Talanta 113 (2013) 26–30

Contents lists available at SciVerse ScienceDirect

Talanta

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

Aptamer functionalized gold nanoparticles based fluorescent probe for the detection of mercury (II) ion in aqueous solution

Daidi Tan, Yue He, Xiaojing Xing, Yu Zhao, Hongwu Tang*, Daiwen Pang

[a] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Research Center for Nanobiology and Nanomedicine (MOE 985 Innovative Platform), Wuhan Institute of Biotechnology, and State Key Laboratory of Virology, Wuhan University, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 22 December 2012
Received in revised form
18 March 2013
Accepted 23 March 2013
Available online 4 April 2013

Keywords:
Gold nanoparticles
Aptamer
Fluorescent probe
Mercury (II) ion

ABSTRACT

A method is proposed for the detection of Hg^{2+} using Hg^{2+} specific DNA (MSD) functionalized gold nanoparticles (AuNPs) based on the formation of T–Hg^{2+}–T complex and the excellent quenching fluorescence property of AuNPs. The MSD is rich in thymine (T) and readily forms T–Hg^{2+}–T configuration in the presence of Hg^{2+}. The MSD which is labeled with a fluorescein (FAM) at the 3′-end and a thiol at the 5′-end is bounded to the AuNPs through Au–S covalent bonds to form the probes (AuNPs–MSD). Hg^{2+} detection can be easily realized by monitoring the change of fluorescence signal of AuNPs–MSD probes. Hg^{2+} can be detected in a range of 0.02–1.0 μM with a detection limit of 16 nM. Besides, the assay shows excellent selectivity for Hg^{2+} over other metal cations such as Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, Ni^{2+}, Cu^{2+}, Co^{2+} and Pb^{2+}. The major advantages of this Hg^{2+} assay are its water-solubility, simplicity, low cost and high sensitivity. Moreover, this method provides a potentially useful method for the Hg^{2+} detection in aqueous solution.

1. Introduction

Contamination of the environment with heavy metal ions has been an important concern throughout the world for decades. Mercuric ion (Hg^{2+}) is one of the most toxic heavy metals and a kind of persistent contaminant that is not biodegradable and thus retained in the ecosystem. Meanwhile the accumulation of Hg^{2+} in organisms has been reported to damage DNA, inhibit ligand–receptor interactions, disable normal functions of liver and kidney, disrupt the immune system homeostasis, and even lead to death [1–3]. Therefore, there is an ever-growing demand to detect Hg^{2+} in drinking water, food, air and soil. Conventional analytical techniques including atomic absorption/emission spectroscopy (AAS/AES), inductively coupled plasma mass spectrometry (ICP-MS) [4], cold-vapor atomic fluorescence spectrometry (CV-AFS) [5], ultraviolet-visible spectrometry, and X-ray absorption spectroscopy [6] have been extensively used for measurement of Hg^{2+} in environmental samples. However, these methods require expensive and sophisticated instrumentation and/or complicated sample preparation processes and their complexity make them unsuitable for use in on-site Hg^{2+} analyses. Therefore, there is a strong incentive to develop a sensitive, reliable, and convenient approach for monitoring Hg^{2+} in the environment [7]. Much effort has been made to develop portable sensors, including fluorescent sensors, scanometric sensors and colorimetric sensors [8–10]. Among which fluorescence based Hg^{2+} detection has attracted a huge interest because of its simple operation, high sensitivity, and adaptability for in-field Hg^{2+} measurement. Fluorescent sensors based on organic chromophores or fluorophores [11–14], conjugated polymers [15], DNA or DNAzyme [16,17] are reported. Although these approaches have made great contributions toward Hg^{2+} assay, limitations, such as insufficient resolution in water, irreversible Hg^{2+} complex, interference with other heavy metal ions, and sophisticated synthesis of the probe materials, still existed. Therefore, it should be desirable to develop a new method to solve these problems.

Another emerging approach for the detection of Hg^{2+} ions involves the use of oligonucleotides. Hg^{2+} ions can specifically interact with thymine bases to form strong and stable thymine–Hg^{2+}–thymine complexes (T–Hg^{2+}–T) [18–20]. Very recently T–Hg^{2+}–T (T=thymine) chemistry has been highlighted in the development of Hg^{2+} sensors because T–T mismatch shows high selectivity to Hg^{2+} against many other metal ions. A mercury specific-aptamer (MSD) has been elegantly designed for Hg^{2+} assays, which has a sequence of 5′-TTCTTTCTTCCCCTTGTTTGTT-3′. It forms a hairpin structure in the presence of Hg^{2+}, and presents a random coil form in the absence of Hg^{2+}. Various Hg^{2+} ion detection assays based on this aptamer MSD have been developed in recent years. Compared with those methods mentioned before, aptamer biosensor-based detection methods are less expensive,

* Corresponding author. Tel.: +87 2768752136.
E-mail address: hwtang@whu.edu.cn (H. Tang).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.03.055

easier to operate and suitable for on-site analyses [21,22]. Special attention has been paid to the sensors based on Förster resonance energy transfer (FRET) between a fluorescent donor and an acceptor. Generally, organic dyes are used as the energy donor and the energy acceptor in FRET sensors. In the absence of Hg^{2+}, fluorescein is separated from dabcyl and the system presents strong fluorescence, while in the presence of Hg^{2+}, the T–Hg^{2+}–T mediated hairpin formation causes dabcyl and fluorescein to come to close proximity for fluorescence quenching [21]. This protocol has shown high selectivity to Hg^{2+} against many other metal ions, however, the sensitivity (40 nM) is still far above the toxic level of Hg^{2+} in drinkable water set by the EPA.

It is well-known that gold nanoparticles (AuNPs) are attractive energy acceptor due to the nanometal surface, leading to a high energy transfer rate from the organic donor to the AuNPs acceptor and a long quenching distance [23–26]. In the present work, FAM and AuNPs are used to create a nanometal surface energy transfer (NSET) sensor for Hg^{2+} detection based on its aptamer MSD in water. It is found that this kind of sensor provides high sensitivity and selectivity for Hg^{2+} detection.

2. Experimental

2.1. Reagents

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 4H_2O$) and the other chemicals were purchased from Sinopharm Chemcial Reagent (Shanghai, China). A mercury specific-DNA (MSD) (5′-SH $(CH_2)_6A_{10}$TTCTTTCTTCCCCTTGTTTGTT-FAM-3′) and the helper DNA (5′-SH $(CH_2)_6A_{10}$) were purchased from Shanghai Sangon Biotechnology (Shanghai, China). The oligonucleotide stock solution (100 μM) were prepared with ultrapure water. All chemicals used in this work were of analytical grade or of highest purity available and used directly without further purification. The water used throughout all experiments was purified through a Millipore system.

2.2. Apparatus

Fluorescent emission spectra were obtained with an F-4600 Fluorescence Spectrophotometer, Hitachi High-Technology (Tokyo, Japan). All experiments were carried out at room temperature. The sample cell was a 700 μL quartz cuvette. The luminescence intensity was monitored by exciting the sample at 480 nm and measuring the emission at 520 nm. The slits for excitation and emission were set at 5 nm and 10 nm respectively. Ultraviolet–visible (UV–vis) absorption spectra were measured by using a Shimadzu UV-2550 spectrophotometer at room temperature. Transmission electron microscopy (TEM) measurements were made on a JEM100CXII (JEOL, Japan) transmission electron microscope at an accelerating voltage of 200 kV. The fitting of the experimental data was accomplished using the software Origin 8.0.

2.3. Preparation of AuNPs

AuNPs of ~13 nm in diameter were synthesized using the method developed by Natan et al. [27]. 50 mL 1.0 mM $HAuCl_4$ solution was boiled and stirred vigorously. 5 mL of 38.8 mM sodium citrate solution was then added into the boiling solution rapidly with concomitant color change from light yellow to wine-red. After boiling for 10 min and followed stirring for 15 min, the solution was allowed to reach room temperature. Then it was filtered through a 0.22 μM membrane filter and stored in a refrigerator at 4 °C before use. The concentration of AuNPs was estimated by UV–vis spectroscopy based on an extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at $\lambda = 520$ nm for 13 nm gold nanoparticles [28].

2.4. MSD-functionalized AuNPs preparation

AuNPs were functionalized [28,29] with oligonucleotides by the MSD to aqueous nanoparticle solution (particle concentration 10 nM) to a final oligonucleotide concentration of 3.0 μM. After 16 h of incubation at room temperature, NaCl solution was added (final concentration of 0.1 M) by the stepwise addition of 1 M NaCl/100 mM phosphate-buffered saline (PBS, pH 7.4). And meanwhile, the short "helper" oligonucleotides, that is, 3′-thiolated oligo-As (10 As), was added into the solution. The solution was allowed to "age" under these conditions for an additional 24 h. Excess reagent was then removed by centrifugation at 13,000 rpm and 4 °C for 30 min. Following the removal of the supernatant, the red oily precipitate was washed once with 10 mM PBS buffer (pH 7.4) by successive centrifugation and redispersion and then finally was suspended in stock solution (25 mM pH 8.2 Tris–HCl buffer and 300 mM NaCl) and stored at 4 °C for further use.

2.5. Procedures for Hg^{2+} detection

The as-prepared DNA–AuNPs was used as the detection probe. For Hg^{2+} assays, different concentration of the samples were added into the probe solutions and then the mixed solution was diluted with Tris–HCl buffer to 500 μL. The above prepared solution was incubated for 30 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm. To confirm the practical application of the probes, the water sample from Donghu Lake in Wuhan was filtered through a 0.22 μm membrane. A 10 μL of 100 μM Hg^{2+} solution and 100 μL lake water were added into the as-prepared probe, and then the mixed solution were diluted with Tris–HCl buffer to 500 μL and incubated for 30 min.

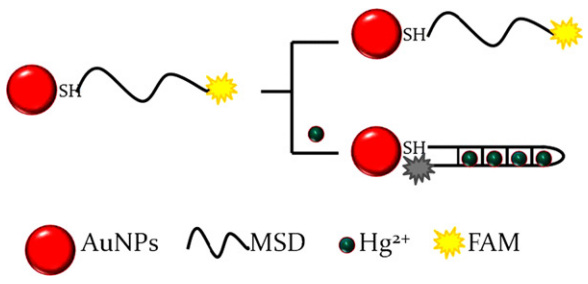
For the sensitivity measurement, different concentrations of Hg^{2+} (0, 0.02, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 4.0, 6.0 μM) were added, and the fluorescence emission spectra were then monitored.

For the selectivity measurement, various metal ions of 100 nM were supplied with other salts. The fluorescence emission spectra were monitored after the introduction of metal ions into the test assay.

3. Results and discussion

3.1. Nanoprobe design

The overall detection strategy is shown in Scheme 1. Hg^{2+} can selectively and strongly bind thymine, so it can specifically link otherwise impossible T–T pairs to form T–Hg–T complex [20,30], and the T–Hg–T complex is thermally stabilized. Based on the



Scheme 1. A schematic illustration of the AuNPs-based fluorescent Hg^{2+} detection based on conformational change of a Hg^{2+} specific oligonucleotides.

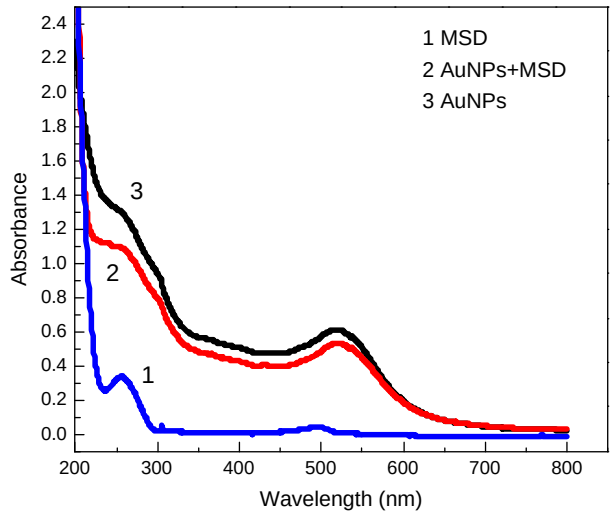


Fig. 1. UV–visible absorption spectra of MSD (1) and AuNPs+MSD (2) in Tris–HCl and AuNPs (3) in water.

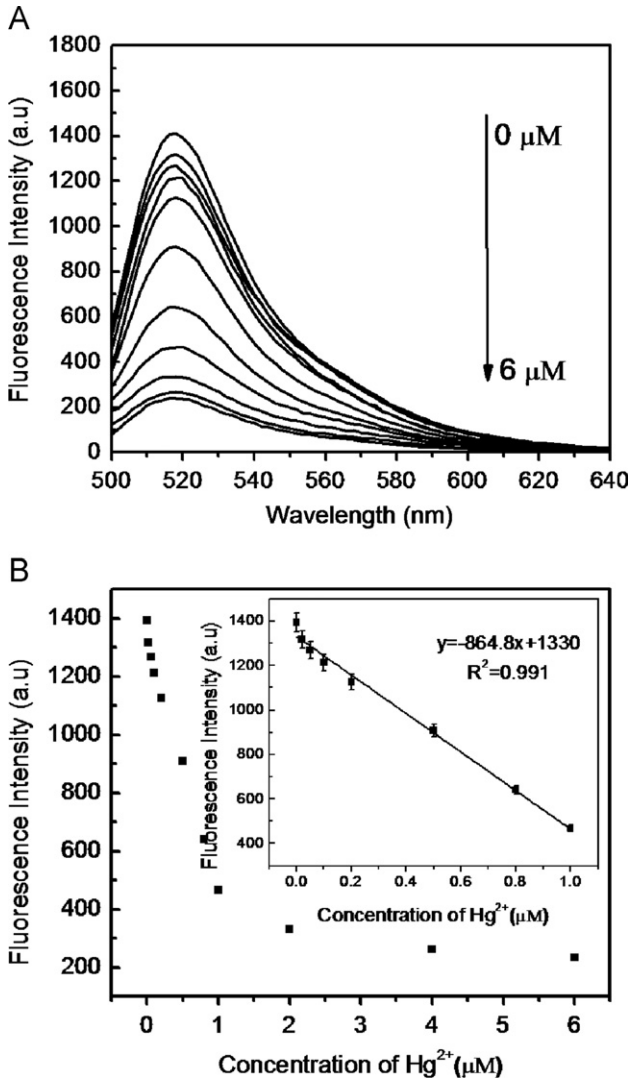


Fig. 2. (A) Fluorescence spectra of AuNPs–MSD in the presence of different Hg^{2+} concentrations (from top to bottom: 0, 0.02, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 4.0, and 6.0 μM) in Tris–HCl buffer (pH 8.2). (B) The fluorescence intensity plotted against the Hg^{2+} concentration. Excitation: 480 nm, emission: 520 nm. The inset is the linear relationship of the fluorescence intensity of AuNPs–MSD in the presence of Hg^{2+}, covering the range from 0 to 1.0 μM.

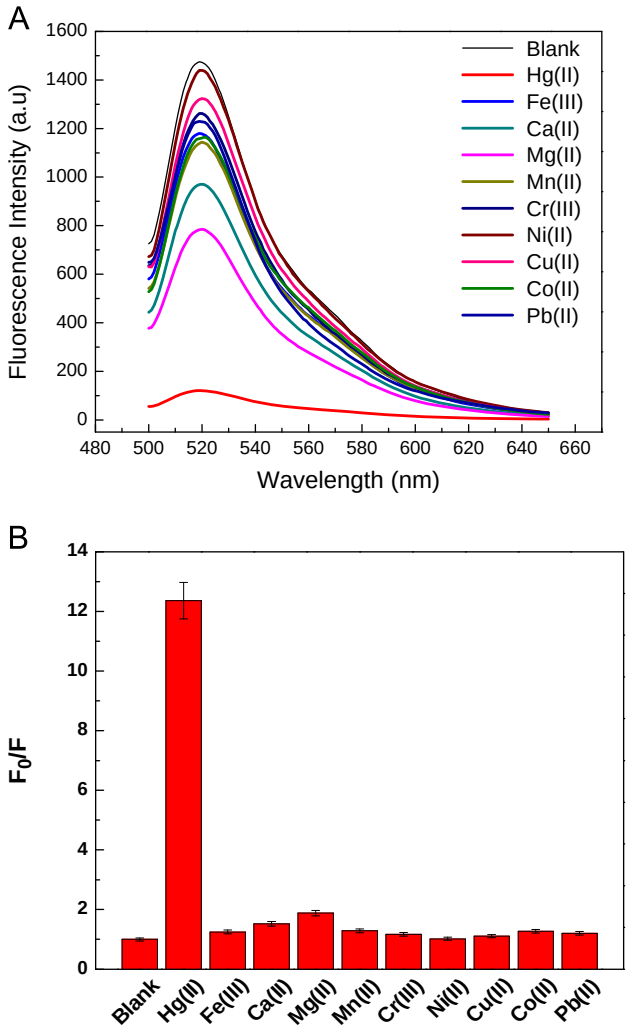


Fig. 3. Fluorescence spectra (A) and fluorescence intensity histograms (B) of AuNPs–MSD in the presence of different metal ions in Tris–HCl buffer (pH 8.2) ([Hg^{2+}]=2.0 mg/L; [other metal ion]=5.0 mg/L). Excitation: 480 nm, emission: 520 nm.

T–Hg–T coordination chemistry, the DNA aptamer of Hg^{2+} is deliberately designed. Here, we used the T-rich single-stranded oligonucleotides (5′-TTCTTTCTTCCCCTTGTTTGTT-3′) as the Hg^{2+} specific DNA (MSD) [21]. The MSD which is labeled with a fluorescein (FAM) at the 3′-end and a thiol at the 5′-end is bounded to the AuNPs through Au–S covalent bonds to form the probes (AuNPs–MSD). When Hg^{2+} ions are present, the MSD which has a random coil structure will change into a hairpin-like structure. As a result, FAM and AuNPs will be brought into close proximity, which enables quenching of the fluorescence of the FAM, thus the fluorescence intensity of FAM in the presence of Hg^{2+} is weaker.

3.2. Characterization of the AuNPs–MSD probe

The optical spectral properties of MSD attached to the surface of AuNPs were investigated. Fig. 1 shows the UV–vis absorption spectra of the MSD (curve 1), AuNPs–MSD (curve 2) and AuNPs (curve 3), respectively. MSD is characterized by an absorption band of FAM (495 nm) and characterized absorption band at 260 nm, while the surface plasmon band (SPB) of AuNPs is at 520 nm.

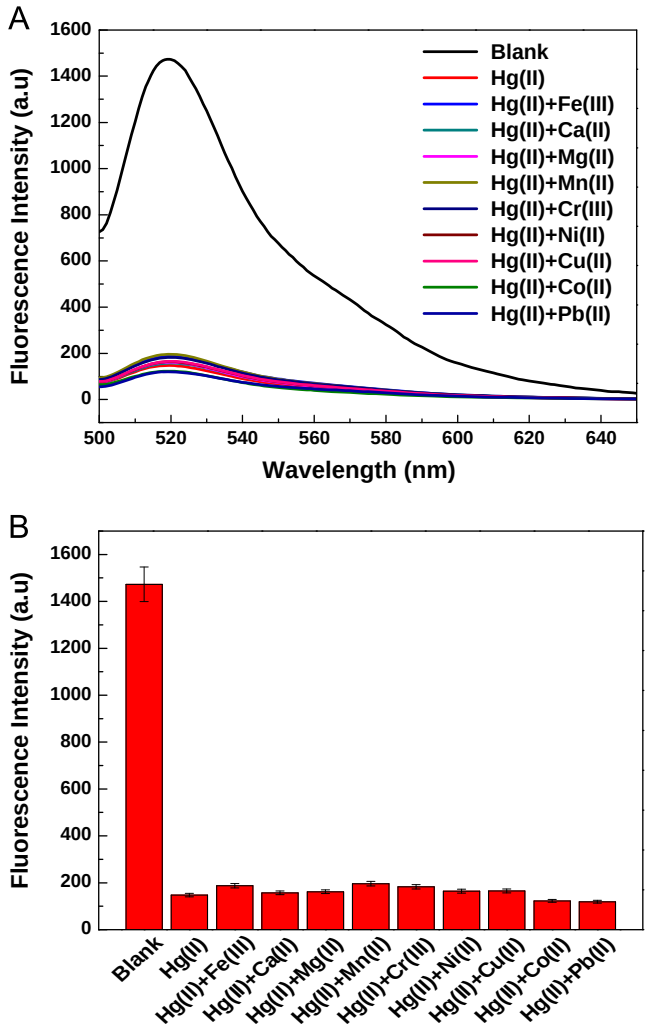


Fig. 4. Fluorescence spectra (A) and fluorescence intensity histograms (B) of AuNPs–MSD in the presence of mixed metal ions in Tris–HCl buffer (pH 8.2) ([Hg^{2+}]=2.0 mg/L; [other metal ion]=5.0 mg/L). Excitation: 480 nm, emission: 520 nm.

The combining of MSD with AuNPs results in no obvious change of the SPB of AuNPs, as shown in Fig. 1.

3.3. Sensitivity of Hg^{2+} ion detection

The fluorescence emission properties of FAM are sensitive to the presence of nanomolar concentrations of Hg^{2+}. For the sensitivity study, different concentrations of Hg^{2+} solutions from 0.02 to 6.0 μM were investigated. Fig. 2A shows the fluorescence spectra of AuNPs–MSD in the presence of different Hg^{2+} concentrations in Tris–HCl buffer solution. Clearly, the fluorescence intensity of the mixture decreases with the increase of Hg^{2+} concentration. Fig. 2B is the fluorescence intensity plotted against the Hg^{2+} concentration. However, when Hg^{2+} concentration is higher than 2.0 μM, the fluorescence intensity was not further enhanced and a plateau is reached, because the interaction between MSD and Hg^{2+} reaches a balance and saturation. The inset in Fig. 2B is the linear relationship between the fluorescence intensity of AuNPs–MSD and Hg^{2+} concentration, covering the range from 0.02 to 1.0 μM. A practically usable range for quantitative determination covers a range from 0.02 to 1.0 μM (R^2=0.991), and the detection limit is 16 nM (three times the standard deviation of the blank solution), which is lower than the toxicity level of Hg^{2+} in drinking water (30 nM) defined by Word Health Organization (WHO). The result indicates that this fluorescent sensor indeed possesses the potential application of detecting Hg^{2+} sensitively in aqueous solution.

Table 1
Assay results of river samples by using the proposed AuNPs–MSD probe and the comparison of our results with the standard Hg^{2+} concentrations.[a]

Sample[b]	Standard Hg^{2+} concentration (nM)	Hg^{2+} concentration detected by AuNPs–MSD probe (nM)	Relative error (%)
1	50	52.4	4.8
2	200	206	3.0
3	800	783	2.1
4	2000	1902	4.9

[a] Each sample was analyzed three times, and the results are the average values.
[b] Samples 1, 2, 3 and 4 were river samples spiked with four different concentrations of Hg^{2+} ions: 50 nM, 200 nM, 800 nM, and 2.0 μM., respectively.

3.4. Selectivity of Hg^{2+} ion detection

The specificity of the present system for Hg^{2+} detection was evaluated. A variety of environmentally relevant metal ions including Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+},Cr^{3+}, Ni^{2+}, Cu^{2+}, Co^{2+}, and Pb^{2+} were examined. We firstly investigated whether these metals alone can quench FAM-labeled AuNPs–MSD probe. As shown in Fig. 3A, the fluorescence ratio, F_0/F, is plotted as a function of different ions interact with AuNPs–MSD probes in Tris–HCl buffer solution, where F_0 and F are FAM intensities at 520 nm in the absence and presence of metal ions, respectively. It can be seen that all the other metal ions presented only slight and negligible effects on the fluorescence of the AuNPs–MSD detection system (Fig. 3B), indicating the sensing system described herein exhibits high specificity for Hg^{2+} against other metal ions. Furthermore, the selectivity of this nanoprobe in the presence of any possible interference ions was evaluated. As demonstrated in Fig. 4, obviously, the present method can still detect Hg^{2+} in the presence of any other possible interference ions. The above results indicated that this AuNPs–MSD fluorescent sensor for Hg^{2+} detection possesses outstanding specificity and selectivity against other interference metal ions.

3.5. Recovery in sample analysis

To investigate whether this method was applicable to natural samples, we tested the spiked river samples with four different concentrations of Hg^{2+} ions: 50 nM, 200 nM, 800 nM, and 2.0 μM. The interfering materials in the river samples did not influence the Hg^{2+} detection with the described method. As shown in Table 1, the average relative error between the assay results and the given concentrations in river samples is less than 5%, demonstrating that detection of Hg^{2+} in river samples is also feasible. Therefore, the proposed method may be of great value for Hg^{2+} assay in real sample applications.

4. Conclusions

In this work, we successfully combined molecular biology with nanoparticle science to develop new tools for analytical chemistry. We proposed a method that combining the advantages of aptamer with the superior properties of AuNPs to detect Hg^{2+} with high sensitivity and selectivity. Dually labeled DNA probe (thiol and dye) are assembled at the surface of AuNPs through the Au–S bond. In the presence of Hg^{2+}, T–Hg^{2+}–T duplex formation between the base T and Hg^{2+} and quench the fluorescence of FAM as a result of the adjacence of the dye to the AuNPs.

Acknowledgments

This work was supported by the National Basic Research Program of China (973 Program, Nos. 2011CB933600 and 2006CB933100), the Science Fund for Creative Research Groups of NSFC (Nos. 20621502 and 20921062), the National Natural Science Foundation of China (21275110; 20833006; 20875071; 21005056), and the Fundamental Research Funds for the Central Universities (20100141110015).

References

[1] H.H. Harris, I.J. Pickering, G.N. George, Science 301 (2003) 1203.
[2] T.W. Clarkson, L. Magos, G.J. Myers, N. Engl. J. Med. 349 (2003) 1731–1737.
[3] F.M.M. Morel, A.M.L. Kraepiel, M. Amyot, Annu. Rev. Ecol. Syst. 29 (1998) 543–566.
[4] N.H. Bings, A. Bogaerts, J.A.C. Broekaert, Anal. Chem. 78 (2008) 3917–3946.
[5] K.E. Lorber, Waste Manage. Res. 4 (1986) 3–13.
[6] A. Bernaus, X. Gaona, J.M. Esbrí, P. Higueras, G. Falkenberg, M. Valiente, Environ. Sci. Technol. 40 (2006) 4090–4095.
[7] E.M. Nolan, S.J. Lippard, Chem. Rev. 108 (2008) 3443–3480.
[8] J. Lee, C.A. Mirkin, Anal. Chem. 80 (2008) 6805–6808.
[9] J. Lee, M.S. Han, C.A. Mirkin, Angew. Chem. Int. Ed. 46 (2007) 4093–4096.
[10] X. Sue, F. Wang, X. Liu, J. Am. Chem. Soc. 130 (2008) 3244–3245.
[11] C.K. Chiang, C.C. Huang, C.W. Liu, H.T. Chang, Anal. Chem. 80 (2008) 3716–3721.
[12] C.W. Liu, C.C. Huang, H.T. Chang, Langmuir 24 (2008) 8346–8350.
[13] H. Wang, Y.X. Wang, J.Y. Jin, R.H. Yang, Anal. Chem. 80 (2008) 9021–9028.
[14] V. Bhalla, R. Tejpal, M. Kumar, A. Sethi, Inorg. Chem. 48 (2009) 11677–11684.
[15] I.B. Kim, U.H.F. Bunz, J. Am. Chem. Soc. 128 (2006) 2818–2819.
[16] J.S. Lee, M.S. Han, C.A. Mirkin, Angew. Chem. Int. Ed. 46 (2007) 4093–4096.
[17] T. Li, E.K. Wang, S.J. Dong, PLOS ONE 4 (2009) e5126.
[18] S. Katz, J. Am. Chem. Soc. 74 (1952) 2238–2245.
[19] 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.
[20] Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, J. Am. Chem. Soc. 129 (2007) 244–245.
[21] A. Ono, H. Togashi, Angew. Chem. Int. Ed. 43 (2004) 4300–4302.
[22] D.S.H. Chan, H.M. Lee, C.M. Che, C.H. Leung, D.L. Ma, Chem. Commun. (2009) 7479–7481.
[23] L. Dyadyusha, H. Yin, S. Jaiswal, T. Brown, J.J. Baumberg, F.P. Booy, T. Melvin, Chem. Commun. (2005) 3201–3203.
[24] G.K. Darbha, A. Ray, P.C. Ray, ACS Nano 1 (2007) 208–214.
[25] B.C. Ye, B.C. Yin, Angew. Chem. Int. Ed. 47 (2008) 8386–8389.
[26] M. Li, R. Li, C.M. Li, N.Q. Wu, Front. Biosci. S3 (2011) 1308–1331.
[27] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Anal. Chem. 67 (1995) 735–743.
[28] L.M. Demers, C.A. Mirkin, R.A. Mucic, R.L. Reynolds, R. Letsinger, R. Elghanian, G. Viswanadham, Anal. Chem. 72 (2000) 5535–5541.
[29] T.A. Taton, C.A. Mirkin, R.L. Letsinger, Science 289 (2000) 1757–1760.
[30] C.K. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Anal. Chem. 67 (1995) 735–743.