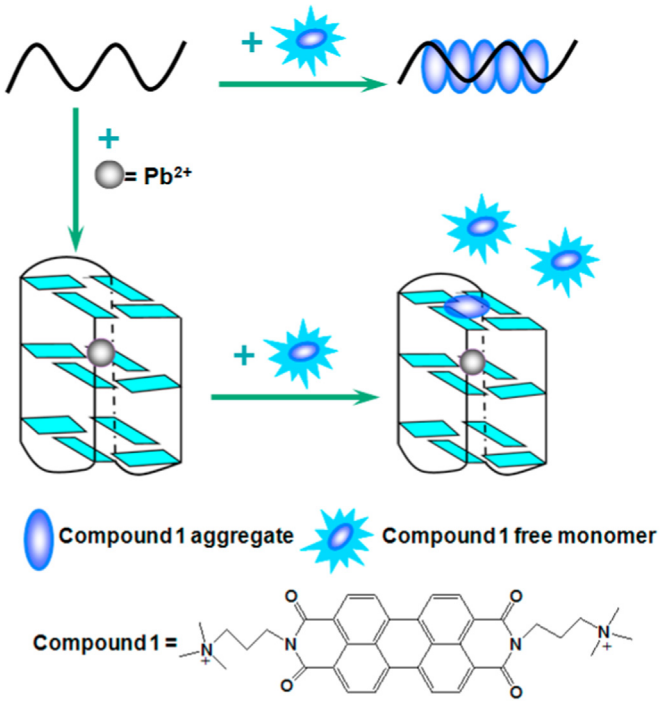

Fig. 1. Schematic illustration of the selective sensing of Pb^{2+}.

could induce strong aggregation of compound 1 and result in the fluorescence quenching. By taking advantage of the aggregation-induced emission change of compound 1, some simple, rapid and label-free fluorescent sensing systems were constructed [28–30]. However, no report is focused on the interaction between G4 structure and the water-soluble cationic perylene derivative (compound 1).

In this work, we reported a simple, rapid and label-free approach for fluorescent "turn-on" detection of Pb^{2+} by using compound 1 as the fluorescence reporter and the PW17 for the specific binding of Pb^{2+}. PW17 has been found to strongly bind hemin to form DNAzymes with peroxidase-like activity under salt conditions. Moreover, Pb^{2+} could cause K^{+}-stabilized PW17 to undergo a parallel-to-antiparallel conformation transition as a result of its unusually high efficiency at stabilizing G-quadruplexes [19,31,32]. Therefore, the biosensor showed a high selectivity to Pb^{2+}. In addition, our proposed biosensor also exhibited a high sensitivity towards Pb^{2+}, with a limit of detection of 5.0 nM observed. Moreover, it was applied for the detection of Pb^{2+} in urine and paint samples, and both showed satisfactory results.

2. Experimental

2.1. Reagents and apparatus

The G-rich DNA probe (PW17: 5'-GGGTAGGGCGGGTTGGG-3') used in this work was synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). 3,4:9,10-Perylenetetracarboxylic dianhydride and N,N′-Dimethyl–1,3-propanediamine were purchased from Alfa Aesar. Methyl iodide was acquired from Shanghai Chemical Reagents (Shanghai). Compound 1 was synthesized as previously reported literature [27]. Lead acetate and all other reagents were of analytical reagent grade, purchased from Sigma-Aldrich Chemical Co., and used without further purification. Milli-Q water (Millipore, 18.2 MΩ cm at 25 °C) was used in all runs.

All fluorescence measurements were carried out on a Hitachi F-4500 Fluorescence Spectrometer (Tokyo, Japan). The instrument settings were chosen as follows: λ_{ex}=494 nm (slit 10 nm), PMT detector voltage=950 V.

2.2. Procedure for fluorescence detection

The solution of PW17 oligonucleotide in Tris-acetate buffer (10 mM, pH 8.0) was heated at 90 °C for 10 min and gradually cooled to room temperature. Then, varying concentrations Pb^{2+} (in the range of 0–10 μM) were added and the mixture was incubated for 40 min. Upon the addition of the compound 1 for 10 min, the fluorescence of the mixture was measured.

3. Results and discussion

3.1. Biosensor design and analytical principle

The design strategy of our proposed sensor is shown in Fig. 1. Compound 1 showed a strong fluorescence emission at 540 nm in aqueous solution, and the addition of Pb^{2+} did not interfere with its fluorescence intensity (see Fig. 2). In the absence of Pb^{2+}, the strong affinity of compound 1 with PW17 induced the aggregation of compound 1, and resulted in the fluorescence quenching. Upon the addition of Pb^{2+}, the conformation of PW17 changed from a random-coil to a Pb^{2+}-stabilized G4 structure. This conformational change weakened the interaction between PW17 and compound 1. As a result, compound 1 monomer molecules were released and fluorescence enhancement was observed, which provided a facile means for Pb^{2+} quantification.

In addition, the conformational switch of PW17 induced by Pb^{2+} was further analyzed with CD spectra (see Fig. 3). The concentration of PW17 and Pb^{2+} were fixed at 10 μM and 100 μM, respectively. In the absence of Pb^{2+}, CD spectra of PW17 was of relatively low amplitude due to PW17 possessing a random structure. Upon the addition of Pb^{2+} ion, the spectra exhibited a long wavelength maximum near 314 nm,indicating the formation and stabilization of the G4 structure by Pb^{2+} ion, which is consistent with previously reported result [33].

3.2. Optimization of assay conditions

In order to achieve the best sensing performance, we investigated the effect of the concentration of compound 1 on the

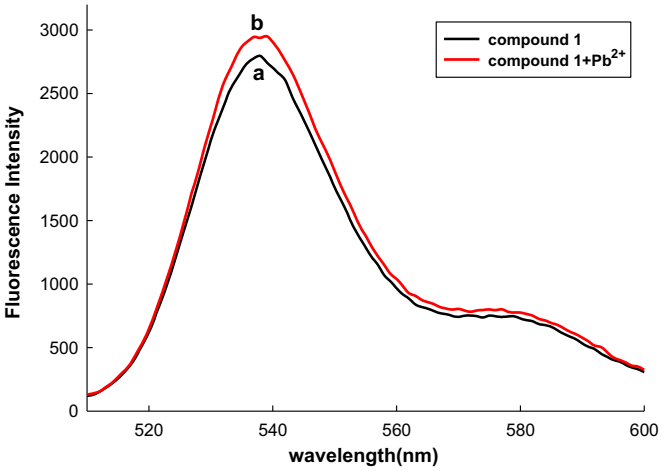

Fig. 2. Fluorescence emission spectra of compound 1 in the presence and absence of Pb^{2+}. The concentrations of compound 1 and Pb^{2+} were 2 μM and 10 μM, respectively.