Talanta 85 (2011) 713–717

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A simple and sensitive fluorescent sensing platform for Hg^{2+} ions assay based on G-quenching

Peng Hu, Lihua Jin, Chengzhou Zhu, Shaojun Dong*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, Jilin, PR China

ARTICLE INFO

Article history:
Received 13 February 2011
Received in revised form 19 April 2011
Accepted 21 April 2011
Available online 29 April 2011

Keywords:
G-quenching
Duplex-like structure
Mercury detection
Fluorescence sensor

ABSTRACT

In this work, a novel fluorescence biosensor was demonstrated for detection of Hg^{2+} ions with relatively high selectivity and sensitivity. The sensing scheme was based on G-quenching induced by Hg^{2+} ions. In the presence of Hg^{2+} ions, the single-stranded signal probe which has carboxylfluorescein (FAM) and guanine segment at its 5′ and 3′ ends, respectively, folded into duplex-like structure via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. It brought guannine segment close to the dye and caused a remarkable decrease of fluorescence signal. The sensor showed a sensitive response to Hg^{2+} ions in a concentration range from 0.5 to 10 μM, and a detection limit of 0.5 nM was given. This homogeneous system required only a single-labeled oligonucleotide, operated by concise procedures, and possessed comparable sensitivity as previous approaches. Furthermore, the sensor exhibits a great perspective for future practical applications.

1. Introduction

Mercury, as one of the most prevalent toxic metals in the environment, could be easily found in ambient air, soil, water and even food [1,2]. It can easily pass through biological membranes, inhibit catalytic activity of different enzymes and lead to damage to the brain, nervous system, endocrine system and the kidneys [3]. Up to now, there have been numerous reports on Hg^{2+} ions detection by using atomic absorption/emission spectroscopy, inductively coupled plasma mass spectrometry (ICPMS) and atomic fluorescence spectrometry [4]. Nevertheless, each of these approaches exhibits some inadequacy, such as step-complicated, time-consuming and relatively high cost, and that makes them unsuitable for the convenient detection of Hg^{2+} ions and limits its practical use. Therefore, it has become an increasing demand to explore new methods to detect Hg^{2+} ions.

Mismatch of T–Hg^{2+}–T base pairs, whose binding mechanism was studied initially by Katz [5], has been attracting more and more attention in recent years. Lots of methods for the assay of Hg^{2+} ions based on the mismatch have been developed. Typical examples include electrochemical [6,7], colorimetric [8–14] and surface plasmon resonance for sensitive detection of Hg^{2+} ions [15]. Among various detection methods, fluorescence biosensors using labeled or unlabeled oligonucleotides, owing to its operational simplicity and high sensitivity, have gotten great interest in different fields, such as the environmental monitoring, biological and clinical analysis [16,17]. To date, a variety of biosensors based on fluorescence have been developed to facilitate the detection of species. For example, Tan et al. designed a novel molecular assembly of quencher molecules to form superquenchers with excellent quenching efficiency to detect DNA [18], Wang et al. developed a fluorescent sensing approach for detection of single-nucleotide polymorphisms (SNPs) based on the ligase reaction and gold nanoparticle (AuNPs)-quenched fluorescent oligonucleotides [19]. Zhang et al. presented a fluorescent sensor for Hg^{2+} detection, which was based on the noncovalent assembly of single-walled carbon nanotubes (SWNTs) and dye-labeled T-rich single-stranded DNA (ssDNA), and the detection limit was 14.5 nM [20]. Liu and his coworkers also developed a technique for the highly selective and sensitive detection of Pb^{2+} and Hg^{2+} using a thrombin-binding aptamer (TBA) probe labeled with the donor carboxyfluorescein (FAM) and the quencher [21]. It should be noted that these methods mentioned above generally used nanomaterials or organic molecules as a quencher. In this article, we utilized guanine as a quencher of fluorophore and proposed a novel and sensitive fluorescence sensing platform for Hg^{2+} ions assay. Based on the G-quenching and the Hg^{2+}-mediated duplex structure, a simple and effective fluorescence sensor for the determination of Hg^{2+} ions was fabricated.

2. Experimental

2.1. Reagents

Signal probe and DNA oligonucleotides were synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

* Corresponding author. Tel.: +86 431 85262101; fax: +86 431 85689711.
E-mail address: dongsj@ciac.jl.cn (S. Dong).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.04.063

Signal probe: 5′FAM-CTTGTTTCTT GCTCGC TTGTTTCTTG GGG-3′.
Control probe: 5′-TTCTTTCTT CCCC TTGTTTGTT-FAM 3′.
Single-stranded DNA: 5′-CTTGTTTCTT GCTCGC TTGTTTCTTG-GGG-3′.
Complementary DNA1: 5′-AAGAAACAAC-3′.
Complementary DNA2: 5′-AGC AAGAAACAAC-3′.
Complementary DNA3: 5′-GCGAGC AAGAAACAAC-3′.

Tris(hydroxymethyl)aminomethane (Tris), the metal salts, and all the other reagents were of analytical grade and used without further purification. Deionized and sterilized water (resistance >18 MΩ cm) was used throughout the experiment. Unless otherwise stated, all the reagents were prepared with Tris–HAc buffer containing 20 mM Tris–HAc, pH 7.4.

2.2. Instrumentation

The fluorescence spectra were measured by a Fluoromax-4 Spectrofluorometer (HORIBA Jobin Yvon, Inc., NJ, USA) using ultraviolet quartz cell at room temperature. The emission spectra were recorded in the wavelength range of 503–650 nm upon excitation at 494 nm. Excitation and emission slits were set at 3 nm and 5 nm, respectively. Circular dichroism (CD) spectra were performed on a JASCO J-820 spectropolarimeter (Tokyo, Japan) equipped with a Peltier temperature controller.

2.3. Homogenous detection of Hg^{2+} ions

The standard stock solution of Hg^{2+} ions was prepared with $Hg(Ac)_2$ in Tris–HAc buffer. Different concentrations of Hg^{2+} ions were obtained by diluting standard stock solutions. The following procedure was used to study the concentration-dependent fluorescence quenching experiments: a 5 μL droplet of signal probe (1 μM) was mixed with 90 μL different concentrations of Hg^{2+} ions, the mixtures were then heated at 85 °C for 5 min and cooled quickly with ice. Subsequently, 200 μL Tris-buffer (20 mM Tris–HAc, 100 mM NaCl, pH 7.4) was added to give final volumes of 300 μL. Then, the fluorescence spectra were recorded at room temperature.

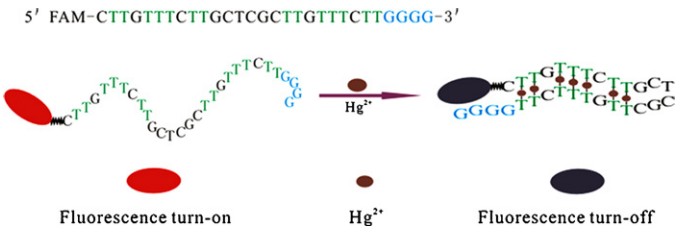
2.4. Circular dichroism (CD)

The CD spectra measurement was carried out and used to confirm the conformational change of the signal probe. 900 μL of 2.78 μM single-stranded DNA solution with or without Hg^{2+} ions was heated at 85 °C for 5 min and cooled quickly with ice. CD spectra were recorded between 210 nm and 320 nm in 1 cm path length cuvettes. Three scans were accumulated and averaged. The scan speed was 100 nm/min, the response time was 0.5 s and the bandwidth was 1.0 nm.

3. Results and discussion

3.1. Design of the fluorescence sensor

Fluorescent dyes, when covalently bound to oligonucleotides, were sensitive to the attachment sites and surrounding nucleotide sequences. Among the four nucleic acid bases, guanine has good electron-donating property and can be used as a quencher of fluorophore [22–27]. The quenching mechanism was thought to involve photo-induced electron transfer from the nucleotide to the singlet excited state of the fluorophore [28,29]. Up to now, many dyes were studied using guanine as a quencher [30–32], and the nucleotide-specific quenching of fluorescent dyes could be used in single-labeled oligonucleotides to detect specific target species. Based on the consideration mentioned above, we constructed a novel fluorescent sensing platform for Hg^{2+} ions assay. The signal probe we designed was labeled with FAM at its 5′ end. It contained a thymine (T)-rich region (bold) and a guanine (G)-rich segment as quencher (italicized region). As can be seen in Scheme 1, in the absence of Hg^{2+} ions, the signal probe was a flexible random-coil in aqueous solution. Fluorophore and quencher were separated from each other and strong fluorescence was obtained when the dyes were excited. In the presence of Hg^{2+} ions, it formed duplex-like structure via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. The structure held the fluorophore and the guanine segment in close proximity to each other and led to a remarkable decrease of fluorescence response. Fig. 1A confirms the feasibility of homogeneous system for screening the target Hg^{2+} ions. Obviously, high fluorescence intensity was observed without target. When adding Hg^{2+} ions to the sample, the target caused a remarkable fluorescence decrease. The result obtained was consistent with our conception. Based on the present signaling scheme, a powerful sensing platform for the highly sensitive detection of Hg^{2+} ions was successfully constructed.



Scheme 1. Schematic illustration for sensitive detection of Hg^{2+} ions based on the G-quenching fluorescence.

To verify the fluorescence signal decrease induced indeed by guanine segment via the formation of duplex structure, a control experiment was implemented by using a control signal probe without the guanine segment at its 5′ end. As shown in Fig. 1B, in contrast to the remarkable signal decrease of the signal probe, there was only a little change of fluorescence signal when adding Hg^{2+} ions to the sample. The experiment result validated directly that the dye could be effectively quenched by guanine segment.

3.2. Stability of T–Hg^{2+}–T mismatch

The coordination between thymine and Hg^{2+} ions is the main driven force of T-rich DNA binding to the Hg^{2+} ions. According to the literature, the interaction between them was more stable than A:T nucleic acid base pairs [5,33]. Herein, we investigated the effect of complementary DNA (cDNA) on the T–Hg^{2+}–T mismatched base pairs. A 5 μL signal probe (1 μM) was mixed with 90 μL Hg^{2+} ions (10 μM). The samples were then heated at 85 °C for 5 min and cooled quickly. 5 μL cDNAs (1 μM) with various base lengths were added to the samples accordingly, and the mixtures were incubated for 40 min at 37 °C. 200 μL Tris-buffer (20 mM Tris–HAc, 100 mM NaCl, pH 7.4) was finally added for the fluorescence detection. As shown in Fig. 2 black bars, there was almost no change of the signal difference upon the addition of cDNAs compared to the absence of them. It indicated that the cDNAs could not hybridize with the signal probe and form double helix, and the T–Hg^{2+}–T mismatches were still the predominant form. While adding Hg^{2+} ions to the mixtures of signal probe and cDNAs, a significant signal drop was obtained (Fig. 2 gray bars). The results clearly demonstrated that the T–Hg^{2+}–T mismatches were stable enough and could be used as a sensing platform to detect Hg^{2+} ions sensitively.

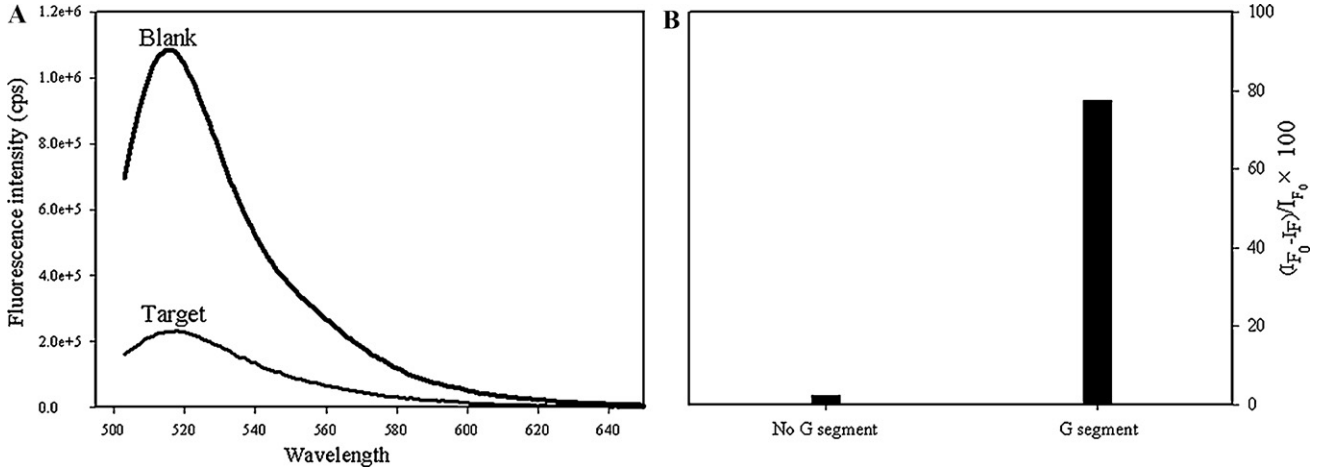


Fig. 1. (A) Fluorescence spectra of signal probe (50 nM) in the absence (Blank) and presence (Target) of Hg^{2+} ions (10 μM). (B) Control experiment. The concentration of the signal probe and the control probe is 50 nM, the Hg^{2+} ions is 5 μM. I_F and I_{F0} represent the fluorescence intensity in the presence and absence of Hg^{2+} ions, respectively. Excitation wavelength is 494 nm and emission wavelength is 516 nm.

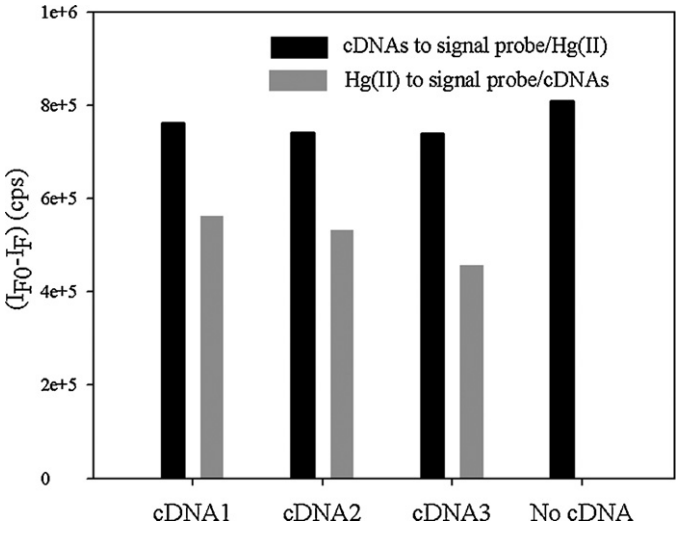


Fig. 2. Effect of complementary DNA on the stability of T–Hg^{2+}–T base pairs. The concentration of signal probe, cDNAs and Hg^{2+} ions is 50 nM, 50 nM and 10 μM, respectively.

3.3. CD spectroscopy characterization

CD spectroscopy could provide the information about structures of DNAs as well as the effects of sequence, cations, chemical modification, and ligand binding. Therefore, the formation of duplex-like structure of DNA due to the introduction of target Hg^{2+} could be readily distinguished via CD spectra. We used the single-stranded DNA (ssDNA) as alternative substances of signal probe to perform this experiment. Without Hg^{2+} ions, the CD spectrum of ssDNA at room temperature exhibited a negative band at 245 nm, and a major positive band at 283 nm (Fig. 3A Blank). Upon the addition of 5.56 μM target species, a dramatic change of CD spectra was observed (Fig. 3A Target). The maximum at 283 nm decreased and shifted to 292 nm. At the same time, a major negative band at about 263 nm appeared, indicating formation of duplex structure [33]. Subsequently, we monitored the CD intensity change with temperature variation at wavelength of 263 nm, 283 nm and 292 nm, respectively. As shown in Fig. 3B, when temperature increased, the positive peak at 263 nm emerged slowly and the negative at 283 nm disappeared gradually. It revealed that the duplex-like DNA gradually dissociated into single strand. However, the CD spectra at 292 nm first decreased slightly and then increased tardily. We speculated that it was due to the maximum of CD spectra gradually shifted from 292 nm to 283 nm accompanying the temperature increase. These results fully evi-

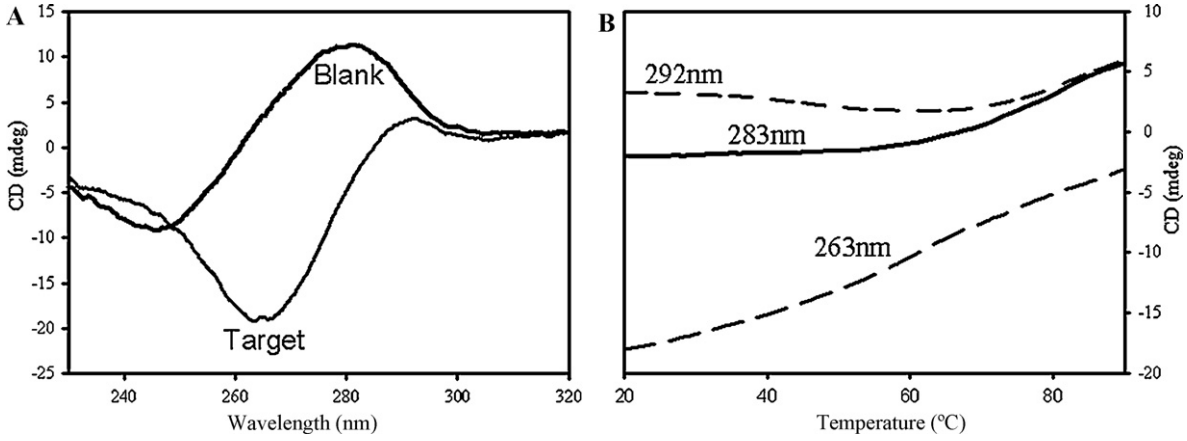


Fig. 3. CD spectra characterization. (A) The single-stranded DNA (2.78 μM) in the absence (Blank) and presence (Target) of Hg^{2+} ions (27.8 μM), respectively. (B) The CD intensity at wavelength of 263 nm, 283 nm and 292 nm with the temperature variation.

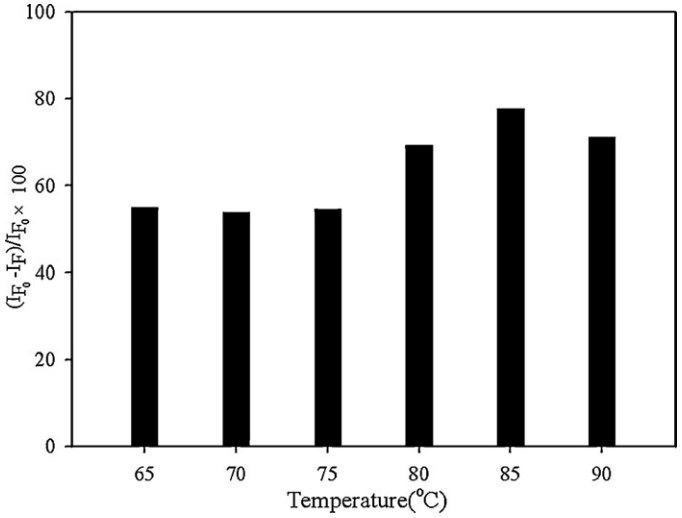


Fig. 4. Effect of reaction temperature on the fluorescence response. The concentration of Hg^{2+} ions is 10 μM.

denced the formation of the duplex structure of signal probe mediated by Hg^{2+} ions, and the signal probe could be used as an effective sensing element for homogenous detection of Hg^{2+} ions.

3.4. Optimization of reaction temperature

In order to obtain a perfect assay performance, the reaction temperature was investigated. In ambient temperature, the signal probe coiled randomly in solution, and there are intra- and inter-chain interactions between them. The interaction could inhibit the formation of duplex-like structure. Therefore, it was necessary to eliminate the interaction through heating. Fig. 4 depicts the relative fluorescence response ($(I_{F0}-I_F)/I_{F0}$) (I_F and I_{F0} are the fluorescence intensity at 516 nm in the presence and absence of Hg^{2+} ions, respectively) at various temperatures. Maximum difference of fluorescence intensity was observed at 85 °C among the reaction temperature we selected. Hence, 85 °C was chosen as the optimized reaction temperature in our experiments.

3.5. Analytical performance of sensing system

To validate whether the fluorescence change could be used for Hg^{2+} ions quantitation, the fluorescence responses of the sensor to Hg^{2+} ions of varying concentrations are shown in Fig. 5A. Under the optimal conditions, a dynamically decreased fluorescence signal was observed in the presence of increasing Hg^{2+} ions concentration within the range from 0.5 nM to 20 μM. A good linear relationship was obtained between the square root of the fluorescence signal change ΔI ($\Delta I = I_{F0} - I_F$) and the logarithm of Hg^{2+} ions concentration in a range from 0.5 to 10 μM for sensitive Hg^{2+} ions quantitation. The regression equation was $\Delta I^{1/2} = 563.13 \log[C_{Hg^{2+}}] + 3731.38$ with a correlation coefficient of 0.9500. Linear calibration curve was shown in the inset of Fig. 5B. The detection limit distinguished from the blank was determined to be 0.5 nM (amounting to 0.1 ppb). Such a low detection limit, to the best of our knowledge, is better than the most sensors previously reported based on the T–Hg^{2+}–T coordination fluorescence and electrochemical methods, and is lower than the toxic level for Hg^{2+} ions defined by the United States Environmental Protection Agency (USEPA) in drinkable water (<10 nM). The sensitivity in present work was favorable compared with several methods

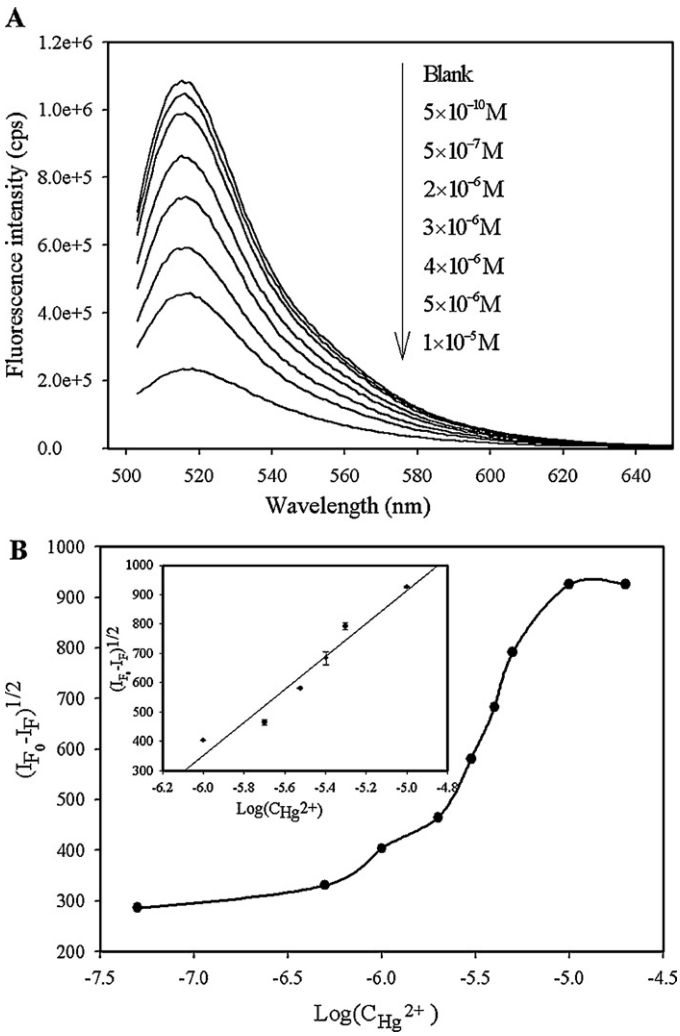


Fig. 5. Linear plots of the sensing system in the presence of target Hg^{2+} ions at different concentrations. Inset: quantitative analysis of the target concentration. The experiment conditions are described in Section 2. The error bars denote the standard deviation of three measurements.

reported [6,8,20,21,34]. The exquisite detection capability of the designed sensor might exhibit great promise in environmental and biological fields.

3.6. Selectivity measurement

We monitored the fluorescence change after adding various metal cations to examine the selectivity of this sensing system for Hg^{2+} ions. As indicated in Fig. 6, there was no significant effects upon the addition of Ca^{2+}, Ba^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Sr^{2+} and Cu^{2+} ions, while Pb^{2+}, Al^{3+}, Cr^{3+} or Ag^{+} ions led to large decrease in the fluorescence intensity. Therefore, to further improve the selectivity toward Hg^{2+} ions, a chelating ligand, citric acid, was added to the solutions. Citric acid was a promising chelating agent due to its biodegradation, low toxicity and amphophilic nature properties. It could form relatively strong complexes with most heavy metal ions at mildly acidic pH compared to other chelator [35]. As seen from the comparison of selectivity upon addition of citric acid in Fig. 6, these results suggested that citric acid could mask interference ions effectively and increased the selectivity toward Hg^{2+} ions greatly.

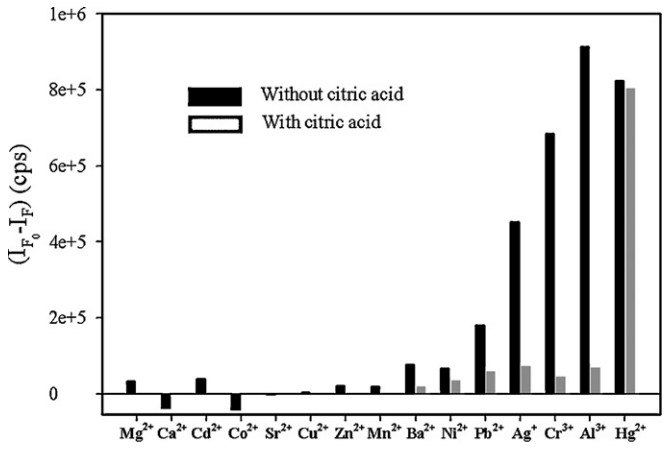


Fig. 6. Fluorescence response to different metal ions (56 μM) in the absence and presence of citric acid (0.28 mM).

Table 1
Analysis of the real sample.

Sample	Added	Found	Recovery (%)	RSD (%)
1	0.50 μM	0.55 μM	110	4.5
2	5.00 μM	5.27 μM	105	4.1
3	10.00 μM	9.25 μM	92	4.4

3.7. Analysis of the sensing system in the real sample

Moreover, in consideration of extending our sensor system for Hg^{2+} ions detection in practical applications, the recovery experiments of Hg^{2+} ions at different concentrations within the linear range were carried out by applying a standard addition method. A pond water sample was filtered through a 0.2 μm membrane and analyzed. A certain amount of target was added to the pond water (85 μL) to make the final concentration of Hg^{2+} ions reach to 0.5, 5 and 10 μM. The target detection was performed according to the procedure mentioned above, and the 'Found' concentration was estimated from corresponding fluorescence intensity according to the regression equation. All the measurements were conducted three times, and the results are shown in Table 1. The recoveries obtained were in the range of 92–110%, and the average RSD was 4.3%.

4. Conclusions

In the present study, a new and facile method for detecting Hg^{2+} ions based on G-quenching fluorescence have been developed. The metal recognition mechanism was based on the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. The fluorescence of FAM could be quenched effectively by guanine via conformational switch induced by Hg^{2+} ions and that resulted in the decrease of fluorescence. In comparison with previous approaches for Hg^{2+} ions detection, the sensor response was fast, and quantitative results could be obtained within 15 min. Additionally, the conformation-switching signaling element could transduce the binding reaction into detectable physical signal efficiently and could be employed to construct a wide range of biosensing schemes for the analysis of various target species via target-mediated oligonucleotides. In the foreseeable future, the biosensor could provide an opportunity for environmental studying, biomolecule analysis and clinical diagnostic tests.

Acknowledgements

This project was supported by the Special Found for Major State Basic Research of China (973 Project, 2011CB911002) and National Natural Science Foundation of China (Nos. 20935003 and 21075116).

References

[1] R. Eisler, Environ. Geochem. Hlth. 25 (2003) 325.
[2] Q.R. Wang, D. Kim, D.D. Dionysiou, G.A. Sorial, D. Timberlake, Environ. Pollut. 131 (2004) 323.
[3] J. Gutknecht, J. Membr. Biol. 61 (1981) 61.
[4] Y. Gao, S. De Galan, A. De Brauwere, W. Baeyens, M. Leermakers, Talanta 82 (2010) 1919–1923.
[5] S. Katz, Nature 194 (1962) 569.
[6] S.J. Liu, H.G. Nie, J.H. Jiang, G.L. Shen, R.Q. Yu, Anal. Chem. 81 (2009) 5724–5730.
[7] Z.Q. Zhu, Y.Y. Su, J. Li, D. Li, J. Zhang, S.P. Song, Y. Zhao, G.X. Li, C.H. Fan, Anal. Chem. 81 (2009) 7660–7666.
[8] J.S. Lee, M.S. Han, C.A. Mirkin, Angew. Chem. Int. Ed. 46 (2007) 4093–4096.
[9] L. Li, B.X. Li, Y.Y. Qi, Y. Jin, Anal. Bioanal. Chem. 393 (2009) 2051–2057.
[10] N. Lu, C.Y. Shao, Z.X. Deng, Analyst 134 (2009) 1822–1825.
[11] T. Li, S.J. Dong, E.K. Wang, Anal. Chem. 81 (2009) 2144–2149.
[12] X.B. Zuo, H.A. Wu, J. Toh, S.F.Y. Li, Talanta 82 (2010) 1642–1646.
[13] D. Li, A. Wieckowska, I. Willner, Angew. Chem. Int. Ed. 47 (2008) 3927–3931.
[14] Y. Wang, F. Yang, X.R. Yang, Appl. Mater. Interfaces 2 (2010) 339–342.
[15] L. Wang, T. Li, Y. Du, C.G. Chen, B.L. Li, M. Zhou, S.J. Dong, Biosens. Bioelectron. 25 (2010) 2622–2626.
[16] F. Samain, S. Ghosh, Y.N. Teo, E.T. Kool, Angew. Chem. Int. Ed. 49 (2010) 7025–7029.
[17] M.E. Ostergaard, P. Cheguru, M.R. Papasani, R.A. Hill, P.J. Hrdlicka, J. Am. Chem. Soc. 132 (2010) 14221–14228.
[18] C.Y.J. Yang, H. Lin, W.H. Tan, J. Am. Chem. Soc. 127 (2005) 12772–12773.
[19] H. Wang, J.S. Li, Y.X. Wang, J.Y. Jin, R.H. Yang, K.M. Wang, W.H. Tan, Anal. Chem. 82 (2010) 7684–7690.
[20] L.B. Zhang, T. Li, B.L. Li, J. Li, E.K. Wang, Chem. Commun. 46 (2010) 1476–1478.
[21] C.W. Liu, C.C. Huang, H.T. Chang, Anal. Chem. 81 (2009) 2383–2387.
[22] Y. Saito, E. Mizuno, S.S. Bag, I. Suzukaa, I. Saito, Chem. Commun. (2007) 4492–4494, doi:10.1039/B709715H, Communication.
[23] J.E. Noblea, L. Wang, K.D. Coleb, A.K. Gaigalasb, Biophys. Chem. 13 (2005) 255–263.
[24] T. Heinlein, J.P. Knemeyer, O. Piestert, M. Sauer, J. Phys. Chem. B 107 (2003) 7957–7964.
[25] J.P. Knemeyer, N. Marme, M. Sauer, Anal. Chem. 72 (2000) 3717–3724.
[26] I. Nazarenko, R. Pires, B. Lowe, M. Obaidy, A. Rashtchian, Nucleic Acids Res. 30 (2002) 2089–2195.
[27] L. Wang, J.Q. Tian, H.L. Li, Y.W. Zhang, X.P. Sun, J. Fluoresc, doi:10.1007/s10895-010-0785-z.
[28] L. Edman, U. Mets, R. Rigler, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6710–6715.
[29] F.D. Lewis, R.L. Letsinger, M.R. Wasielewski, Acc. Chem. Res. 34 (2001) 159–170.
[30] L. Edman, U. Mets, R. Rigler, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6710.
[31] I. Nazarenko, B. Lowe, M. Darfler, P. Ikonomi, D. Schuster, A. Rashtchian, Nucleic Acids Res. 30 (2002) e37.
[32] C.A.M. Seidel, A. Schulz, M.J. Sauer, Phys. Chem. 100 (1996) 5541.
[33] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, J. Am. Chem. Soc. 128 (2006) 2172–2173.
[34] N. Dave, M.Y. Chan, P.J.J. Huang, B.D. Smith, J. Liu, J. Am. Chem. Soc. 132 (2010) 12668–12673.
[35] D. del Mundo Dacera, S. Babel, Water Sci. Technol. 54 (2006) 129–135.