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RAPID COMMUNICATION

A Novel Single-Labeled Fluorescent Oligonucleotide Probe for Mercury(II) Ion Detection: Using the Inherent Quenching of Deoxyguanosines

Lei Wang · Jingqi Tian · Hailong Li · Yingwei Zhang · Xuping Sun

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Abstract A novel single-labeled fluorescent oligonucleotide (OND) probe for the detection of nanomolar mercury (II) ion in aqueous solution is developed based on the inherent quenching of deoxyguanosines. The formation of hairpin structure of OND-Hg^{2+} complex brings deoxyguanosines close to dye, resulting in decreased dye fluorescence due to photoinduced electron transfer from dye to deoxyguanosines.

Keywords Single-labeled probe · Fluorescence · Oligonucleotide · Mercury(II) ion detection · Quenching

Introduction

Mercury(II) ion is a highly toxic heavy metal ion and recognized as one of the most dangerous and ubiquitous pollutants [1], and its contamination is widespread and arises from a variety of natural sources [2]. It is a demonstrated fact that Hg^{2+} can easily pass through skin, respiratory, and gastrointestinal tissues into the human body and damage the central nervous and endocrine systems [3], which raises serious environmental and health concerns. So it is highly desirable to develop effective analytical methods to detect Hg^{2+} sensitively and selectively. Indeed, the past years have witnessed increasing research efforts in this direction and many detection methods have been established. Traditionally, Hg^{2+} is detected by atomic absorption/emission spectroscopy, Auger-electron spectroscopy, inductively coupled plasma Mass Spectrometry, or ion selective electrode or polarography [4, 5]; however, these methods require sophisticated instrumentation and/or sample preparation, which limits their practical applications. To solve these problems, alternative methods based on small-molecules-based fluorescent probe [6–11], DNAzymes [12–15], protein [16, 17], conjugated polymers [18], gold nanoparticles [19, 20], and semiconductor quantum dots [21] have also been developed in the past years. However, most of these methods still have some limitations such as poor selectivity, insufficient resolution in aqueous media, and sophisticated synthesis of the probe materials. As a result, it still remains a great challenge to develop new methods to solve these issues.

On the other hand, OND has also been proven to be a versatile tool for the detection of metal ions due to their specific interactions. It has been established that certain OND sequences can be induced to fold into a hairpin structure by metal ion such as Hg^{2+} owing to the formation of T–Hg^{2+}–T base pairs [22], which provides a rationale to design T-rich OND-based sensor for Hg^{2+}. Indeed, Ono et al. have developed a fluorescent Hg^{2+} sensor using a dual-labeled, T-rich OND with a fluorescent dye and a quencher at its 3′- and 5′-end, respectively [23]. In the presence of Hg^{2+}, T–Hg^{2+}–T coordination chemistry induces OND to fold into hairpin structure which brings both termini close to each other, resulting in significant quenching of the dye fluorescence. More recently, Zhang et al. have demonstrated sensitive and selective Hg^{2+} detection with the use of single-walled carbon nanotube (SWCNT) as a quencher [24]. In their study, single-labeled T-rich fluorescent OND

L. Wang · J. Tian · H. Li · Y. Zhang · X. Sun (✉)
State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry,
Chinese Academy of Sciences,
Changchun 130022, Jilin, China
e-mail: sunxp@ciac.jl.cn

J. Tian · H. Li
Graduate School of the Chinese Academy of Sciences,
Beijing 100039, China

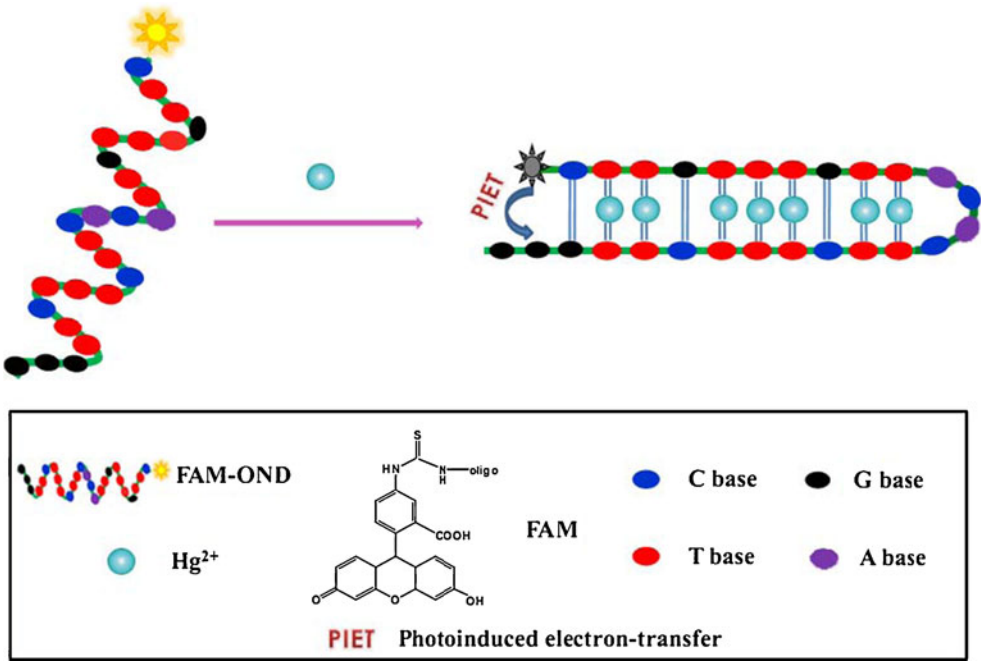
wrapped around SWCNT and then was quenched by SWCNT due to energy transfer between them [25]; however, upon addition of Hg^{2+}, the DNA and Hg^{2+} form a double helical structure which will detach from the nanotube surface, resulting in an increase of fluorescence. Although these two detection methods are sensitive and selective, they have some drawbacks: the first strategy requires labeling at both ends of the OND probe with specific dyes that suffer in overall yield and are expensive [26] and the second one is time-consuming.

In this communication, we develop a novel single-labeled fluorescent OND probe for the sensitive and selective detection of Hg^{2+} in aqueous solution. In our design,, a T-rich OND (5′-GGGTTCTTTCTTCACATTGTTTGTTC-3′, sequence specific for Hg^{2+} binding underlined) is fluorescently labeled with FAM, a fluorescein-based dye, at its 3′-end (FAM-OND) and used as a probe to report the presence of Hg^{2+} by a strong decrease in fluorescence intensity. Three extra Gs and one C have been introduced at its 5′- and 3′-end, respectively. The present strategy takes advantage of the fact that the used FAM is efficiently quenched by the Gs owning to photoinduced electron transfer between the dye and the Gs [27] when OND is induced to fold into a hairpin structure by Hg^{2+}. Scheme 1 shows a schematic diagram to illustrate the concept of our proposed single-labeled fluorescent OND-based Hg^{2+} detection. In the absence of Hg^{2+}, the FAM-OND forms a random coil, which separates the FAM and Gs from each other and thus FAM exhibits strong fluorescence emission. However, in the presence of Hg^{2+}, mercury-mediated base pairs are formed between thymine residues in the ODN to give rise to a hairpin structure. The fluorescent dye and the Gs are brought close to each other upon formation of the hairpin structure, leading to significant quenching of the fluorescence emission relative to the random coil.

Results and Discussion

Figure 1 shows the fluorescence emission spectra recorded on a PerkinElmer LS55 Luminescence Spectrometer (PerkinElmer Instruments, UK) of FAM-OND at different conditions. In the absence of Hg^{2+}, FAM-OND exhibits strong fluorescence emission at 520 nm which can be attributed to the presence of the fluorescein-based dye (curve a). Given that the maximum binding capacities of one OND for Hg^{2+} is seven, we first examined the FAM-OND-Hg^{2+} system with 7:1 molar ratio of Hg^{2+} to OND. In the presence of Hg^{2+}, about 26.1% quenching of the fluorescence emission is observed (curve b), indicating that the Gs at the 5′-end of the OND can effectively quench the fluorescent dye. Such observation also provides clear evidence to support the Hg^{2+}-induced formation of double helical structure. It should be pointed out that our measurement was performed right after the addition of Hg^{2+} into the OND solution and the involvement of longer incubation time does not lead to an observable increase of the fluorescence emission, indicating that the process of detection is very rapid.

To evaluate the sensitivity of this detection system, we collected emission spectra of FAM-OND in the presence of different concentrations of Hg^{2+} ranging from 0.005 to 5 μM, as shown in Fig. 2. It is clearly seen that the fluorescence



Scheme 1 A scheme diagram (not to scale) to illustrate single-labeled fluorescent OND-based Hg^{2+} detection

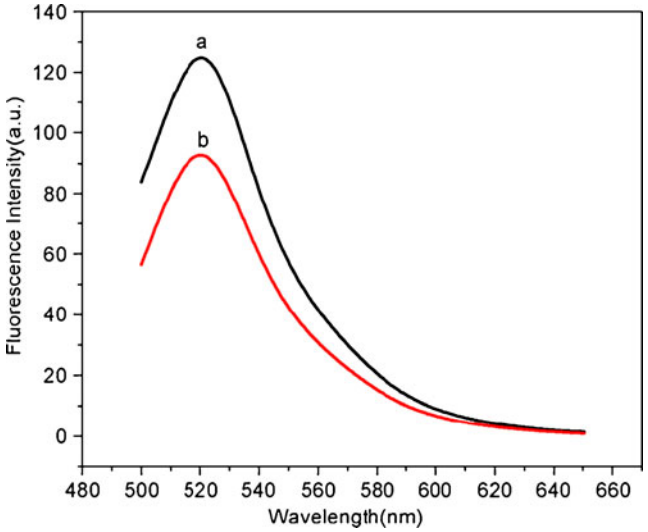


Fig. 1 Fluorescence emission spectra of FAM-OND (100 nM) at different conditions: **a** FAM-OND; **b** FAM-OND + 700 nM Hg^{2+}. Excitation was at 480 nm, and the emission was monitored at 520 nm. All measurements were performed in Tris–HCl buffer (pH: 7.4)

intensity of FAM-ODN is sensitive to Hg^{2+} and decreases as the concentration of Hg^{2+} increases. Figure 2 inset shows the fluorescence intensity changes ($1-F/F_0$) of FAM-OND in the presence of different concentrations of Hg^{2+}, where F and F_0 are FAM fluorescence intensities at 520 nm in the presence and absence of Hg^{2+}, respectively. However, there is no further enhancement of the fluorescence intensity when a higher Hg^{2+} concentration (>5 μM) is used, indicating the interaction between OND and Hg^{2+} reaches a balance of saturation. Note that this detection system exhibits different response sensitivities depending on the Hg^{2+} concentration range probably due to the requirement of several Hg^{2+} ions bound to one OND for the hairpin formation [23] and thus a linear correlation between the emission intensity and the Hg^{2+} concentration has been proven to be unsuccessful. It is worthwhile mentioning that we can observe distinct and reproducible fluorescence intensity difference from time to time at a low Hg^{2+} concentration of 5 nM. These observations indicate that our probe is highly sensitive to Hg^{2+} and its sensitivity is higher than those of the previously reported OND-based Hg^{2+} sensors [23, 24].

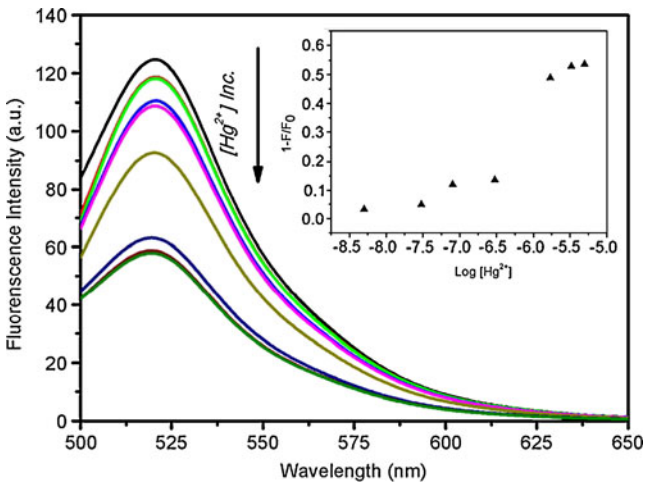


Fig. 2 Fluorescence emission spectra of FAM-OND (100 nM) in the presence of different concentration of Hg^{2+} (from top to bottom: 0, 0.005, 0.03, 0.08, 0.3, 0.7, 1.7, 3.3, and 5 μM). Excitation was at 480 nm, and the emission was monitored at 520 nm. Inset: fluorescence intensity ratio of FAM-OND with $1-F/F_0$ (where F and F_0 are the fluorescence intensity with and without the presence of Hg^{2+}, respectively) plotted against the logarithm of the concentration of Hg^{2+}

We also evaluated the selectivity of the FAM-OND probe for Hg^{2+} detection. To do this, a variety of environmentally relevant metal ions including Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} were examined. Figure 3a shows the difference in fluorescence intensity between the blank and solutions containing Hg^{2+} (5 μM) and other metal ions (5 μM). It can be clearly seen

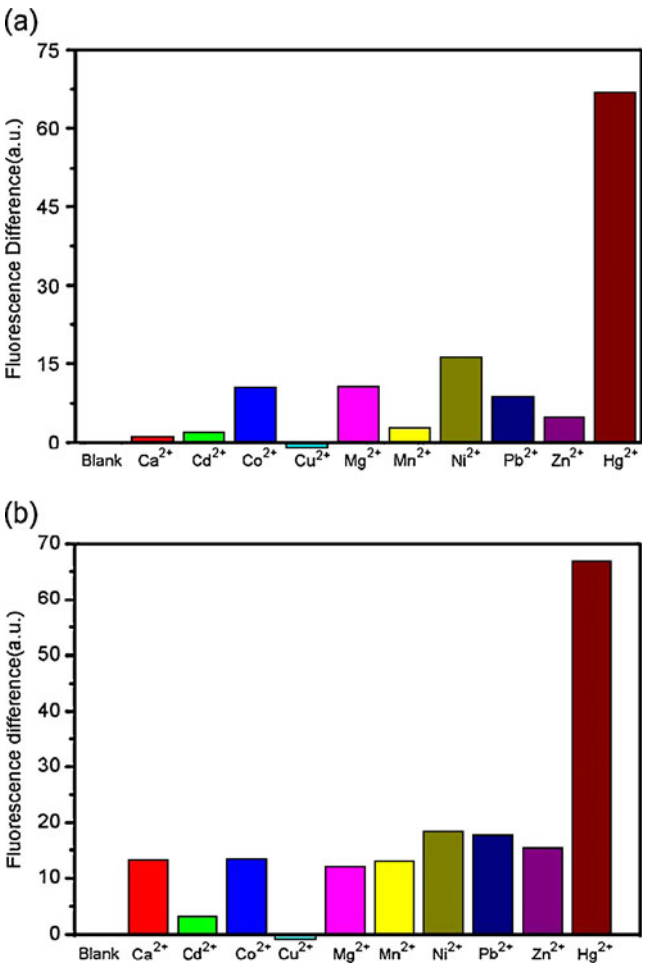


Fig. 3 The difference in fluorescence intensity between the blank and solutions containing different metal ions: **a** the concentration of all metal ions is 5 μM; **b** the concentration of Hg^{2+} and other metal ions is 5 and 50 μM, respectively

that all the other metal ions have small influence on the fluorescence of the FAM-OND. We further examined the influence of these metal ions with a 10-fold concentration of Hg^{2+} on the FAM-OND fluorescence, as shown in Fig. 3b. Under such conditions, although FAM-OND produces a little bit bigger fluorescence differences in most cases than those observed at 5 μM, the use of Hg^{2+} still gives the best result. All these observations indicate that the FAM-OND probe described herein exhibits high selectivity for Hg^{2+}.

Conclusion

In conclusion, a novel single-labeled fluorescent OND probe based on the inherent quenching of deoxyguanosines is developed for sensitive and selective detection of Hg^{2+} in aqueous solution. Our design is simple and the detection process is cost-effective and fast. We believe that our observations will help to improve the direct detection of Hg^{2+} in the environment without the interference from other excess metal ions coexisted.

Experimental

Chemically synthesized OND was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA concentration was estimated by measuring the absorbance at 260 nm. All the other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. The water used throughout all experiments was purified through a Millipore system. Fluorescent emission spectra were recorded on a PerkinElmer LS55 Luminescence Spectrometer (PerkinElmer Instruments, U.K.).

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