Biosensors and Bioelectronics 29 (2011) 178–183

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Time-resolved fluorescence biosensor for adenosine detection based on home-made europium complexes

Da-Wei Huang, Cheng-Gang Niu*, Guang-Ming Zeng, Min Ruan

College of Environmental Science and Engineering, Hunan University, Key Laboratory of Environmental Biology and Pollution Control, Ministry of Education, Hunan University, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 22 April 2011
Received in revised form 27 July 2011
Accepted 10 August 2011
Available online 19 August 2011

Keywords:
Time-resolved fluorescence
Aptamer
Adenosine
Biosensor
Europium complexes

ABSTRACT

In this protocol, the authors report a time-resolved fluorescence biosensor based on home-made europium complexes for highly sensitive detection of small molecules using adenosine as a model analyte. The fluorophore that used is europium complexes. Its signal can be measured in a time-resolved manner that eliminates most of the unspecific fluorescent background. The amino modified aptamer probe, which is designed to specifically recognize adenosine, is combined to the aldehyde-group modified glass slide by covalent bond. Europium complex-labeled a short ssDNA, designed to segment hybridize with aptamer probe is immobilized on the glass slide by hybridization reaction. In the presence of adenosine, the aptamer part is more inclined to bounds with adenosine and triggers structure-switching of the aptamer from aptamer/ssDNA duplex to aptamer/target complex. As a result, europium complexes-labeled ssDNA is forced to dissociate from the sensor interface, resulting in time-resolved fluorescence intensity decrease. The decrement intensity is proportional to the amount of adenosine. Under optimized assay conditions, a linear range (1.0×10^{-8} M to 1.0×10^{-7} M) is got with low detection limit of 5.61 nM. The biosensor exhibits excellent selectivity and can provide a promising potential for aptamer-based adenosine detection.

1. Introduction

Adenosine is a nucleoside composed of a molecule of adenine and an endogenous nucleoside with potent vasodilator and antiarrhythmic activities. What's more, adenosine plays a vitally important signaling function in both the peripheral and central nervous system. In the peripheral nervous system, it is involved in the control of smooth muscle contraction and is a powerful vasodilator (Phillis, 1989; McMillan et al., 1999). In the central nervous system, adenosine is used as the neuroprotection during ischemia, regulation of spinal motor pattern generation, and it is also an inhibitory neurotransmitter (Dunwiddie1 and Masino, 2001), believed to play a role in promoting sleep and suppressing arousal, with levels increasing with each hour an organism is awake (Wu et al., 2007; Zhang et al., 2008; Baraldi et al., 2008). Furthermore, adenosine plays an important role in energy transfer as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (Wu et al., 2007). Therefore, the detection of adenosine is of great significance.

Aptamers, which obtained from random-sequence nucleic acid libraries by an in vitro evolution process called SELEX (systematic evolution of ligands by exponential enrichment), are synthetic single-strand nucleic acids with high specificity and affinity to some given targets ranging from small molecules to large proteins and even cells (Ellington and Szostak, 1990; Tuerk and Gold, 1990; Hermann and Patel, 2000; Pavlov et al., 2004; Herr et al., 2006; Wu et al., 2007; Tuleuova et al., 2010). To date, a large number of aptamers have been reported as probes for proteins and small-molecule metabolites detection with high affinity and specificity (Cruz-Aguado and Penner, 2008; Centi et al., 2008; Huang and Zhu, 2009; Xu and Lu, 2010; Guo et al., 2006). Aptamers can form a three dimensional aptamer/target complex, and aptamer/ssDNA duplex with the complementary ssDNA. When the target molecule and the complementary ssDNA are introduced into aptamer, they are competed to interact with aptamer. Actually, aptamer is more inclined to form aptamer/target complex but not to form aptamer/ssDNA duplex (Huizenga and Szostak, 1995; Rupcich et al., 2005; Green et al., 1996). Consequently, in recent years, a new strategy based on structure-switching signaling aptamers, in which fluorescence signal was generated through target-induced switching between an aptamer/ssDNA duplex and an aptamer/target complex, was developed (Wu et al., 2007; Zhang et al., 2008; Nutiu and Li, 2003, 2005a; Liu and Lu, 2006; Zayats et al., 2006; Chen et al., 2008; Wang et al., 2010). The most popular assays for small molecules using structure-switching signaling aptamers are based on electrochemistry, colorimetric or fluorescence. The electrochemistry methods for the monitoring of the interaction between aptamer

* Corresponding author. Tel.: +86 731 88823820; fax: +86 731 88822829.
E-mail addresses: cgniu@hotmail.com, cgniu@hnu.edu.cn (C.-G. Niu).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.08.014

and specific targets have recently been reported by many investigators and some have achieved good results (Wu et al., 2007; Zhang et al., 2008; Zayats et al., 2006; Kim et al., 2009; Baker et al., 2006). Nonetheless, these methods have the shortages of time-consuming, special analytical instruments needed as well as the difficulty in the modification of electroactive group to aptamers. The colorimetric sensors of adenosine detection have the advantages of simple and eliminate the use of analytical instruments and the disadvantages of an appropriate dye has to be found, time-consuming to observe a color change and relatively low detection limit (Liu and Lu, 2006; Stojanovic and Landry, 2002; Ho and Leclerc, 2004; Liu and Lu, 2004). Compared to the two popular methods for small molecules detection, the fluorescence sensor well solved these problems the electrochemistry and colorimetric methods encountered. It is without the need of a complicated modification step to link the fluorescence molecular to aptamers, without time-consuming and complex operation steps during the fluorescence signal acquiring process and the sensitivity is competitive with or better than these methods (Nutiu and Li, 2003, 2004; Achenbach et al., 2005). What's more, fluorescence technique is highly compatible with nucleic acid aptamers because there are a large number of fluorophores can be used to modify nucleic acids using simple schemes (Nutiu and Li, 2005b).

Considering all these, the authors prefer to design fluorescence biosensor because of the simple detection procedures and the minimal usage of analytical instruments. In conventional fluorescence biosensor, there is a problem of how to eliminate background noises caused by autofluorescence from biological samples, the scattering light from solid substrates, and the luminescence from the optical components. For this purpose, one approach is to use time-resolved fluorescence with lanthanide complexes as the labels to decrease the background noises (Diamandii and Christopoubs, 1990; Egorova et al., 1999; Lode et al., 2003). Compared to many conventional fluorophores, fluorescent europium chelates present long fluorescence lifetimes (in the order of milliseconds for Eu^{3+} compared with 5–100 ns for conventional fluorophores) allow use of microsecond time-resolved fluorescence measurements, which further reduce the observed background signals (Diamandii and Christopoubs, 1990). To the best of the authors' knowledge, time-resolved fluorescence-based assay for adenosine detection has not been reported.

Herein, in the present study, the authors report a novel sensitive method for the detection of adenosine using time-resolved fluorescence biosensor. A europium complex, $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ (ETN) with long fluorescence lifetime synthesized by our group, was used as the fluorescent label (Qin et al., 2009, 2010). Using this biosensor, the adenosine could be specifically detected, and a relatively low detection limit of 5.61 nM, as well as a linear range could be achieved. The optimal conditions for the detection of adenosine were investigated.

2. Experimental

2.1. Materials and reagents

Oligonucleotides designed according to the literature (Wu et al., 2007; Huizenga and Szostak, 1995; Liu and Lu, 2006; Chen et al., 2008) in the present study were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), the sequence of the adenosine-binding aptamer was 5′-NH_2–$(CH_2)_6$–AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′ (aptamer); the sequence of its part complementary strand was 5′-NH_2–$(CH_2)_6$–CCCAGGTTCTCT-3′ (ssDNA). Adenosine, cytidine and uridine, were all purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). 3-Aminopropyltrimethoxysilane (APTES) was purchased from Acros organics (Geel, Belgium). Glycine was purchased from Jiehui Biological Technology Co. Ltd. (Changsha, China). Sodium cyanoborohydride, 95%, was purchased from J&K chemical Ltd. (Beijing, China). Glutaraldehyde (25% aqueous solution) was supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals used were of analytical grade and were used without further purification. Doubly distilled water was used throughout the experiments.

2.2. Preparation of buffer solutions

Tris–HCl buffer solutions (20 mM) of different pH were prepared by mixing an appropriate 0.10 M Tris base solution with 0.10 M hydrochloric acid. PBS buffer (100 mM) was prepared by mixing an appropriate content of 200 mM Na_2HPO_4 and 200 mM NaH_2PO_4. The binding buffer was included appropriate of 20 mM Tris–HCl (pH = 7.4), 150 mM NaCl, 4 mM $MgCl_2$. The composition of immobilization and label buffer was 100 mM PBS buffer (pH = 7.3) containing 0.9% NaCl. The rinse buffer was 100 mM PBS buffer (pH = 7.3) containing 0.9% NaCl and 0.05% Tween 20.

2.3. Instrumentation

All fluorescence measurements were conducted on a Perkin-Elmer LS-55 spectrofluorimeter (United Kingdom) and controlled by a personal computer data processing unit. A home-made poly (tetrafluoroethylene) detection-cell and two arms of the bifurcated optical fiber were fixed in the detecting chamber of the spectrofluorimeter to carry the excitation and emission light. The excitation light was carried outside the spectrofluorimeter to the biosensor fixed in the detection-cell through one arm of the fiber and the emission light was collected inside the spectrofluorimeter through the other. A glass plate (side length 12.5 mm, thickness 1.5 mm) covered with adenosine–aptamer complex was fixed at the bottom of the chamber by the mounting screw nut. The parameters of spectrofluorimeter were set as follows: both excitation and emission slits 8.0 nm, delay time 0.1 ms, gate time 1.0 ms, and excitation/emission wavelength 360/611 nm. All assays were performed in triplicates and all measurements were performed at room temperature (25 °C) and atmospheric pressure (101 kPa).

2.4. Preparation of activated glass slides

Glass slides were activated according to the literature (Stadtherr et al., 2005; Lee and Walt, 2000) with slight modifications. Glass slides were cleaned in chromic acid washer liquid for 12 h, and then rinsed in doubly distilled water, followed by an immersion in ammonia water (25%) for another 12 h. The glass slides were then washed with doubly distilled water. Precleaned glass slides were immersed in a 95% alcohol/water solution containing 2% APTES, and adjusted to the desired pH of 4.5 with glacial acetic acid for 30 min. Slides were cleaned with ethanol and doubly distilled water and finally dried at room temperature. Silanized slides were incubated in PBS buffer containing 2.5% glutaraldehyde for 3 h, and washed with rinse buffer and doubly distilled water, respectively, and then dried at 37 °C.

2.5. Adenosine aptamer coupling on activated glass slides

The aptamer and ssDNA were heated at 95 °C for 3 min and then cooled in ice for 10 min prior to coupling steps. The thermal treatment made the aptamer unfold and allowing the proper conformation for molecular recognition. 5′-Amino-modified aptamer (20 μl, 1.0 μM in PBS) was dropped onto the activated glass surface and incubated overnight at 20 °C. The droplets were allowed to

dry, and any non-covalently bond aptamer was removed by washing in rinse buffer. After rinsing the glass samples three times with rinse buffer, remaining non-reacted aldehyde groups were blocked with 0.1 M glycine at 37 °C (2 h) and the aptamer modified glass slides were then immersed in a sodium cyanoborohydride solution for 30 min to reduce the carbon–nitrogen double bond. Finally, the slides were dried at room temperature after washing with rinse buffer and double-distilled water. The aptamer probe was then covalently immobilized on the glass surface.

2.6. Preparation of Eu(TTA)$_3$(5-NH$_2$-phen) (ETN) labeled probe

The probe ssDNA was combined with the functionalized ETN via cross-linking by glutaraldehyde. The complex was first dispersed in PBS buffer containing 2.5% glutaraldehyde and thermal agitation for 3 h at room temperature. After centrifuging and washing with PBS buffer and double-distilled water, a certain volume ssDNA (the final concentration of ssDNA is 2 μM) was added to the solution of aldehyde combined complex, the mixture was stirred at 20 °C overnight. After centrifuging and dialysising with rinse buffer to remove the unbound substances, the final concentration of the DNA labeled ETN was 1.6 μM and the final product was stored at 4 °C for future usage.

2.7. Time-resolved fluorescence detection of adenosine

Firstly, the adenosine aptamer modified glass slides were hybridized with ETN-labeled ssDNA 3.0 h at 37 °C, followed by the addition of adenosine of a fixed volume (10 μl) at series of concentrations (for blank sample, 10 μl binding buffer was added). The dsDNA-modified glass slides were incubated with adenosine in binding buffer at 37 °C for 2.0 h. Then the glass slides were washed with rinse buffer for 3 times to eliminate the unbounded substances. Finally, all glass slides were dried at room temperature. The detection was accomplished by monitoring time-resolved fluorescence signals of the ETN-labeled ssDNA left on the glass slides surface. The main steps of the assay are shown in Scheme 1.

3. Results and discussion

3.1. Experimental principle of the proposed biosensor

In the present study, the authors fabricated a biosensor for highly sensitive detection of adenosine, using an amino-modified aptamer, an ETN-labeled ssDNA sequence as the capture probe and the detection probe, respectively. Scheme 1A shows the fundamental of aptamer-based adenosine assay in the present paper. The aptamer probe comprises two major segments: the first segment (in boldface) is a five-base segment close to the 5′-terminal that could hybridize with short ssDNA sequence close to the 3′-terminal; the second segment (in italic) is the aptamer sequence for adenosine, whose seven-base segment could hybridize with the 5′-terminal seven-base segment of ssDNA sequence. Scheme 1B displays the adenosine detection procedures and principle. The surface of glass slide was first modified with APTES, followed by incubated in 2.5% glutaraldehyde. Subsequently, 5′-amino-modified aptamer was immobilized on the activated glass surface by covalent bond, and then glycine and freshly prepared sodium cyanoborohydride solutions were used to block the non-reacted aldehyde group and reduce the imine group of Schiff base, respectively. Finally, the ETN-labeled ssDNA dropped onto the glass surface to hybridize with aptamer, and the sensor interface was prepared for adenosine test. As the aptamer/target complex is more stable than the aptamer/ssDNA duplex, the aptamers prefer to form the aptamer/target complex rather than the aptamer/ssDNA duplex when the target is introduced, triggering the release of the ETN-ssDNA from the aptamer/ssDNA duplex and made the intensity decreased. That is to say, the change of time-resolved fluorescence intensity was obtained.

3.2. Experimental conditions for adenosine detection

In the present work, the concentration of aptamer, the hybridization time and the incubation time played crucial roles for the detection sensitivity. Firstly, the optimal concentration of aptamer coupling onto the activated glass slides was investigated. Five different concentrations of aptamer, 10.0 μM, 5.0 μM, 1.0 μM, 0.5 μM and 0.1 μM, were employed to coupling onto the activated glass slides. The hybridization time and incubation time were 200 min in this experiment. The results are recorded in Fig. 1A. The concentration of the aptamer at 1.0 μM presents a lower noise and a stable signal than others. It may be the appropriate concentration for coupling with the aldehyde group on the glass slides. Thus, the concentration of aptamer used for all experiments was 1.0 μM. One could suppose that too much aptamer would induce an insufficient displacement between adenosine and ETN-labeled ssDNA, resulting in high background. In contrast, not enough amount of aptamer would lead to weak time-resolved fluorescence signal. Both of the two conditions would reduce signal to noise ratio and sensitivity of the sensor. In addition, in order to ensure the completeness hybridization between aptamer and ssDNA and decrease the background signal from excess ETN-labeled ssDNA, the authors choose 2.0 μM as the ssDNA concentration.

The hybridization time between aptamer and ETN-labeled probe was investigated as it may influence the hybridization efficiency. Fig. 1B shows that the fluorescence intensity increased with increasing the hybridization time and got stable over 150 min. To ensure the completeness of hybridization between aptamer and ETN-labeled probe, we chose 180 min as the hybridization time in all experiments. The concentration of aptamer and the incubation time used in the experiment were 1.0 μM and 200 min, respectively.

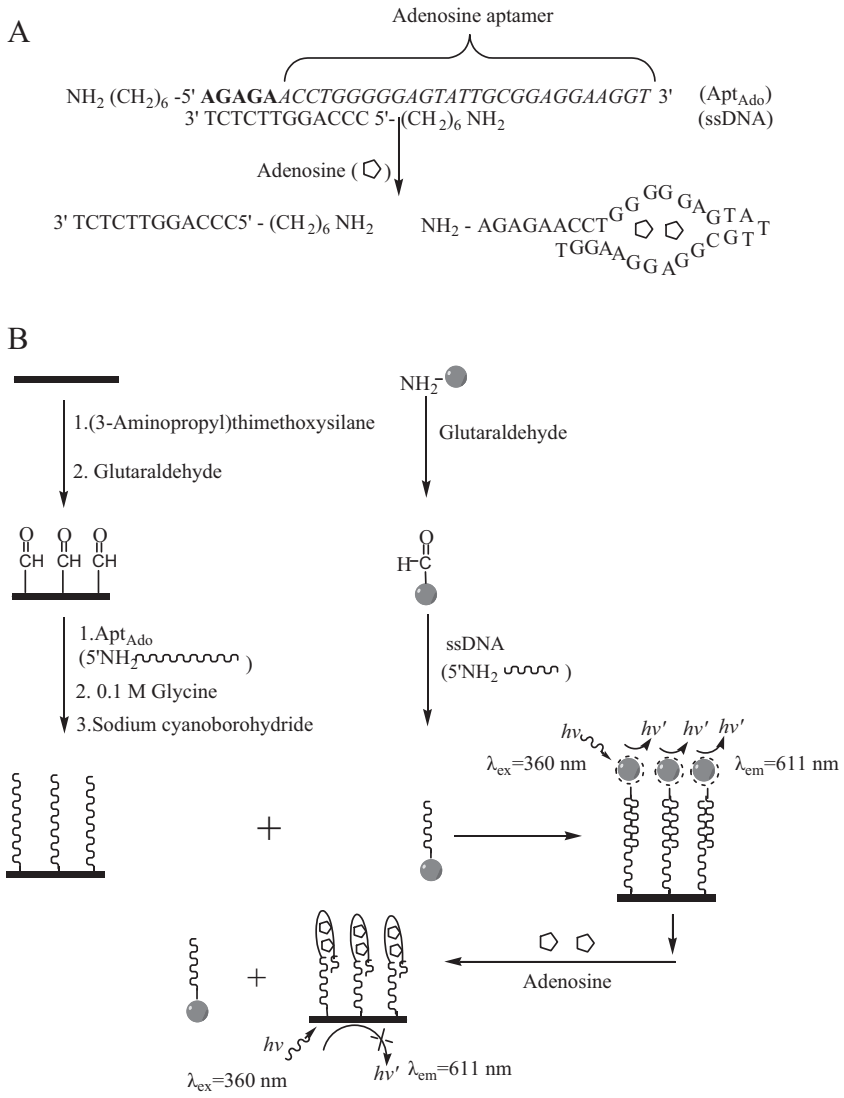
Also, the incubation time after the introduction of adenosine onto the sensor surface is investigated and the results are shown in Fig. 1C. The fluorescence intensity decreased when the adenosine was introduced and then tended to stabilize after more than 120 min. On the basis of this, all the experiments were carried out for the incubation time of 120 min. The concentration of aptamer and the hybridization time used in the experiment were 1.0 μM and 180 min, respectively.

Herein, 250 nM was used as the adenosine concentration to optimize the experiment conditions.

3.3. Detection of adenosine

Under the optimized assay conditions, the biosensor was employed to detect adenosine in binding buffer. Time-resolved fluorescence intensities of different concentrations of adenosine detected from glass surfaces were recorded at λ_{ex} = 360 nm, λ_{em} = 611 nm. As shown in Fig. 2A, the time-resolved fluorescence intensity was decreased with the increasing concentration of adenosine (from 75.56 with blank to 7.17 with 1000 nM). The decrement intensity is proportional to the amount of adenosine. In speculating on the results, the proposed assay can be used to detect trace adenosine with lower background signal.

The reproducibility and stability of fluorescent signal of this assay were tested by measuring three adenosine concentrations in three independent measurements. The results are shown in Table 1.



Scheme 1. Design of adenosine detection biosensor and the schematic diagram of this method.

3.4. The calibration curve of adenosine detection

The sensitivity of the time-resolved fluorescence biosensor was investigated. Under the optimized assay conditions, the decrement fluorescence intensity was found to be linear with the concentration of adenosine in the range from 1×10^{-8} M to 1×10^{-7} M. The equation for the resulting calibration plot was $y = -0.3853x + 67.8789$ (x was the concentration of adenosine, y was the time-resolved fluorescence intensity) with correlation coefficient of 0.9761. According to the standard deviation 0.72 for the blank signal with 20 parallel measurements, a detection limit of approximate 5.61 nM was estimated according to the three times standard deviation rule. The lower limit of detection under 10 nM was sufficient to monitor adenosine changes from basal levels (Kumara Swamy and Jill Venton, 2007), which was estimated to be 50-200 nM in the brain (Latini and Pedata, 2001). The calibration equation can serve as the quantitative basis for the determination of trace adenosine content in buffer.

The linear relationship between the time-resolved fluorescence intensity and adenosine concentration is shown in Fig. 2B.

Table 1
The reproducibility of adenosine.

$C_{adenosine}$ (nM)	Intensity (λ_{em} = 611 nm)	Average	RSD (%)
0	75.56, 75.92, 72.51	74.66	2.5
10	66.31, 64.45, 72.22	67.66	6.0
25	58.14, 57.41, 61.32	58.95	3.5
50	45.25, 49.39, 49.22	47.95	4.9
75	35.77, 35.40, 37.62	36.25	3.3
100	31.54, 29.20, 34.12	31.62	7.8
250	25.40, 26.78, 24.78	25.65	4.0
500	11.32, 12.78, 14.55	12.88	12.0
1000	6.64, 8.24, 7.17	7.35	11.0

3.5. Selectivity of the biosensor

To investigate the selectivity of the biosensor, the authors have compared the fluorescence signal brought by adenosine and other two analogues, cytidine and uridine. Guanosine was not tested because of its poor solubility at room temperature. As shown in Fig. 3, a significant decrease induced by the interaction of the aptamer probe and adenosine was observed compared to the other two nucleoside samples after the addition of 500 nM adenosine, cytidine, or uridine under the same experimental conditions, the time-resolved fluorescence intensities of buffer, cytidine, uridine and adenosine were 72.33, 67.70, 60.78 and 12.57, respectively.

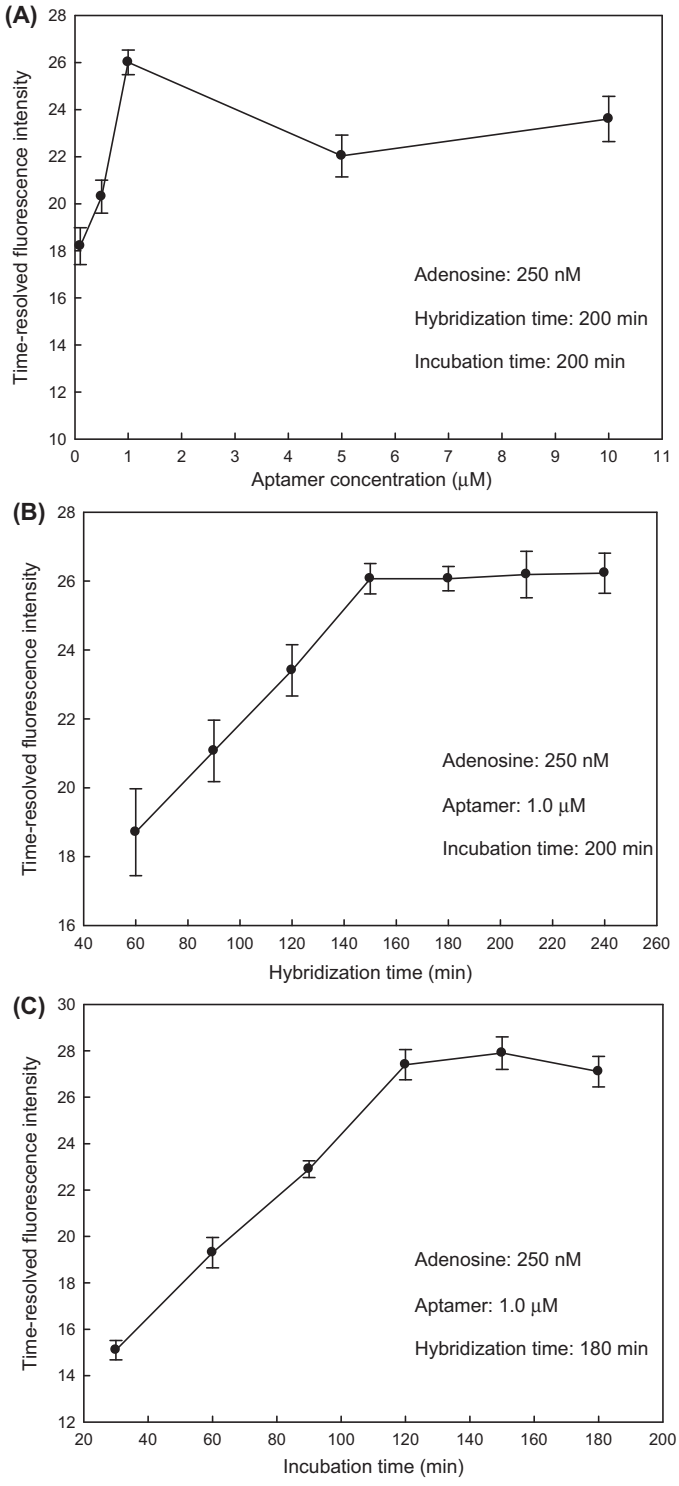


Fig. 1. The optimization of experimental conditions for adenosine detection. A–C show the effect of different aptamer concentration, hybridization time, incubation time to time-resolved fluorescence intensities. Every data point was the mean of three measurements. The error bars are the standard deviation.

These results indicated that the developed strategy had a sufficient specificity and adenosine could be unequivocally identified.

3.6. Determination of adenosine in human serum

The following experiments were focused on evaluating the ability of the sensor to detect adenosine in complex matrixes such as

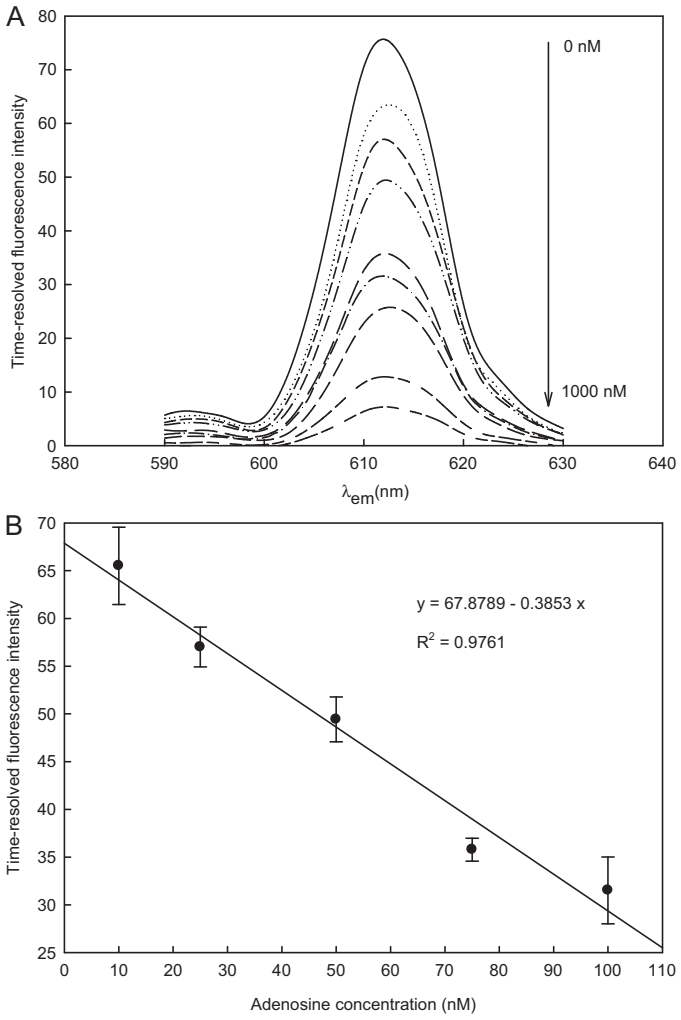


Fig. 2. (A) Time-resolved fluorescence emission spectra for different concentrations of adenosine (from top to bottom: 0 nM, 10 nM, 25 nM, 50 nM, 75 nM, 100 nM, 250 nM, 500 nM, 1000 nM), for time-resolved spectra: λ_{ex} = 360 nm, λ_{em} = 611 nm, delay time, 0.1 ms, gate time, 1.0 ms. A: adenosine. (B) The linear relationship between the time-resolved fluorescence intensity and adenosine concentrations. The concentrations of adenosine were 10 nM, 25 nM, 50 nM, 75 nM, and 100 nM. Every data point was the mean of three measurements. The error bars are the standard deviation.

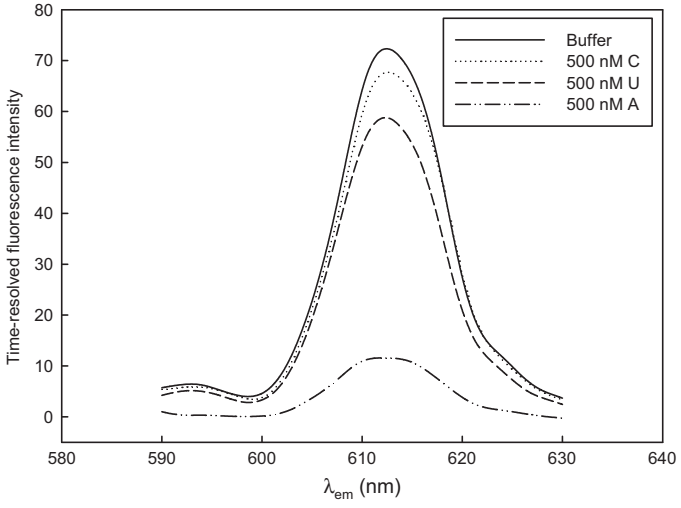


Fig. 3. The time-resolved fluorescence intensity of the sensor after being incubated with three nucleosides at 500 nM in binding buffer. A: adenosine, C: cytidine, and U: uridine.

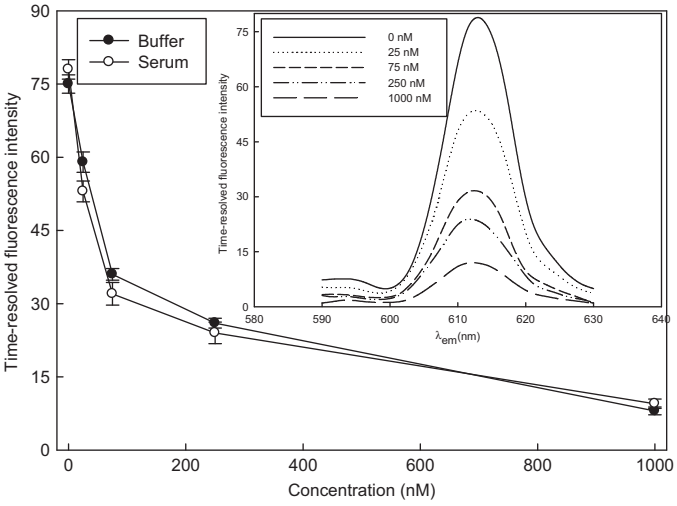


Fig. 4. Results obtained with serum samples spiked with different concentrations of adenosine and comparison with the same concentrations tested in buffer. The concentrations of adenosine were 0 nM, 25 nM, 75 nM, 250 nM, and 1000 nM. (Inset) The fluorescence spectra of serum samples spiked with different concentrations of adenosine. Every data point was the mean of three measurements. The error bars are the standard deviation.

serum of normal human blood. Human serum, diluted ten times with PBS buffer, was used to prepare adenosine solution and other experimental conditions were the same as described previous. The results are shown in Fig. 4, and the recoveries and the relative standard derivations of 25 nM, 75 nM, 250 nM, and 1000 nM were 90%, 90%, 92%, 112% and 4.1%, 6.9%, 8.8%, 9.5%, respectively. These results suggested that the sensor can still work in the complex serum matrix.

4. Conclusions

The present study has described a novel sensitive method for the detection of adenosine using time-resolved fluorescence biosensor based on home-made europium complexes and structure-switching signaling aptamer. The ETN modified ssDNA exhibited extremely long lifetime and excellent signaling ability of a trace amount of target small molecules. This biosensor exhibited excellent sensitivity and selectivity. Using this method, the authors have successfully detected adenosine over a range of 1×10^{-8} M to 1×10^{-7} M with a detection limit of 5.61 nM. The sensor shows an obvious fluorescence change and the scheme could be applicable to any aptamer of choice. The proposed method has been successfully applied to determine adenosine in human serum samples. Additionally, the present biosensor exhibits other advantages, such as easy to operate, a low dosage of material, and low-cost measurement. The authors believed that the proposed technique provides a promising method for aptamer-based small-molecule detection.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (20977026 and 51039001), the National 863 High Technology Research Foundation of China (2006AA06Z407), the Research Fund for the Doctoral Program of Higher Education of China (20090161110009), the Fundamental Research Funds for the Central Universities of Hunan University, the Hunan Key Scientific Research Project (2009FJ1010), and the Equipment Foundation of Hunan University.

References

Achenbach, J.C., Nutiu, R., Li, Y.F., 2005. Anal. Chim. Acta 534, 41–51.
Baker, B.R., Lai, R.Y., Wood, M.S., Doctor, E.H., Heeger, A.J., Plaxco, K.W., 2006. J. Am. Chem. Soc. 128, 3138–3139.
Baraldi, P.G., Tabrizi, M.A., Gessi, S., Borea, P.A., 2008. Chem. Rev. 108, 238–263.
Centi, S., Messina, G., Tombelli, S., Palchetti, I., Mascini, M., 2008. Biosens. Bioelectron. 23, 1602–1609.
Chen, J.W., Liu, X.P., Feng, K.J., Liang, Y., Jiang, J.H., Shen, G.L., Yu, R.Q., 2008. Biosens. Bioelectron. 24, 66–71.
Cruz-Aguado, J.A., Penner, G., 2008. Anal. Chem. 80, 8853–8855.
Diamandii, E.P., Christopoubs, T.K., 1990. Anal. Chem. 62, 1149A–1157A.
Dunwiddie1, T.V., Masino, S.A., 2001. Annu. Rev. Neurosci. 24, 31–55.
Egorova, A., Beltyukova, S., Teslyuk, O., 1999. J. Pharm. Biomed. 21, 585–590.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
Green, L.S., Jellinek, D., Jenison, R., Ostman, A., Heldin, C.H., Janjic, N., 1996. Biochemistry 35, 14413–14424.
Guo, X.H., Liu, Z.Q., Liu, S.Y., Bentzley, C.M., Bruist, M.F., 2006. Anal. Chem. 78, 7259–7266.
Hermann, T., Patel, D.J., 2000. Science 287, 820–825.
Herr, J.K., Smith, J.E., Medley, C.D., Shangguan, D.H., Tan, W.H., 2006. Anal. Chem. 78, 2918–2924.
Ho, H.A., Leclerc, M., 2004. J. Am. Chem. Soc. 126, 1384–1387.
Huang, H.P., Zhu, J.J., 2009. Biosens. Bioelectron. 25, 927–930.
Huizenga, D.E., Szostak, J.W., 1995. Biochemistry 34, 656–665.
Kim, J., Kim, I.Y., Choi, M.S., Wu, Q., 2009. Chem. Commun., 4747–4749.
Kumara Swamy, B.E., Jill Venton, B., 2007. Anal. Chem. 79, 744–750.
Latini, S., Pedata, F., 2001. J. Neurochem. 79, 463–484.
Lee, M., Walt, D.R., 2000. Anal. Biochem. 282, 142–146.
Liu, J.W., Lu, Y., 2004. Anal. Chem. 76, 1627–1632.
Liu, J.W., Lu, Y., 2006. Angew. Chem. Int. Ed. 45, 90–94.
Lode, P., Rosenberg, J., Pettersson, K., Takalo, H., 2003. Anal. Chem. 75, 3193–3201.
McMillan, M.R., Burnstock, G., Haworth, S.G., 1999. Br. J. Pharmacol. 128, 543–548.
Nutiu, R., Li, Y.F., 2003. J. Am. Chem. Soc. 125, 4771–4778.
Nutiu, R., Li, Y.F., 2004. Chem. Eur. J. 10, 1868–1876.
Nutiu, R., Li, Y.F., 2005a. Angew. Chem. Int. Ed. 44, 1061–1065.
Nutiu, R., Li, Y.F., 2005b. Methods 37, 16–25.
Pavlov, V., Xiao, Y., Shlyahovsky, B., Willner, I., 2004. J. Am. Chem. Soc. 126, 11768–11769.
Phillis, J.W., 1989. Cerebrovasc. Brain Metab. Rev. 1, 26–54.
Qin, P.Z., Niu, C.G., Ruan, M., Zeng, G.M., Wang, X.Y., 2010. Analyst 135, 2144–2149.
Qin, P.Z., Niu, C.G., Zeng, G.M., Ruan, M., Tang, L., Gong, J.L., 2009. Talanta, 10752–10756.
Rupcich, N., Nutiu, R., Li, Y., Brennan, J.D., 2005. Anal. Chem. 77, 4300–4307.
Stadtherr, K., Wolf, H., Lindner, P., 2005. Anal. Chem. 77, 3437–3443.
Stojanovic, M.N., Landry, D.W., 2002. J. Am. Chem. Soc. 124, 9678–9679.
Tuerk, C., Gold, L., 1990. Science 249, 505–510.
Tuleuova, N., Jones, C.N., Yan, J., Ramanculov, E., Yokobayashi, Y., Revzin, A., 2010. Anal. Chem. 82, 1851–1857.
Wang, X.Y., Dong, P., He, P.G., Fang, Y.Z., 2010. Anal. Chim. Acta 658, 128–132.
Wu, Z.S., Guo, M.M., Zhang, S.B., Chen, C.R., Jiang, J.H., Shen, G.L., Yu, R.Q., 2007. Anal. Chem. 79, 2933–2939.
Xu, W.C., Lu, Y., 2010. Anal. Chem. 82, 574–578.
Zayats, M., Huang, Y., Gill, R., Ma, C., Willner, I., 2006. J. Am. Chem. Soc. 128, 13666–13667.
Zhang, S.S., Xia, J.P., Li, X.M., 2008. Anal. Chem. 80, 8382–8388.