Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 146 (2015) 1–6

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

A turn-off fluorescent biosensor for the rapid and sensitive detection of uranyl ion based on molybdenum disulfide nanosheets and specific DNAzyme

HongYan Zhang [a,b], YaJuan Ruan [a], Ling Lin [a], Minggui Lin [c], Xiaoxue Zeng [a], Zhiming Xi [a], FengFu Fu [a,*]

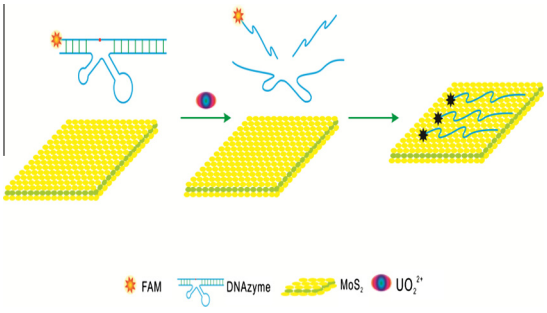
[a] Key Lab of Analysis and Detection for Food Safety of Ministry of Education, Fujian Provincial Key Lab of Analysis and Detection for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China
[b] College of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian 350122, China
[c] Fujian Environmental Radiation Supervision Station, Fuzhou, Fujian 350012, China

HIGHLIGHTS

- A turn-off fluorescent biosensor for detecting UO_2^{2+} in aqueous environment has been developed.
- The biosensor employs DNAzyme as UO_2^{2+} recognition and MoS_2 nanosheets as fluorescence quencher.
- It has obvious analytical advantage such as high sensitivity, short analytical time and low cost.
- It can be used to detect ultra-trace UO_2^{2+} in water with a recovery of 96–102% and a RSD < 5%.

GRAPHICAL ABSTRACT

A novel fluorescent biosensor for uranyl ion (UO_2^{2+}) detection in aqueous environment has been developed based on the specific recognition of DNAzyme and the fluorescence quenching ability of molybdenum disulfide (MoS_2) nanosheets.



ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 25 February 2015
Accepted 26 February 2015
Available online 10 March 2015

Keywords:
DNAzyme
Molybdenum disulfide
Uranyl ion
Fluorescent biosensor

ABSTRACT

A novel fluorescent biosensor for detecting uranyl ion (UO_2^{2+}) in aqueous environment has been developed based on the specific recognition of DNAzyme and the fluorescence quenching ability of molybdenum disulfide (MoS_2) nanosheets. The DNAzyme contains a DNA enzyme strand and a 6-carboxylfluorescein (FAM)-labeled DNA substrate strand. We demonstrated that MoS_2 nanosheets have low affinity to the substrate-enzyme complex DNAzyme. Whereas, in the presence of UO_2^{2+}, UO_2^{2+} can specifically cleave DNAzyme to release FAM-labeled single-strand DNA and the released FAM-labeled single-strand DNA can be firmly adsorbed on the surface of MoS_2 nanosheets, which resulted in an obvious decrease of fluorescence intensity. This provided a sensing platform for the rapid, simple and sensitive fluorescent detection of UO_2^{2+}. By using the sensing platform, a sensitive and selective fluorescent method for the rapid detection of UO_2^{2+} has been developed. In comparison with previous biosensor, the proposed method has obvious analytical advantage such as relatively high sensitivity and good stability, short analytical time and low cost. It can be used to detect as low as 2.14 nM of UO_2^{2+} in aqueous environment with a recovery of 96–102% and a RSD < 5% (n = 6). The success of this study provides a promising alternative for the rapid and on-site detection of UO_2^{2+} in environmental monitoring.

* Corresponding author. Tel./fax: +86 591 22866135.
E-mail address: fengfu@fzu.edu.cn (F. Fu).

http://dx.doi.org/10.1016/j.saa.2015.02.113
1386-1425/

Introduction

As the main fuel of nuclear energy, the uranium has been widely used in nuclear power station and nuclear weapons [1]. With the continuous development of nuclear energy, the worldwide uranium consumption is also increasing. However, mining and processing of uranium mineral resources has also brought a large area of uranium pollution. In the past few decades, the pollution of uranium has caused increasing attention due to its high toxicity and radioactivity. It was reported that high doses exposure of uranium could give rise to kidney damage, urinary system disease and lung cancer [2], and chronic low-dose exposure of uranium could give reproductive toxicity, maternal toxicity, embryo/fetal toxicity and postnatal negative effects [3]. Furthermore, uranium was also found to have a chemical genotoxicity, which is similar to that of hexavalent chromium [2]. For the reasons mentioned above, it is thus crucial to develop a simple method for the rapid and on-site detection of uranyl ion in the aqueous environment.

Traditional techniques for the uranyl ion analysis included inductively coupled plasma mass spectrometry (ICP-MS) [4], energy dispersive X-ray fluorescence spectrometry [5], laser-atomic absorption spectrometry [6], and laser-induced kinetic phosphorimetry [7]. These methods are very sensitive and accurate, however, they usually require sophisticated and expensive instruments, which makes on-site and real time detection difficult. The previously mentioned limitations of traditional techniques have pushed researchers to develop novel methods for the rapid, sensitive and specific detection of uranyl ion in the field. Along this goal, a few chemical sensors have been developed for the rapid detection of UO_2^{2+} [8–10]. However, these methods have lower sensitivity and relatively poor selectivity, which do not meet the requirement of rapid and on-site detection of trace UO_2^{2+} in aqueous environment. DNAzymes are catalytic DNA sequences, which was isolated via in vitro selection and has high specificity for a number of metal ions [11]. Compared to protein enzymes, DNAzymes are easier to synthesize, more economic and more thermally stable [12,13]. The afore-mentioned advantages make DNAzyme an attractive recognition probe for detecting metal ions [12,14,15]. So far, a number of highly specific DNAzymes have been isolated and used for detecting metal ions such as Pb^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and so on [16–19]. Not long ago, a uranyl ion-specific DNAzyme was reported by Lu and his co-workers [20]. Encouraged by the discovery of uranyl ion-specific DNAzyme, some DNAzyme-based biosensors have been developed for the rapid and specific detection of UO_2^{2+} [21–23]. For example, Lu and his co-workers have reported a catalytic beacon fluorescent biosensor for detecting UO_2^{2+} by using uranyl ion-specific DNAzyme as recognition probe [21]. A colorimetric biosensor for detecting uranyl ion was also developed by them based on the DNAzyme and gold nanoparticles [22]. Although some progress has been made over the past few years, it still is a significant challenge to design simple and portable sensors for UO_2^{2+} detection with both high sensitivity and selectivity.

MoS_2 is a type of transition metal sulfides, constructed by stacking covalently bound S–Mo–S through weak vander Waals interactions. As a 2D layer structure analogous of graphene, thin layer 2D MoS_2 nanosheets were reported can effectively adsorb dye-labeled single-stranded DNA (ssDNA) and quench the fluorescence of the dye, just like graphene oxide (GO) [24]. Additionally, thin layer 2D MoS_2 nanosheets have more excellent water solubility, more robust mechanical properties and superior electrical performance [25]. In view of the above properties of MoS_2 nanosheet, it has been used for fabricating fluorescent and electrochemical DNA biosensor [24,26,27]. Herein, we developed a high sensitive and selective fluorescent biosensor for UO_2^{2+} detection by taking the advantage of uranyl ion-specific DNAzyme and thin layer 2D MoS_2 nanosheets, in hope of providing a reliable and simple method for rapid and on-site detection of UO_2^{2+} in aqueous environment to ensure human health.

Materials and methods

Reagent and materials

The uranyl ion-specific DNAzyme used in the experiment is the same as that reported by Lu and his co-workers [22]. High-performance liquid chromatography (HPLC) purified enzyme strand (39E) and FAM-labeled substrate strand (39S) were synthesized by Takara BioInc (Dalian, China). Their concentrations were quantified by OD260 based on their individual absorption coefficients. Their base sequences were as follow: 39E: 5′-CAC GTC CAT CTC TGC AGT CGG GTA GTT AAA CCG ACC TTC AGA CAT AGT GAG T-3′; 39S: 5′-FAM-ACT CAC TAT rA GGA AGA GAT GGA CGT G-3′. Thin layer molybdenum disulfide (MoS_2) nanosheets solution (1–8 monolayers, 100–400 nm, 18 mg/L) was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China), and was treated by ultrasonic agitation for 3 h before use. The stock standard solution of 1 μg/mL UO_2^{2+} was provided by Fujian Environmental Radiation Supervision Station. More dilute standard solutions of UO_2^{2+} were prepared by serially diluting the stock standard solution (each diluted 10-fold) to the desired concentration with buffer solution. The buffer solution used in the experiment is 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) containing 300 mM $NaNO_3$ (pH 5.5). All other reagents were of analytical grade and used without further purification. The sterilized Milli-Q water (18 MΩ/cm) was used in all experiment.

A simple F-4600 fluorescence spectrometer (Hitachi, Japan) was used to determine fluorescence spectra and intensity. The fluorescence emission spectra were obtained in the wavelength range of 510–600 nm under an excitation wavelength of 495 nm and the room temperature. The atomic force microscopic (AFM) image was obtained with a Nanoscope(R) IIIa multimode atomic force microscope (Bruker, USA).

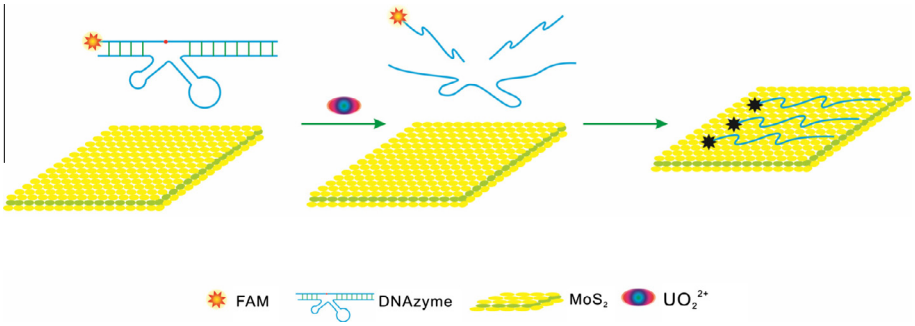
The detection of UO_2^{2+} with the proposed fluorescent biosensor

For the fluorescent detection of UO_2^{2+}, firstly, 230 μL of buffer solution in which containing 500 nM FAM-labeled substrate strand (39S) and 1 μM enzyme strand (39E) was pre-annealed by heating the whole at 85 °C for 2 min and then cooling the whole to room temperature with a rate of 1 °C/min to form substrate–enzyme complex DNAzyme. Subsequently, 20 μL of molybdenum disulfide solution (18 μg/mL) was added into above resulting DNAzyme solution, and the whole was fully mixed for 5 min. Then, 220 μL of the above prepared DNAzyme–MoS_2 nanosheets solution was taken, and 30 μL different concentrations of UO_2^{2+} standard solution or sample solution were added. The whole was incubated for 10 min to finish the specific cleavage, then, the fluorescence emission spectra were determined in the wavelength range of 510–600 nm under an excitation wavelength of 495 nm. The difference of fluorescence intensity at 516 nm ($\Delta F = F_0 - F$, F_0 is the fluorescence intensity in the absence of UO_2^{2+}, F is the fluorescence intensity in the presence of UO_2^{2+}) was used to calculate the concentration of UO_2^{2+}.

Results and discussion

The detailed principle of the fluorescent biosensor for detecting UO_2^{2+}

As we mentioned previously, thin layer 2D MoS_2 nanosheets can firmly adsorb dye-labeled single-stranded DNA (ssDNA) and



Scheme 1. Schematic illustration of the detection principle of the proposed fluorescent biosensor.

quench the fluorescence of the dye, whereas its affinity toward double-strand DNA is weaker [24]. Thus, a turn-off fluorescent biosensor of the rapid, specific and sensitive detection of UO_2^{2+} by using DNAzyme as UO_2^{2+} recognition and MoS_2 as fluorescence quencher was designed. As shown in Scheme 1, the substrate strand (39S) was labeled with FAM as the fluorescence reporter. In the 50 mM MES-300 mM $NaNO_3$ buffer solution (pH 5.5), the FAM-labeled substrate strand (39S) can completely hybridize with enzyme strand (39E) to form UO_2^{2+}-specific substrate–enzyme complex DNAzyme. In the absence of UO_2^{2+}, the substrate–enzyme complex DNAzyme is stable and cannot be effectively adsorbed on the surface of MoS_2 nanosheets since MoS_2 nanosheets have low affinity toward double-strand DNA [24,27]. Therefore, the DNAzyme-MoS_2 nanosheets system showed strong fluorescence intensity. However, in the presence of UO_2^{2+}, the UO_2^{2+} can specifically cleave substrate–enzyme complex DNAzyme to release FAM-labeled single-strand DNA. The FAM-labeled single-strand DNA can be firmly adsorbed on the surface of MoS_2 nanosheets, which leading to the quench of FAM fluorescence. Therefore, the fluorescence intensity of the DNAzyme-MoS_2 nanosheets system will remarkably reduce when the UO_2^{2+} was added into it. The reducing of fluorescence intensity directly depended on the amount of UO_2^{2+}, which provide a sensing platform for rapid, specific and sensitive detection of UO_2^{2+}.

Fluorescent characterization of the proposed UO_2^{2+} biosensor

In order to verify whether the biosensor worked as expected, the fluorescence emission spectra of DNAzyme–MoS_2 nanosheets system was investigated in the absence and presence of UO_2^{2+}, respectively. From the results shown in the Fig. 1, it was clearly observed that the pure substrate–enzyme complex DNAzyme has a highest fluorescence intensity owing to the FAM-labeling of substrate strand (curve a). Upon the addition of MoS_2 nanosheets (its size see Fig. 2), the fluorescence intensity of substrate–enzyme complex DNAzyme decreased slightly (curve b), this should be attributed to that MoS_2 nanosheets can adsorb a little of DNAzyme on the surface and quench its fluorescence since some bases of substrate-enzyme complex DNAzyme is in the single stranded state (see Scheme 1). When the UO_2^{2+} was added into the mixture of DNAzyme and MoS_2 nanosheets solution, the fluorescence intensity of the system remarkably decreased (curve c), indicating that the substrate–enzyme complex DNAzyme has been specifically cleaved to release FAM-labeled single-strand DNA and the released FAM-labeled single-strand DNA has been firmly adsorbed on the surface of MoS_2 nanosheets by the strong π–π stacking interaction, which resulting in the fluorescence quenching. All above facts suggested that the proposed UO_2^{2+} biosensor indeed worked as expected.

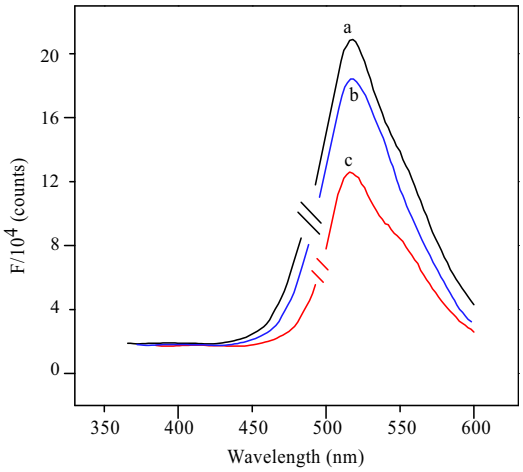


Fig. 1. Fluorescence emission spectra under an excitation wavelength of 495 nm. (a) The mixture of 500 nM of 39S and 1 μM of 39E; (b) the mixture of 500 nM 39S, 1 μM 39E and 1.5 μg/mL MoS_2; (c) the mixture of 500 nM 39S, 1 μM 39E, 1.5 μg/mL MoS_2 and 50 nM UO_2^{2+}.

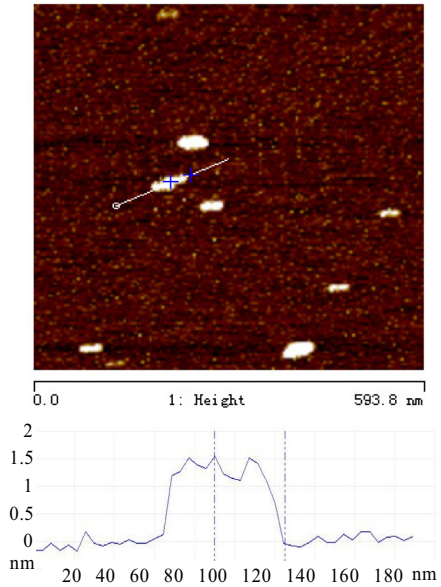


Fig. 2. AFM height image of MoS_2 used in the experiment.

Optimization of the proposed UO_2^{2+} biosensor

MoS_2 nanosheets play a key role in the experiment, the quenching ability of MoS_2 nanosheets directly affect the sensitivity of the

biosensor. In order to obtain the highest sensitivity, the concentration of MoS_2 nanosheets was optimized in detail. From the results shown in Fig. 3A, it was clearly observed that there is a most stable and highest quenching effect when the concentration of MoS_2 nanosheets is in the range of 1.4–1.6 μg/mL. Finally, 1.5 μg/mL of MoS_2 nanosheets were selected as the fluorescence quencher in this work.

The activity of DNAzyme to UO_2^{2+} greatly depends on the pH. The effect of pH on the interaction between DNAzyme and UO_2^{2+} was also investigated in the range of pH 4.0–pH 6.5. As illustrated in Fig. 3B, when the pH = 5.5, DNAzyme has a highest activity to UO_2^{2+}. Our results are consistent with the conclusion reported previously [20]. The most likely explanation of this result is that pH has an impact on the uranyl ion speciation in solution, and pH 5.5 produced a uranyl species that is the most effective cofactor for carrying out catalysis of DNAzyme [20]. If the pH is higher than 5.5, hydrolyzed species such as $UO_2(OH)^+$ might begin to dominate, which might not be the optimal cofactor for catalysis [28]. Therefore, pH 5.5 was selected as the optimal condition for the detection of UO_2^{2+} in this work.

In order to ensure all FAM-labeled substrate strand 39S completely hybridized with enzyme strand 39E to form active substrate–enzyme complex DNAzyme, moderate excess 39E was used in the experiment. The experimental results showed that the fluorescent biosensor has a highest sensitivity when the concentration ratio of 39S/39E = 1/2. Inadequate 39E will lead to the incomplete hybridization of FAM-labeled substrate strand 39S and finally decreased the sensitivity. Whereas, more excess of 39E will occupy the surface of MoS_2 nanosheets and reduce MoS_2 nanosheet's ability of adsorbing/quenching FAM-labeled single-strand DNA, which eventually decreased the sensitivity too.

For a rapid and on-site method, the rate of interaction between DNAzyme and UO_2^{2+} is another key consideration. The effect of the reaction time on the fluorescence intensity was investigated in detail in the range of 0–60 min. The experimental results (see Fig. 3C) clearly revealed that the fluorescence intensity rapidly decreased to bottom within 5 min and then the fluorescence intensity turn to keep no change, indicating that the reaction between DNAzyme and UO_2^{2+} reached to equilibrium within 5 min. Thus, 10 min was chosen as the optimal reaction time.

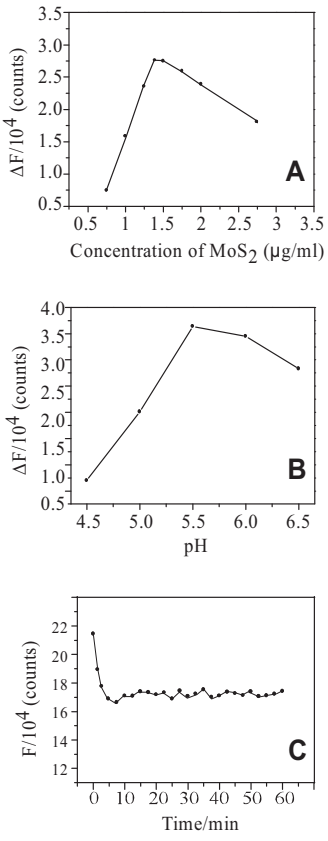


Fig. 3. (A) The relationship between the concentration of MoS_2 and the sensitivity (ΔF) of the proposed method. (B) The relationship between the pH and the sensitivity (ΔF) of the proposed method. (C) Time-dependent fluorescence intensity of the DNAzyme–MoS_2–UO_2^{2+} system at λ_{em} = 516 nm and λ_{ex} = 495 nm. The concentration of DNAzyme, MoS_2 and UO_2^{2+} are 500 nM, 1.5 μg/mL and 50 nM, respectively.

The selectivity of the proposed fluorescent biosensor

It was well known, there are various metallic ions existing in the aqueous environment, and it is possible that the existence of these metallic ions will interfere with the detection of UO_2^{2+}. In order to investigate whether other metallic ions would disturb the detection of UO_2^{2+}, we challenge various metal ions including 1000-folds (in comparison with UO_2^{2+}) of Mg^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Cr^{3+}, Zn^{2+}, Ba^{2+}, Mn^{2+}, K^+, Na^+ and 100-folds of La^{3+}, Lu^{3+} and Eu^{3+} with our biosensor, respectively. As the results shown in Fig. 4, only UO_2^{2+} can cleave DNAzyme to release FAM-labeled single-strand DNA and thus results in the fluorescence quenching, which gives rise to a remarkable reducing of fluorescence intensity (ΔF). Only a negligible reducing of fluorescence intensity (ΔF) was observed for other metallic ions, even if their concentrations were 1000-folds or 100-folds higher than that of UO_2^{2+}, indicating that other metallic ions do not cleave DNAzyme and thus interfere with the detection of UO_2^{2+}. The experimental results above revealed that the proposed biosensor, which is based on the DNAzyme and MoS_2 nanosheets, has excellent specificity.

The linear range, sensitivity and reproducibility of the proposed biosensor

Under the previously discussed optimum conditions, the fluorescence intensity of the DNAzyme–MoS_2 nanosheets system was monitored in the presence of different concentrations of UO_2^{2+} (0, 5, 10, 25, 50, 75, and 100 nM) to obtain calibration curve. As results shown in Fig. 5, upon the addition of UO_2^{2+} from 0 to 100 nM, the fluorescence intensity at 516 nm gradually decreased. The decreased fluorescence intensity (ΔF) showed a good linear correlation with the concentration of UO_2^{2+} in the range of 0–100 nM. The regression equation was: $\Delta F = 522.1 \times C - 838.2$ ($r = 0.9987$), where C is the concentration of UO_2^{2+} (nM). The detection limit ($3\sigma/S$, the concentration necessary to yield a net signal equal to three times the standard deviation of the background) was calculated to be 2.14 nM for UO_2^{2+}, and the RSD ($n = 5$) was

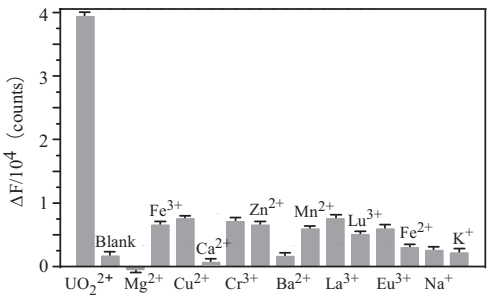


Fig. 4. The decrease of fluorescence intensity of DNAzyme and MoS_2 system in the presence of 50 nM UO_2^{2+}, 50 μM of Mg^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Cr^{3+}, Zn^{2+}, Ba^{2+}, Mn^{2+}, K^+, Na^+ and 5 μM of La^{3+}, Lu^{3+} and Eu^{3+}.

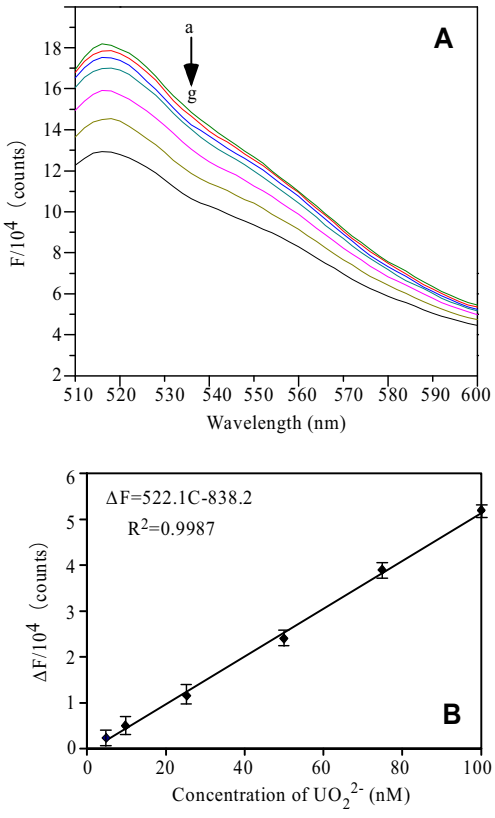


Fig. 5. Fluorescence spectra and calibration curve for detecting UO_2^{2+}. The concentrations of UO_2^{2+} are 0, 5, 10, 25, 50, 75, and 100 nM, respectively.

calculated to be less than 5% for the detection of 20 nM UO_2^{2+}. As we mentioned previously, so far, several rapid methods including colorimetric sensors [8,9,22], fluorescent sensor [21] and magnetoelastic sensor [23] have been developed for detecting UO_2^{2+}. In comparison with previous colorimetric sensors [8,9,22], the proposed biosensor has higher sensitivity, similar or more excellent selectivity and short analysis time. Compared to the previous fluorescent sensor [21], the proposed biosensor is more simple and cost-effective although the sensitivity and selectivity of both are similar. More importantly, the detection limit of our method is also lower than the maximum allowable level of UO_2^{2+} (130 nM) in the drinking water defined by the USA Environmental Protection Agency (EPA) [22], indicating that our method meets the requirement of rapid and on-site detection of UO_2^{2+} in the aqueous environment.

Detection of UO_2^{2+} in river water samples

The applicability and reliability of the biosensor developed in this study was demonstrated by analyzing river water samples, which were collected from the Minjiang river of Fujian, China. The river water was firstly filtered through a 0.22 μm membrane filter to remove any sediment, and then the amount of UO_2^{2+} in the water was directly determined with our method. The results obtained with our method were compared with the results obtained by inductively coupled plasma mass spectrometry (ICP-MS), a standard method for UO_2^{2+} determination. The analytical results are shown in Table 1 together with the data determined with inductively coupled plasma mass spectrometry (ICP-MS). From the results shown in Table 1, it was clearly observed that the results obtained with our biosensor are consistent with those determined with ICP-MS. The recovery was in the range of

Table 1
The analytical results of UO_2^{2+} in water samples collected from Minjiang river, Fujian of China.

Sample	Added con. (nM)	Detected con. (nM)	Recovery (%)	RSD (n = 6) (%)	[a]ICP-MS (nM)
1	0.0	–[b]	–	–	0.77
2	5.0	4.8	96	5	5.61
3	25.0	25.5	102	3	26.1
4	50.0	49.5	99	4	51.2

[a] Data obtained with ICP-MS method.
[b] Concentration is lower than detection limit.

96–102% and the RSD (n = 6) was smaller than 5%. All above facts indicated that the proposed biosensor has a good selectivity, accuracy, reproducibility and robust resistance to the matrix. The success of this study provides a promising approach for the rapid and on-site detection of UO_2^{2+} ions in aqueous environment.

Conclusions

In summary, a "turn-off" fluorescent biosensor for the rapid detection of UO_2^{2+} with high sensitivity and selectivity was developed by using DNAzyme as UO_2^{2+} recognition and MoS_2 nanosheets as fluorescence quencher. We demonstrated that MoS_2 nanosheets have low affinity to the substrate–enzyme complex DNAzyme, and UO_2^{2+} can specifically cleave DNAzyme to release FAM-labeled single-strand DNA. The released FAM-labeled single-strand DNA can be firmly adsorbed on the surface of MoS_2 nanosheets and thus resulted in an obvious decrease of fluorescence intensity, which provided a sensing platform for the rapid, simple and sensitive fluorescent detection of UO_2^{2+}. By using the sensing platform, a sensitive and selective fluorescent biosensor for the rapid detection of UO_2^{2+} has been developed. The proposed method has outstanding analytical advantage such as higher sensitivity, short analytical time, lower cost and robust resistance to the matrix. It can be used to detect as low as 2.14 nM of UO_2^{2+} in aqueous environment with a recovery of 96–102% and a RSD < 5% (n = 6). The success of this study provides a promising alternative for the rapid and on-site detection of UO_2^{2+} in environmental monitoring.

Acknowledgments

The authors gratefully acknowledge NSFC (21377025), Ministry of Education, Science and Technology Development Center (20123514110001), Fujian Provincial Department of Science and Technology (2014Y0042), National Key Technologies R & D Program of China during the 12th Five-Year Plan Period (No. 2012BAD29B06) and the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1116) for financial support.

References

[1] J. Li, Y. Zhang, Proc. Environ. Sci. 13 (2012) 1609–1615.
[2] M. Yazzie, S.L. Gamble, E.R. Civitello, D.M. Stearns, Chem. Res. Toxicol. 16 (2003) 524–530.
[3] J.L. Domingo, Reprod. Toxicol. 15 (2001) 603–609.
[4] A. Lorber, Z. Karpas, L. Halicz, Anal. Chim. Acta 334 (1996) 295–301.
[5] Y.B. Rao, B.V.V. Ramana, P.G. Raghavan, R.B. Yadav, J. Radioanal. Nucl. Chem. 294 (2012) 371–376.
[6] A. Quentmeier, M. Bolshov, K. Niemax, Spectrochim. Acta Part B 56 (2001) 45–55.
[7] R. Brina, A.G. Miller, Anal. Chem. 64 (1992) 1413–1418.
[8] J.L. Sessler, P.J. Melfi, D. Seidel, A.E.V. Gorden, D.K. Ford, P.D. Palmer, C.D. Tait, Tetrahedron 60 (1097) (2004) 11089–11097.
[9] A. Safavi, M. Bagheri, Anal. Chim. Acta 530 (2005) 55–60.
[10] P.A. Greene, C.L. Copper, D.E. Berv, J.D. Ramsey, G.E. Collins, Talanta 66 (2005) 961–966.
[11] S.K. Silverman, Chem. Commun. 48 (2008) 3467–3485.

[12] I. Willner, B. Shlyahovsky, M. Zayats, B. Willner, Chem. Soc. Rev. 37 (2008) 1153–1165.
[13] J. Kosman, B. Juskowiak, Anal. Chim. Acta 707 (2011) 7–17.
[14] L.H. Tan, H. Xing, Y. Lu, Acc. Chem. Res. 47 (2014) 1881–1890.
[15] K. Sefah, J.A. Phillips, X. Xiong, L. Meng, D.V. Simaeys, H. Chen, J. Martin, W. Tan, Analyst 134 (2009) 1765–1775.
[16] C. Li, L. Wei, X. Liu, L. Lei, G. Li, Anal. Chim. Acta 831 (2014) 60–64.
[17] Y. Qian, C. Wang, F. Gao, Anal. Chim. Acta 845 (2014) 1–6.
[18] Y.L. He, J. Tian, J. Zhang, S. Chen, Y. Jiang, K. Hu, Y. Zhao, S. Zhao, Biosens. Bioelectron. 55 (2014) 285–288.
[19] Z. Liu, S.H.J. Mei, J.D. Brennan, Y. Li, J. Am. Chem. Soc. 125 (2003) 7539–7545.
[20] A.K. Brown, J. Liu, Y. He, Y. Lu, ChemBioChem 10 (2009) 486–492.
[21] J. Liu, A.K. Brown, X. Meng, D.M. Cropek, J.D. Istok, D.B. Watson, Y. Lu, Proc. Natl. Acad. Sci. USA 104 (2007) 2056–2061.
[22] J.H. Lee, Z. Wang, J. Liu, Y. Lu, J. Am. Chem. Soc. 130 (2008) 14217–14226.
[23] J.C. Yin, Y.-S. Wang, B. Zhou, X.-L. Xiao, J.-H. Xue, J.-C. Wang, Y.-S. Wang, Q.-M. Qian, Sens. Actuators B 188 (2013) 147–155.
[24] C. Zhu, Z. Zeng, H. Li, F. Li, C. Fan, H. Zhang, J. Am. Chem. Soc. 135 (2013) 5998–6001.
[25] A. Splendiani, L. Sun, Y. Zhang, T. Li, J. Kim, C.-Y. Chim, G. Galli, F. Wang, Nano Lett. 10 (2010) 1271–1275.
[26] K.-J. Huang, Y.-J. Liu, H.-B. Wang, Y.-Y. Wang, Y.-M. Liu, Biosens. Bioelectron. 55 (2014) 195–202.
[27] X. Wang, F. Nan, J. Zhao, T. Yang, T. Ge, K. Jiao, Biosens. Bioelectron. 64 (2014) 386–391.
[28] C. Moulin, P. Decambox, Anal. Chem. 67 (1995) 348–353.