Biosensors and Bioelectronics 39 (2013) 231–235

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

A highly sensitive and selective optical sensor for Pb^{2+} by using conjugated polymers and label-free oligonucleotides

Yan Lu*, Xiang Li, Gongke Wang, Wen Tang

School of Chemistry and Environmental Science, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, Henan Normal University, Xinxiang, Henan 453007, PR China

ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
21 July 2012
Accepted 23 July 2012
Available online 27 July 2012

Keywords:
Lead(II) detection
G-quadruplex
Water-soluble conjugated polymers
Mercury(II) interference
Label-free

ABSTRACT

The detection of Pb^{2+} with DNA-based biosensor is usually susceptible to severe interference from Hg^{2+} because of the T–Hg^{2+}–T interaction between Hg^{2+} and T residues. In this study, we developed a rapid, sensitive, selective and label-free sensor for the detection of Pb^{2+} in the presence of Hg^{2+} based on the Pb^{2+}-induced G-quadruplex formation with cationic water-soluble conjugated polymer (PMNT) as a "polymeric stain" to transduce optical signal. We selected a specific sequence oligonucleotide, TBAA (5′-GGAAGGTGTGGAAGG-3′), which can form a G-quadruplex structure upon the addition of Pb^{2+}. This strategy provided a promising alternative to Pb^{2+} determination in the presence of Hg^{2+} instead of the universal masking agents of Hg^{2+} (such as CN^-, SCN^-). Based on this observation, a simple "mix-and-detect" optical sensor for the detection of Pb^{2+} was proposed due to the distinguishable optical properties of PMNT–ssDNA and PMNT–(G-quadruplex) complexes. By this method, we could identify micromolar Pb^{2+} concentrations within 5 min even with the naked eye. Furthermore, the detection limit was improved to the nanomolar range by the fluorometric method. We also successfully utilized this biosensor for the determination of Pb^{2+} in tap water samples.

1. Introduction

Lead ion (Pb^{2+}), which is a major environmental pollutant, may affect the environment adversely and pose severe risks to human health. For example, lead ion can cause serious damage to the brain and central nervous system (Needleman, 2004). Therefore, routine detection of Pb^{2+} in environmental and biological samples is of considerable significance and has become an important subject of current chemical research. The conventional methods, such as atomic absorption/emission spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), have been widely used in detecting trace amount of metal ions (Butler et al., 2006). However, the costly apparatus and complicated pretreatment procedures prevent them from being used.

Over the past decade, DNA-based biosensors for the detection of Pb^{2+} have been paid high attention (Zhang et al., 2011a). Of particular interest has been focused on two types of sensors. One is Pb^{2+}-dependent 8–17 DNAzyme, which reveals cleavage activity at the scissile ribo-adenosine position with Pb^{2+} as the cofactor (Brown et al., 2003). It has been extensively explored for Pb^{2+} detection via fluorescence (Kim et al., 2011; Nie et al., 2012; Wen et al., 2011; Zhang et al., 2011b; Zhao et al., 2011), colorimetric (Liu and Lu, 2003, 2004) and electrochemical techniques (Xiao et al., 2007; Yang et al., 2010). However, many of these systems have limited practical use because of, for example, high cost (e.g., enzymes), complicated processing and the using of unstable molecules (e.g., RNA). The other is based on the Pb^{2+} induced G-quadruplex (G4) formation through the DNA sequences reacting with Pb^{2+} (Fu et al., 2012; Li et al., 2009, 2010, 2011a, 2011b; Liu et al., 2009). G-quadruplexes are unique higher order structures, in which G-rich nucleic sequence form stacked arrays of G-quartets connected by Hoogsteen-type base pairing (Phan et al., 2006). For example, Chang's group reported a fluorescence resonance energy transfer (FRET)-based DNA senor for Pb^{2+} detection (Liu et al., 2009). Dong's group developed a novel Pb^{2+} sensor with a G-quadruplex DNAzyme by colorimetry and chemiluminescence (Li et al., 2010). However, most of these systems were often susceptible to severe interference from Hg^{2+} because a few T residues of the G-rich single-strand DNA usually used can interact with Hg^{2+} through the T–Hg^{2+}–T interaction. At present, almost all assays employed the masking agents of Hg^{2+} (such as CN^- and SCN^-) to solve this problem, but the masking agents are usually highly toxic, resulting in additional environmental contamination. Tang's group developed an amperometric method for Pb^{2+} detection based on Pb^{2+} induced G-quadruplex with crystal violet as a G4-binding indicator (Li et al., 2011b). This method is sensitive and selective. However, it needs to immobilize DNA probe on gold electrode, which increases the processing steps and analytical costs. Therefore,

* Corresponding author. Tel./fax: +86 373 3325249.
E-mail address: yanlu2001@sohu.com (Y. Lu).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.07.045

there is an urgent need for the development of inexpensive and label-free DNA-based sensors to rapidly, sensitively and selectively detect Pb^{2+} without using any masking agents of Hg^{2+}.

In recent years, water-soluble conjugated polymers have provided a unique platform for chemical and biological sensors due to their superior electronic property, light-harvest nature and optical signal amplification effect (Duan et al., 2010; Feng et al., 2010; Liu et al., 2011; Thomas et al., 2007). For example, Leclerc et al. developed some new water-soluble polythiophene derivatives, which exhibit interesting optical properties owing to the conformational changes in their flexible conjugated backbones (Ho et al., 2002). It is well known that water-soluble polythiophene derivatives can adopt different conformations upon forming interpolyelectrolyte complexes with DNA of different conformations such as signal- and double-stranded DNA and G-quadruplexes. The induced conformational changes can be conveniently monitored by using absorption or fluorescence spectroscopy. Taking advantage of these interesting properties, some schemes have been developed with polythiophene derivatives as optical probes to detect DNA (Ho et al., 2005, Zheng and He, 2009), proteins (Aberem et al., 2006), enzyme activity (Tang et al., 2006; Zhang et al., 2009), small molecules (Li et al., 2005; Yao et al., 2008; Yao et al., 2009; Zhan et al., 2010) and metal ions (Ho and Leclerc, 2004; Liu et al., 2007).

Inspired by these studies, we developed a simple, high sensitive, label-free and G4-based selective optical sensor for the detection of Pb^{2+} with the distinguishable optical properties of PMNT–ssDNA and PMNT–(G-quadruplex) complexes. Using this novel sensor, we could identify micromolar Pb^{2+} concentrations within 5 min with the naked eye, and nanomolar sensitivity was achieved by using a fluorometric method. More importantly, the interference from Hg^{2+} in detection of Pb^{2+} could be eliminated by using the specific sequence DNA without using masking agents of Hg^{2+} (such as CN^- and SCN^-).

2. Experimental

2.1. Materials

Cationic polythiophene (PMNT) was prepared according to the literature (Ho et al., 2002). The concentration of polymer was given using its monomer. The TBAA (3'–GGAAGGTGTGGAAGG-5′) and TBA (3′-GGTTGGTGTGGTTGG-5′) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The DNA stock solution was heated to 90 °C for 5 min and cooled slowly to room temperature. The concentration of DNA stock solution was calculated according to the absorbance at 260 nm. All other reagents are analytical grade and used without further purification. Doubly distilled water was used throughout the work. All stock solutions were stored in the dark at 0–4 °C.

2.2. Instrumentation

All fluorescence spectra were performed on a Cary Eclipse fluorescence spectrophotometer (Varian, America). The UV-absorption spectra were recorded at room temperature on a TU-1810 UV spectrophotometer (Beijing Puxi Analytic Instruments Co., Ltd.). The photographs were taken with a FUJIFFILM FinePix F605 digital camera.

2.3. General procedure for Pb^{2+} detection

2.3.1. Colorimetric measurement

In the typical procedure, 10 μL of ss-DNA (50 μM), 10 μL of Pb^{2+} (50 μM) were added to 50 μL of the aqueous solution in a 1.5 mL eppendorf cup, and then the mixed solution was incubated for 5 min. After incubation, 10 μL of PMNT (1.5 mM) was added. The control experiments were done under the identical conditions. The color change was recorded using a digital camera. The UV–visible absorption spectra were measured in a 200 μL quartz cuvette at room temperature.

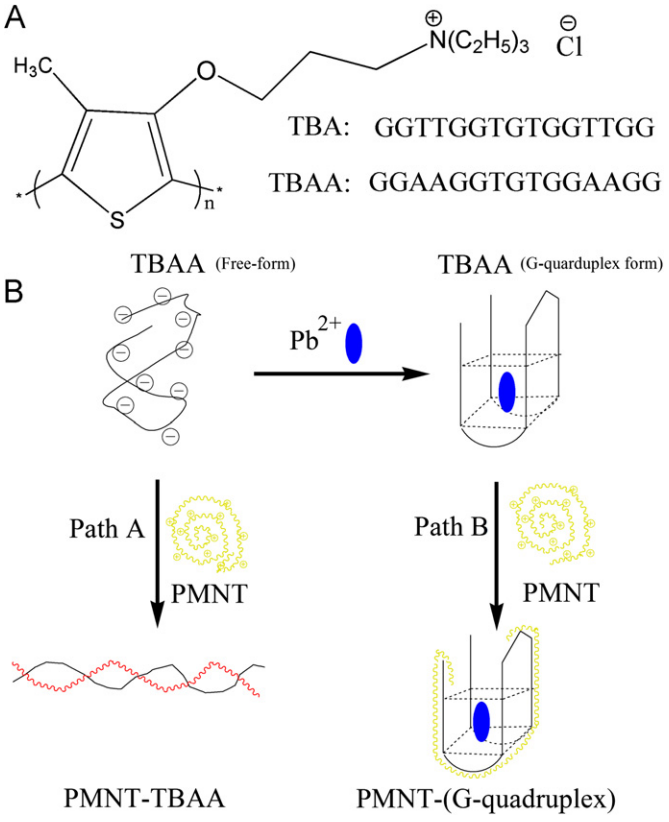
2.3.2. Fluorescent measurement

In a 1.0 cm path length fluorescence cell, 2 mL pure water containing TBAA (125 nM), different concentrations of Pb^{2+} were equilibrated for 5 min at room temperature, and 2.5 μL of 1.5 mM PMNT solution was added. The fluorescence spectra of mixtures were recorded at room temperature. The excitation wavelength was made at 395 nm.

3. Results and discussion

3.1. Principle of sensing Pb^{2+}

As shown in Scheme 1, our detection system consists of a single-stranded oligonucleotide (TBAA) and a polythiophene derivative, poly [3-(3′-*N*,*N*,*N*-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride] (PMNT, structure shown in Scheme 1A). TBA (5′-GGTTGGTGTGGTTGG-3′), as a specific thrombin binding aptamer (Ho and Leclerc, 2004; So et al., 2005), could also be used as a G-rich oligonucleotide for the detection of Pb^{2+} (Smirnov, Shafer, 2000; Kotch et al., 2000; Liu et al., 2009). However, a problem produced from the use of TBA is that Hg^{2+} can interact with TBA to form a hairpin-like structure through T–Hg^{2+}–T interactions. So it has a potential influence on the detection of Pb^{2+}. To solve this problem, a new sequence of oligonucleotide, TBAA (5′-GGAAGG-TGTGGAAGG-3′), was employed in this work. TBAA is a 15-mer single-stranded oligonucleotide and usually has a random coil



Scheme 1. (A) The chemical structure of PMNT and the sequence of ss-DNA. (B) Schematic illustration of the assay for the detection of Pb^{2+}.

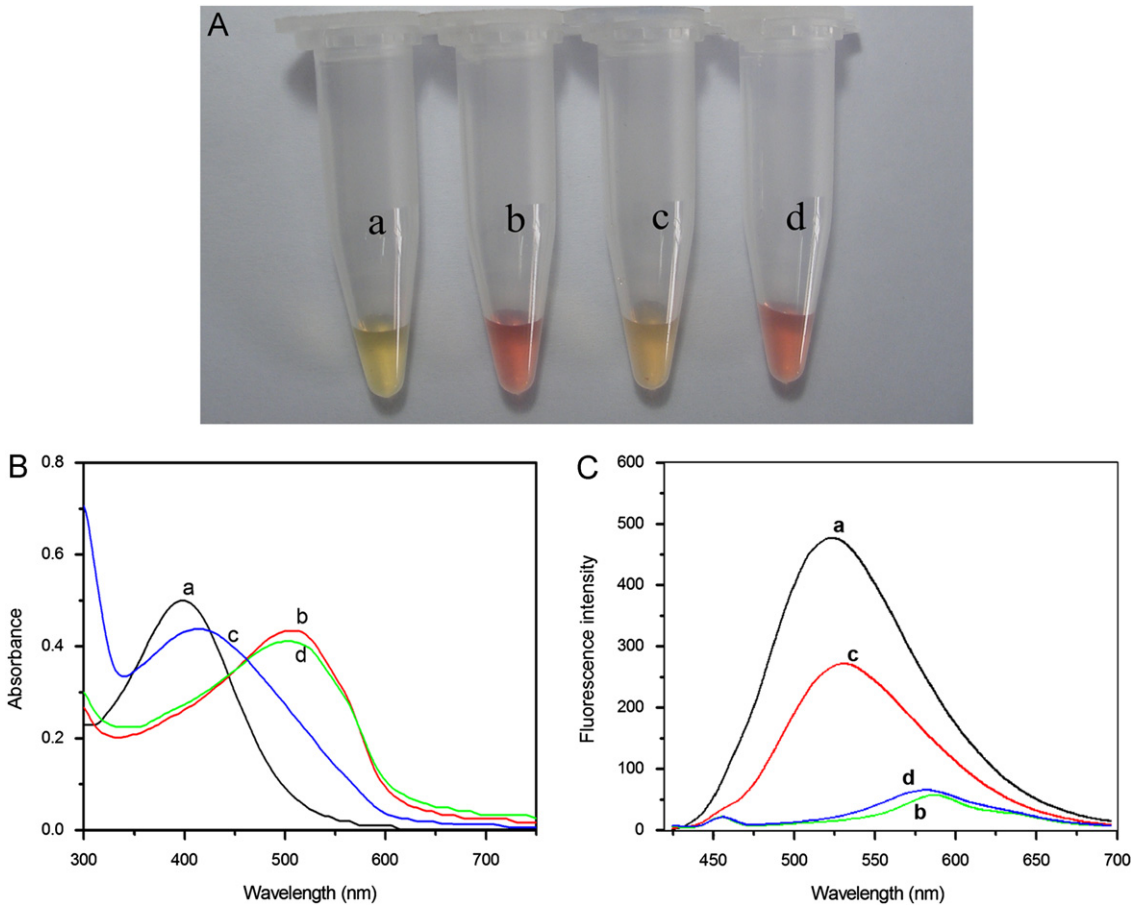


Fig. 1. (A) Photographs, (B) UV–vis absorption spectra, and (C) fluorescence spectra of aqueous solutions of PMNT (a); PMNT/TBAA (b); PMNT/TBAA/Pb^{2+} (c); PMNT/TBAA/Hg^{2+} (d). In (A), [PMNT]=0.19 mM, [TBAA]=12.5 μM, [Pb^{2+}]=12.5 μM, [Hg^{2+}]=125 μM; in (B) and (C), the concentrations of the samples were diluted 2.5 and 25 times, respectively. (The pH value of aqueous solution is 6.5.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structure. This DNA sequence can form a G-quadruplex structure upon the addition of Pb^{2+} (Smirnov et al., 2002). Whereas TBAA cannot bind with Hg^{2+} through T–Hg^{2+}–T interaction because of lacking –TT– unit, so the interference of Hg^{2+} is completely eliminated. With the above analysis, we determined to use TBAA as a probe to detect Pb^{2+}.

The aqueous solution of PMNT is yellow-colored (Fig. 1A, a) with a maximum absorption (λ_{max}) at 395 nm (Fig. 1B, a), its absorption maximum at a short wavelength is related to a flexible and nonplanar conformation of polymer backbone in the solution (Ho et al., 2002). Cationic PMNT and TBAA probe readily formed an electrostatic PMNT–TBAA complex in aqueous solution (Scheme 1B, path A). In the complex, PMNT took a highly conjugated and planar conformation with a characteristic red color (λ_{max}=520 nm) (Fig. 1A, b and 1B, b). In the presence of Pb^{2+}, the TBAA probe formed a G-quadruplex structure through the specific Pb^{2+} binding G-quartets. We observed a color change from red to yellow and the λ_{max} shifting to shorter wavelength upon addition of Pb^{2+} to the PMNT–TBAA complex (Fig. 1A, c and 1B, c). This optical change possibly arises from the wrapping of PMNT onto the G-quadruplex structure, resulting in the twisting of conjugated backbone (Scheme 1B, path B) (Ho and Leclerc, 2004). It should be noted that the solution still kept red color in the presence of Hg^{2+} (Fig. 1A, d and 1B, d), which implies that Hg^{2+} cannot specifically interact with TBAA. However, when TBA was used as the DNA probe, both Pb^{2+} and Hg^{+} could change the solution color (Fig. S1). So the results indicate that TBAA possesses higher selectivity for Pb^{2+} by comparison with TBA in the presence of Hg^{2+}.

3.2. Sensitivity and selectivity

To investigate the selectivity of the system for the determination of lead(II) ions, control experiments were performed using other metal ions, such as K^{+}, Li^{+}, Na^{+}, Mg^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Cu^{2+}, Ca^{2+} and Hg^{2+}. The results are shown in Fig. 2. From Fig. 2(a), we can see that these nonspecific ions did not lead to the characteristic color change even at high concentrations, which implies that this sensor has a high specificity for Pb^{2+} detection. Additionally, the fluorometric detection also showed high selectivity. As illustrated in Fig. 2(b), Pb^{2+} led to a significant fluorescence increase, while other nonspecific metal ions only showed small fluorescence changes.

Based on these observations, we then used the PMNT–TBAA system as a colorimetric sensor for Pb^{2+} detection. The color change of PMNT/TBAA solution upon the concentration of Pb^{2+} is shown in Fig. S2. As shown in Fig. S2, the color of the solution changed from red to yellow gradually with the increasing concentration of Pb^{2+}. In the present work, we could identify the red to yellow color change by naked eye even at a Pb^{2+} concentration as low as 2 μM. More importantly, the reaction was fairly fast, and we could observe an obvious color change within 5 min.

The colorimetric detection of Pb^{2+} is very simple and rapid, but the detection sensitivity is usually quite limited. The sensitivity of the Pb^{2+} detection could be further improved by using a fluorescence method. The random-coil form of PMNT with yellow color is fluorescent with a maximum of emission at 527 nm (Fig. 1C, a). With the addition of ssDNA, the fluorescence of PMNT

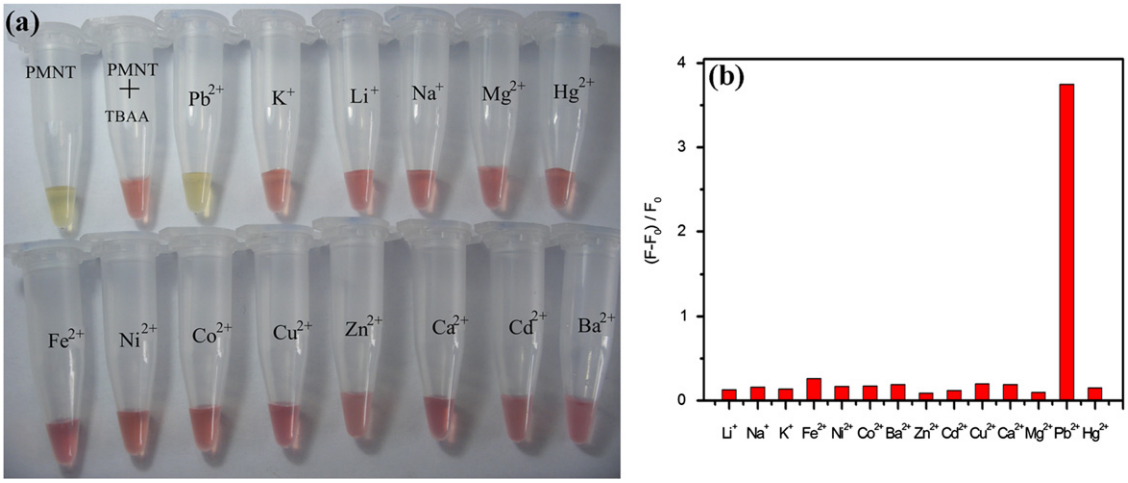


Fig. 2. (a) Visual color changes upon treatment of the PMNT/TBAA system with or without (blank) various metal ions. The concentration of PMNT is 0.19 mM and TBAA (12.5 μM), the concentration of metal ions were: 12.5 μM Pb^{2+}; 12.5 mM K^+, Li^+, Na^+, Mg^{2+}; 1.25 mM Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}; 125 μM Cu^{2+}, Ca^{2+} and Hg^{2+}. (b) The selectivity of Pb^{2+} analysis using the proposed sensor by fluorescence method. In (b), the concentrations of the samples were diluted 25 times, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

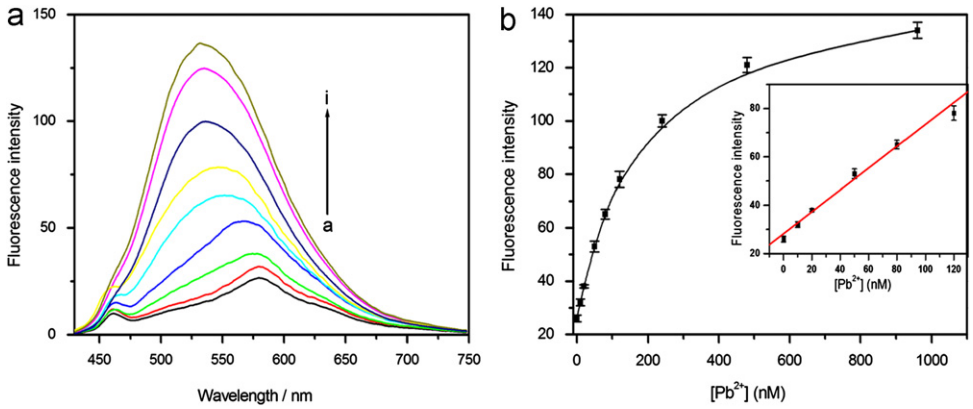


Fig. 3. (a) Fluorescence spectra of PMNT–TBAA aqueous solutions in the presence of different concentrations of Pb^{2+} (from a to i): 0, 10, 20, 50, 80, 120, 240, 480 and 960 nM. (b) The relationship between the fluorescence intensity and the concentration of Pb^{2+}. The inset shows a linear relationship in the concentration range from 0 to 120 nM. The concentration of TBAA is 125 nM. In this fluorescence experiment, the slits of excitation and emission were all 10 nm.

is quenched and the maximum of emission is red-shifted to 587 nm (Fig. 1C, b), corresponding to a planar, highly conjugated aggregated form of the polythiophene. However, we observed a distinct fluorescence enhancement in the presence of Pb^{2+} (Fig. 1C, c). The enhancement of the fluorescence intensity could be related to a partially planar conformation of the polythiophene chain but with less aggregation of the chains (Ho and Leclerc, 2004). By measuring the fluorescence intensity of the solution upon adding different concentrations of Pb^{2+} (Fig. 3a), we obtained the working curve for Pb^{2+} concentration ranging from 0 to 960 nM. The inset in Fig. 3(b) reveals a linear correlation to the concentration of Pb^{2+} over the range from 0 nM to 120 nM. The linear regression equation is $Y=28.138+0.45C$ (Pb^{2+}, nM) with the correlation coefficient $R=0.9937$. The lowest detection limit for Pb^{2+} was estimated to be 6 nM based on 3σ. The maximum contamination level for lead in the drinking water defined by the U.S. Environmental Protection Agency is 72 nM. So the proposed Pb^{2+} sensor is sufficient for Pb^{2+} monitoring in the environment.

3.3. Application

To demonstrate the potential application of our sensor in environment analysis, we applied the sensor to evaluate the tap water samples. The results are shown in Fig. 4. It was found that the Pb^{2+} content in the tap water is too low to be detected by the sensor. However, there is an obvious increase in the read signal if different concentrations of Pb^{2+} are added into the sample, indicating that this sensor can be utilized to analyze Pb^{2+} in the environmental samples.

4. Conclusions

In summary, based on a target-induced conformational change of oligonucleotide and PMNT, a simple, sensitive and selective sensor for the detection of Pb^{2+} has been developed. This sensor has several important features. Firstly, it is a signal-on mode, which is able to effectively reduce background noise and increase detection sensitivity. Secondly, the usage of a Pb^{2+}-responsive oligonucleotide probe (TBAA) provides a promising alternative to Pb^{2+} determination in the presence of Hg^{2+} instead of the universal masking agents of Hg^{2+}. Finally, this sensor does not require any labels or chemical modification on the oligonucleotide probe, which can provide an inexpensive mean to detect Pb^{2+}. We believe that this simple, rapid, sensitive and specific sensor will make it promising for monitoring lead pollution in the environment.

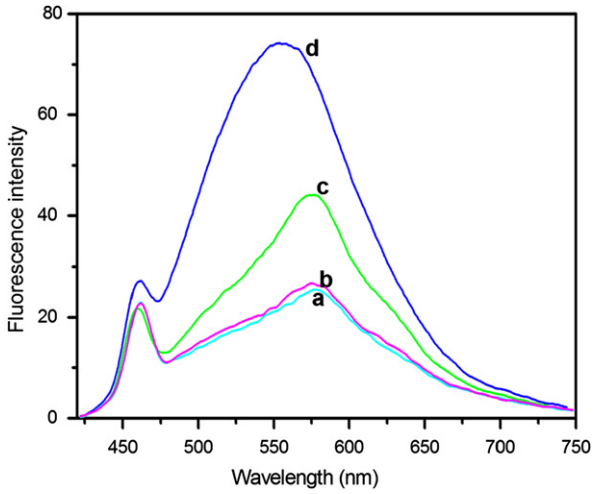


Fig. 4. Application of our sensor to the detection of Pb^{2+} in tap water: (a) in pure water, (b) in tap water, (c) tap water+30 nM [Pb^{2+}] and (d) tap water+100 nM [Pb^{2+}].

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21173071, 20673034).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.07.045.

References

Aberem, M.B., Najari, A., Ho, H.A., Gravel, J.F., Nobert, P., Boudreau, D., Leclerc, M., 2006. Advanced Materials 18, 2703–2707.
Brown, A.K., Li, J., Pavot, C.M.B., Lu, Y., 2003. Biochemistry 42, 7152–7161.
Butler, O.T., Cook, J.M., Harrington, C.F., Rieuwerts, S.J.J., Miles, D.L., 2006. Journal of Analytical Atomic Spectrometry 21, 217–243.
Duan, X.R., Liu, L.B., Feng, F.D., Wang, S., 2010. Accounts of Chemical Research 43, 260–270.
Fu, X.B., Qu, F., Li, N.B., Luo, H.Q., 2012. Analyst 137, 1097–1099.
Feng, X.L., Liu, L.B., Wang, S., Zhu, D.B., 2010. Chemical Society Reviews 39, 2411–2419.
Ho, H.A., Boissinot, M., Bergeron, M.G., Corbeil, G., Dore, K., Boudreau, D., Leclerc, M., 2002. Angewandte Chemie International Edition 41, 1548–1551.
Ho, H.A., Leclerc, M., 2004. Journal of the American Chemical Society 126, 1384–1387.
Ho, H.A., Dore, K., Boissinot, M., Bergeron, M.G., Tanguay, R.M., Boudreau, D., Leclerc, M., 2005. Journal of the American Chemical Society 127, 12673–12676.
Kim, J.H., Han, S.H., Chung, B.H., 2011. Biosensors & Bioelectronics 26, 2125–2129.
Kotch, F.W., Fettinger, J.C., Davis, J.T., 2000. Organic Letters 2 (21), 3277–3280.
Li, C., Numata, M., Takeuchi, M., Shinkai, S., 2005. Angewandte Chemie International Edition 44, 6371–6374.
Li, C.L., Liu, K.T., Lin, Y.W., Chang, H.T., 2011a. Analytical Chemistry 83 (1), 225–230.
Li, F., Feng, Y., Zhao, C., Tang, B., 2011b. Chemical Communications 47, 11909–11911.
Li, T., Wang, E.K., Dong, S.J., 2009. Journal of the American Chemical Society 131, 15082–15083.
Li, T., Wang, E.K., Dong, S.J., 2010. Analytical Chemistry 82 (4), 1515–1520.
Liu, C.W., Huang, C.C., Chang, H.T., 2009. Analytical Chemistry 81 (6), 2383–2387.
Liu, J.W., Lu, Y., 2003. Journal of the American Chemical Society 125, 6642–6643.
Liu, J.W., Lu, Y., 2004. Journal of the American Chemical Society 126, 12298–12305.
Liu, X.F., Tang, Y.L., Wang, L.H., Zhang, J., Song, S.P., Fan, C.H., Wang, S., 2007. Advanced Materials 19, 1471–1474.
Liu, X.F., Fan, Q.L., Huang, W., 2011. Biosensors & Bioelectronics 26, 2154–2164.
Needleman, H., 2004. Annual Review of Medicine 55, 209–222.
Nie, D.D., Wu, H.Y., Zheng, Q.S., Guo, L.Q., Ye, P.R., Hao, Y.L., Li, Y.N., Fu, F.F., Guo, Y.H., 2012. Chemical Communications 48, 1150–1152.
Phan, A.T., Kuryavyi, V., Patel, D.J., 2006. Current Opinion in Structural Biology 16, 288–298.
So, H.M., Won, K., Kim, Y.H., Kim, B.K., Ryu, B.H., Na, P.S., Kim, H., Lee, J.O., 2005. Journal of the American Chemical Society 127, 11906–11907.
Smirnov, I., Shafer, R.H., 2000. Journal of Molecular Biology 296 (1), 1–5.
Smirnov, I.V., Kotch, F.W., Pickering, I.J., Davis, J.T., Shafer, R.H., 2002. Biochemistry 41, 12133–12139.
Tang, Y.L., Feng, F.D., He, F., Wang, S., Li, Y.L., Zhu, D.B., 2006. Journal of the American Chemical Society 128, 14972–14976.
Thomas III, S.W., Joly, G.D., Swager, T.M., 2007. Chemical Reviews 107, 1339–1386.
Wen, Y.Q., Peng, C., Li, D., Zhuo, L., He, S.J., Wang, L.H., Huang, Q., Xu, Q.H., Fan, C.H., 2011. Chemical Communications 47, 6278–6280.
Xiao, Y., Rowe, A.A., Plaxco, K.W., 2007. Journal of the American Chemical Society 129, 262–263.
Yang, X.R., Xu, J., Tang, X.M., Liu, H.X., Tian, D.B., 2010. Chemical Communications 46, 3107–3109.
Yao, Z.Y., Li, C., Shi, G.Q., 2008. Langmuir 24 (22), 12829–12835.
Yao, Z.Y., Feng, X.L., Hong, W.J., Li, C., Shi, G.Q., 2009. Chemical Communications 31, 4696–4698.
Zhan, R.Y., Fang, Z., Liu, B., 2010. Analytical Chemistry 82 (4), 1326–1333.
Zhao, X.H., Kong, R.M., Zhang, X.B., Meng, H.M., Liu, W.N., Tan, W.H., Shen, G.L., Yu, R.Q., 2011. Analytical Chemistry 83 (13), 5062–5066.
Zhang, Y.L., Li, Z.P., Cheng, Y.Q., Lv, X.C., 2009. Chemical Communications 22, 3172–3174.
Zhang, X.B., Kong, R.M., Lu, Y., 2011a. Annual Review of Analytical Chemistry 4, 105–128.
Zhang, L.B., Han, B.Y., Li, T., Wang, E.K., 2011b. Chemical Communications 47, 3099–3101.
Zheng, W.M., He, L., 2009. Journal of the American Chemical Society 131, 3432–3433.