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An aptazyme-based electrochemical biosensor for the detection of adenosine

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ABSTRACT

In this work, an aptazyme-based electrochemical biosensor for the detection of adenosine is reported. Aptazyme activity was modulated by appending an "inhibitor" oligonucleotide strand containing a 32-base adenosine aptamer to the 8-17 DNAzyme. In the absence of adenosine, the DNAzyme could not form appropriate catalytic structure due to the binding with the inhibitor strand. Upon adenosine binding to the aptamer, the inhibitor strand was dissociated from the DNAzyme sequence. This allowed the DNAzyme to open and bind with the hairpin substrate, and DNAzyme activity was thereby induced, cleaving the substrate at its ribonucleotide site in the presence of Pb^{2+}. Cleavage of the substrate yields two single-stranded products, one of which was ferrocene-tagged and acted as the signal probe. The thiolated probe modified on the gold electrode could capture the signal probe. As a result, the ferrocene (Fc) moiety was brought in close proximity to the electrode surface and the Faradaic current was observed. This electrochemical biosensor was proved to have a wide dynamic range from 5 nM to 2000 nM with a detection limit of 5 nM. The fabricated sensor is shown to exhibit high sensitivity and desirable selectivity, which might be promising for the rational construction of aptazyme-based biosensors and the determination of adenosine in clinical examination.

1. Introduction

DNAzymes and DNA aptamers are functional nucleic acids obtained through in vitro selection [1] or SELEX technique [2], which have recently received considerable attention in biosensing research [3–5]. Numerous DNAzymes have been isolated to display high specificity toward a variety of metal ions [6–11] and synthesized for the catalysis of diverse chemical reactions, such as the cleavage and ligation of DNA or RNA [12–14], and stimulating the activity of peroxidase [15]. Also, a large number of DNA aptamers have been developed for the recognition of targets ranging from small molecules (cocaine or adenosine) to proteins (thromin or PDGF) and complex molecular assemblies (cells) [16–18]. Various electrochemical [19–21] or optical [22–24] aptasensors have been developed.

More recently, the principle of allosteric ribozymes [25,26] has been applied to the design of allosteric DNAzymes or aptazymes, in which a DNA aptamer is connected to a DNAzyme in such a way that the DNAzyme can only be activated by the ligand that binds to the aptamer [27–30]. Aptazymes are very useful as biosensing tools, because the use of DNAzymes with high rate enhancements, high specificity and the ability to perform multiple substrate turnovers [31] allows for the engineering of effective aptazymes for practical applications where rapid enzymatic action is essential. To take advantage of the high sensitivity of DNAzymes for detection of a broad range of analytes, aptamers have been coupled with DNAzymes, the molecular-recognition event between an aptamer and its specific ligand can be translated into the activity of a DNAzyme for signal generation and amplification. For example, a general approach [32] was described for generating versions of ribozymes and DNAzymes whose catalytic activity could be controlled by the binding of small molecule effectors. Another simple yet general DNA aptazyme rational design strategy [33] has been described for the design of structure-switching signaling aptamers that change structural states from a DNA–DNA duplex to a DNA-target complex upon target binding, which triggers the catalytic action of the aptazyme.

To date, many aptazyme sensors for small molecules have been successfully developed, among which the analysis based upon optical methods has been extensively studied. For example, the action of an RNA-cleaving allosteric DNAzyme in response to ligand binding was coupled to a rolling circle amplification process to generate long single-stranded DNA molecules for colorimetric sensing [34]. The horseradish peroxidase (HRP)-mimicking DNAzyme was used for amplified colorimetric readout of the formation of the aptamer–analyte complex [35–37]. Aptazyme-directed assembly of gold nanoparticles was also reported for colorimetric detection of adenosine [38].

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Some of these optical methods, however, suffer from possible drawbacks including high background absorbance that prohibits the detection of the analytes at low concentrations [36] or relatively complex handling procedures. Electrochemical methods, in contrast, are superior because of their significant advantages such as simplicity, high sensitivity and stability. Electrochemical sensors that utilize oligonucleotide probes covalently linked with a redox active reporter have become an essential technique in the biosensing research [39–41]. Among them, ferrocene-based redox active species have received much attention due to their special structural and unique electronic properties [42], making it an attractive reporter moiety in electrochemical sensors.

Here, we developed a simple, sensitive and signal-on electrochemical aptazyme biosensor based on target-induced conformational change for the detection of adenosine. Based on the principle of allosteric DNAzyme technology, we simultaneously exploited DNA aptamer and DNAzymes for the biosensing events. A specially engineered DNA molecule contained two distinct functional domains within the same molecule: a ligand-binding motif (an adenosine aptamer) that is responsive to a specific ligand (adenosine) and a catalytic element (an 8-17 DNAzyme) that can induce hydrolytic reactions under appropriate conditions. This DNAzyme–aptamer hybrid is designed in such a way that the function of its catalytic domain is dependant on the binding state of the aptamer element. In the absence of the cognate ligand for the aptamer, the enzymatic activity of the catalytic element cannot be manifested due to inappropriate structural binding. The presence of the ligand causes adaptive folding of the aptamer, which, in turn, frees the entire sequence of the DNAzyme and leads to the correct structural formation of the catalytic domain. Consequently, the catalytic domain is activated. After the cleavage of the ribonucleotide, one of the short fragments could be captured by the complementary thiolated probe on the electrode, using ferrocene as the signaling moiety. The redox current of ferrocene was employed to quantify the amount of analyte. Because DNAzyme could cleave multiple copies of the hairpin substrate [43], the adenosine could be detected specifically with a low detection limit and a wide dynamic range.

2. Experimental

2.1. Materials

The DNA oligonucleotides used in this paper (sequences shown in Table 1) were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). N-Hydroxysulfosuccinimide (sulfo-NHS), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), and ferrocenecarboxylic acid were purchased from Sigma–Aldrich Chemical Co. All other reagents were of analytical-reagent grade. Diethypyrocarbonate (DEPC) treated DNase/RNase-free ultrapure water and other laboratory utensils were used for all buffers and stock solutions of oligonucleotides throughout this work.

2.2. Instrumentation

The electrochemical experiments were performed with a model CHI 760B electrochemical workstation (Shanghai Chenhua Equipments, China). The three electrode system consists of a KCl saturated calomel electrode (SCE) as the reference electrode, a platinum electrode as the counter electrode, and a gold electrode (2 mm diameter) as the working electrode.

2.3. Procedures

2.3.1. Preparation of ferrocene-labeled oligonucleotides

The ferrocene label was conjugated to the 3′ NH_2-moiety substrates by the succinimide coupling (EDC-NHS) method [44]. Briefly, 100 μL of 10 μM oligonucleotide was mixed with 100 μL of 10 mM PBS (pH 7.4) containing 10 mM ferrocenecarboxylic acid, 1 mM EDC and 5 mM sulfo-NHS and then incubated at 37 °C for 2 h. The conjugate was dialyzed against 10 mM PBS (500 mL) for 24 h to remove excessive ferrocenecarboxylic acid. Finally, the mixture was stored at −20 °C in the refrigerator.

2.3.2. Gold electrode treatment and oligonucleotide immobilization

The gold electrodes (99.99% polycrystalline gold, ~2 mm diameter, i.e., 3.14 mm^2 area) were polished sequentially with 0.3 μm and 0.05 μm alumina power, ultrasonicated in ultrapure water, ethanol, and ultrapure water for 5 min each, and cleaned in piranha solution (30% H_2O_2 and 70% H_2SO_4 in volume) for 40 min. Then the electrodes were rinsed thoroughly in ultrapure water and dried in nitrogen. Afterwards, 15 μL of 1 μM thiolated oligonucleotide solution was pipetted onto the surface of gold electrode and kept 12 h at room temperature.

2.3.3. DNA hybridization

In a typical experiment, a 80 μL of reaction mixture containing buffered Tris (50 mM Tris-acetate, 0.3 M NaCl, pH 8.2) and 10 μL 1 μM aptazyme in the absence or in the presence of a given concentration of adenosine was incubated at 37 °C. After 1 h, cleavage reactions were initiated by the addition of 10 μL of 5 μM ferrocene-tagged substrate (5-fold molar excess) and 10 μL of 100 μM Pb^{2+} to a total volume of 100 μL and the incubation of the system at 37 °C water bath for 1 h. Then the thiolated capture probe functionalized electrode was incubated in the reaction mixture at 37 °C for 1 h.

2.3.4. Electrochemical detection

The electrochemical sensors were measured via cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (ESI). CV was performed in 10 mM PBS (pH 7.4) containing 0.1 M $NaClO_4$ and 0.3 M NaCl within the potential range from −0.1 V to 0.5 V under a scan rate of 0.01 V s^{-1}. DPV was recorded in 10 mM PBS (pH 7.4) containing 0.1 M $NaClO_4$ and 0.3 M NaCl within the potential range from −0.1 V to 0.5 V under a modulation amplitude of 50 mV. ESI was performed in 10 mM $K_3Fe(CN)_6$ solution containing 0.2 M KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude. All the experiments were performed at room temperature. The reported DPV curves were background-subtracted using ORIGIN 7.0 (Microcal Software, Northampton, MA) through extrapolation to the baseline in the regions far from the peaks.

Table 1
Sequence of synthesized oligonucleotides used in this work.[a].

Oligonucleotide	Sequence (5′→3′)
Allosteric DNAzyme	**TGGAAGGAGGCGTTATGAGGGGGTCCA**TTCGTCTGTTTGATTCCGAGCCGGACGAATGGACCCAG
Hairpin substrate	NH_2-CGTCTGGGTCCAT*ra*GATCAAACAGACG
Capture probe	ATGGACCCAGACG-$(CH_2)_6$-SH

[a] Adenosine aptamer sequences (bold), inhibitory segment (underlined), *ra* represents an adenine ribonucleotide.

3. Results and discussion

3.1. Scheme of the electrochemical sensor

The 8-17 DNAzyme contains three domains: one catalytic core and two target recognition arms. The typical DNAzyme system consists of a catalytic DNA strand and a DNA substrate strand containing an adenine ribonucleotide (ra). In the presence of Pb^{2+}, the catalytic strand carries out catalytic reactions to hydrolytic cleavage of the substrate strand at the scissile ra. Then the substrate is broken into two fragments and dissociated from the catalytic strand. According to previous report [45], the 8-17 DNAzyme is much more active in the presence of Pb^{2+} than any other metal ions. As shown in Table 1, we linked an inhibitor DNA strand which contains a 32-base adenosine aptamer (bold) and a 5-base inhibitory segment (underlined) to the 5′ end of the 33-base 8-17 DNAzyme sequence. The 5-base inhibitory segment could bind to part of the catalytic core of the 8-17 DNAzyme, while the aptamer could bind to one recognition arm of the DNAzyme, which assembled the whole allosteric DNAzyme into the hairpin configuration and prevented the DNAzyme from binding to and cleaving its hairpin substrate. Thus, the engineered DNAzyme was inactive.

As shown in Scheme 1, when the adenosine is added to the solution, the aptamer (the light gray parts in the aptazyme sequence) will bind to it and then gradually pulls the inhibitory DNA strand from the part of DNAzyme domain through strand displacement [46], which freed the DNAzyme and thus made it become active. The sequences of the two binding arms were complementary to the hairpin substrate, resulting in the hairpin opening and the hybridization of the two binding arms and the substrate. In the presence of Pb^{2+}, the hydrolytic cleavage of the ra site in the hairpin substrate took place. When the substrate was cleaved into two fragments, the short fragments would dissociate from the enzyme, one of which was ferrocene-labeled and could be captured by the complementary thiolated probes modified on the electrode. As a result, the ferrocene moiety was brought close to the electrode surface and a strong Faradaic current was obtained. After the cleavage reaction, the sensing reaction was first to dissociative, where the fragmented products left the aptazyme and thus regenerated the active site for another substrate. Because of the lower affinity of the cleaved substrate at a temperature of 37 °C, the DNAzyme could bind to yet another substrate so that it could be recycled for hydrolysis of multiple substrates in a manner similar to that of enzymes with their substrate target. The multiple turnover reactions gave rise to significantly increased signal probes and thus realized the signal enhancement.

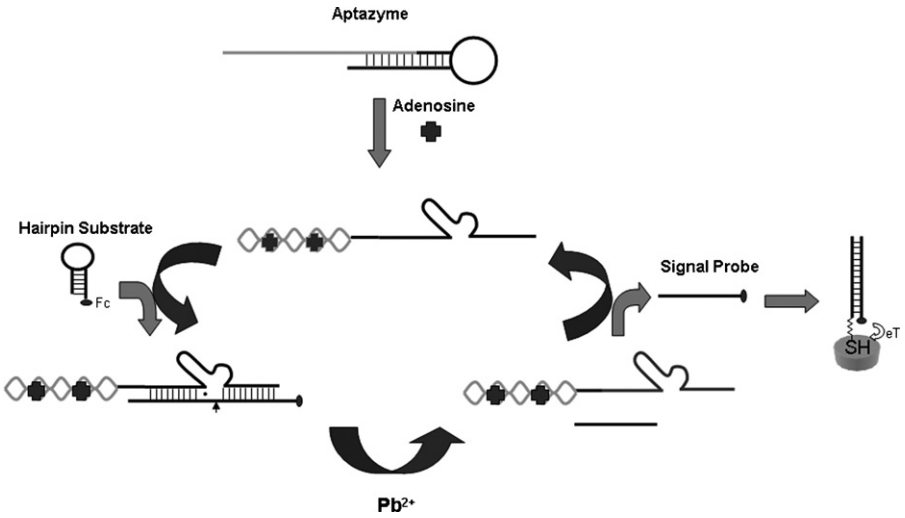
The hairpin substrate adopted a stem-loop structure in the buffer solution. When the modified electrode was immersed into the solution, the capture probe could not stably hybridize with one-half of the loop sequence of the hairpin substrate at a temperature of 37 °C, but instead would capture the signal probe to form a more stable duplex. Although the capture probe could not open the hairpin structure completely, it might slightly destabilize the stem [47], which might contribute to one source of background signal.

Typical voltammetric characteristics of the sensor, in response to 2000 nM adenosine, are shown in Fig. 1. Fig. 1(A) shows that in the presence of adenosine, a couple of strong redox peaks appeared at 0.162 V and 0.273 V (versus SCE) in CVs, characteristic of the electrochemistry of Fc at the electrode. In the absence of adenosine, the couple of redox peak almost disappeared. Fig. 1(B) shows a remarkable reduction peak at ~0.230 V (versus SCE) in the DPV curve in the presence of adenosine, about 10 times more than the blank background. Both observations implied that the sensor was sensitively responsive to adenosine.

3.2. Electrochemical characterization of the sensor

Impedance spectroscopy is a common and effective method for probing the features of surface-modified electrodes. Thus, electrochemical impedance spectroscopy (EIS) was carried out to study the gold electrodes modified by different procedures. The impedance plot for bare gold electrode (Fig. 2, curve a) exhibits an almost straight line, as characteristics of an electrochemical diffusional limiting process. Faraday impedance increased significantly because of the thiolated DNA capture probe modification (Fig. 2, curve b). Electron transfer became more difficult because of the capture probe modification resulting in negative charge enhancement on the gold electrode surface. However, hybridization with Fc-tagged signal probe induced sharply decreased electrochemical impedance (Fig. 2, curve c) in the presence of 1000 nM adenosine. The above results gave the immediate evidence for surface confinement of the ferrocene labels that exhibited facilitated electron transfer kinetics when the signal probe hybridized with the capture probe.

To study the controlled factor of the electrochemical process on the electrode surface, the effect of scan rates on the peak current by cyclic voltammetry was investigated (Fig. 3). At scan rates rang-



Scheme 1. Schematic representation of the biosensor and the principle of adenosine detection.

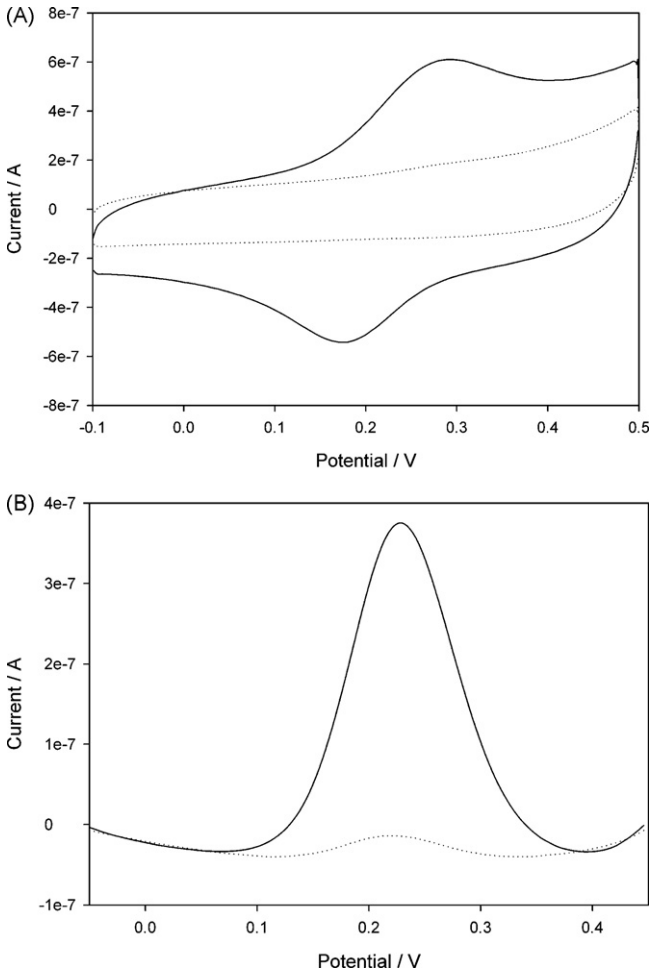


Fig. 1. Typical voltammetric characteristics of the sensor: (A) cyclic voltammograms recorded in 10 mM PBS (pH 7.4) containing 0.1 M $NaClO_4$ before (dotted line) and after (solid line) the addition of 2000 nM adenosine. (B) Differential pulse voltammetry responses of the biosensor recorded in 10 mM PBS (pH 7.4) containing 0.1 M $NaClO_4$ before (dotted line) and after (solid line) the addition of 2000 nM adenosine. Reaction conditions: 50 mM Tris-acetate (pH 8.2, 0.3 M NaCl) incubation at 37 °C for 2 h. DPV: pulse amplitude, 50 mV; pulse period, 0.2 s.

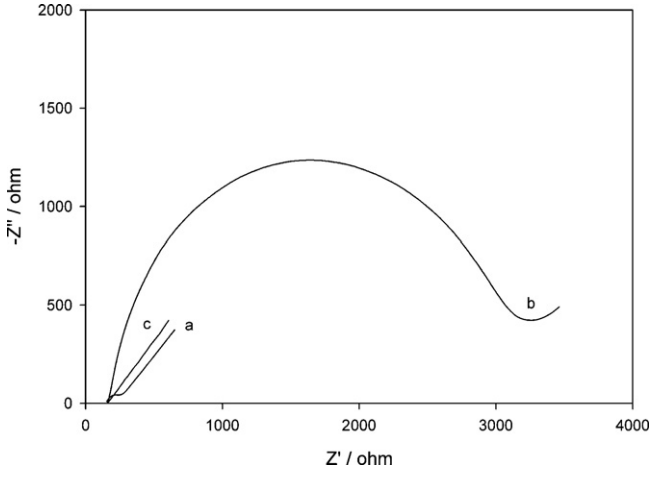


Fig. 2. Nyquist plots in PBS buffer (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ at 240 mV for (a) a bare gold electrode, (b) the thiolated DNA modified gold electrode, (c) the modified electrode after hybridization with the Fc labeled signal probe in the presence of 1000 nM adenosine. Frequency range, 0.1–100 kHz; ac amplitude, 5 mV.

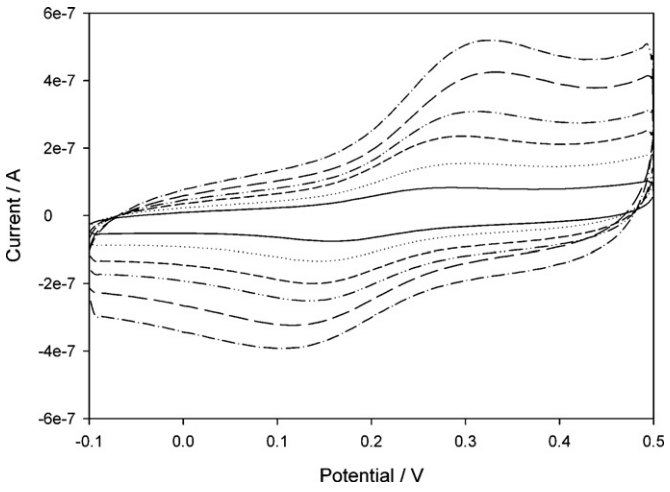


Fig. 3. The effect of scan rates on the CV peak currents: cyclic voltammograms obtained after incubation with 1000 nM adenosine at different scan rates: 20 mV s^{-1}, 40 mV s^{-1}, 60 mV s^{-1}, 80 mV s^{-1}, 100 mV s^{-1}, and 120 mV s^{-1}, in 10 mM PBS (pH 7.4) containing 0.1 M $NaClO_4$.

ing from 20 mV s^{-1} to 160 mV s^{-1}, the reduction peak currents of the electrochemical sensor in the presence of 1000 nM adenosine increased linearly with the scan rate, indicating that the process is controlled by the surface reaction. The resulting linear regression curve shows the linear relationship between the peak currents and scan rates, with the equation $y = 11.414 + 0.9287x$ ($R = 0.998$), where y and x stand for the peak current (nA) and scan rate (mV s^{-1}) respectively. These observations indicated that the sensor demonstrated a typical surface-bound electrochemical process and confirmed that Fc labels were confined to the gold electrode surface.

3.3. Optimization of Na^+ concentration, the incubation time and the capturing time

The hybridization efficiencies were strongly influenced by the ion concentration. So the effect of Na^+ concentration was investigated. Fig. 4(A) shows the effect of Na^+ concentration from 0 M to 0.5 M on the electrochemical readout of the sensor for 100 nM adenosine detection. The signal increased significantly as the Na^+ concentration increased up to 0.3 M, reflecting improved hybridization performance. However, the signal was decreased after the Na^+ concentration was up to 0.3 M, which indicated that a high Na^+ concentration is disadvantageous for the dissociation of the two fragmented oligos after DNAzyme cleavage. Therefore, 0.3 M was selected as the optimum salt ion concentration throughout the experiments.

The incubation time of the cleavage reaction also influence the performance of the biosensor. In order to optimize the incubation time, the cleavage reaction was allowed to proceed for different periods of time. As shown in Fig. 4(B), the peak current rose gradually with the increasement of the incubation time and then tended to be constant after about 1 h. If the reaction duration were elongated to 1.5 h or longer, there would be no obvious signal enhancement. To obtain the optimum hybridization efficiencies and the maximum cleavage of the substrate, 1 h was selected for the cleavage reaction.

Considering that the capture probe might destabilize the stem of the hairpin structure and then bind with one-half of the substrate sequence to generate background signal, the capturing time was investigated. Fig. 4(C) shows the effect of capturing time from 0 h to 2 h on the electrochemical readout of the biosensor for 100 nM adenosine detection. The ratio of the signal to the blank

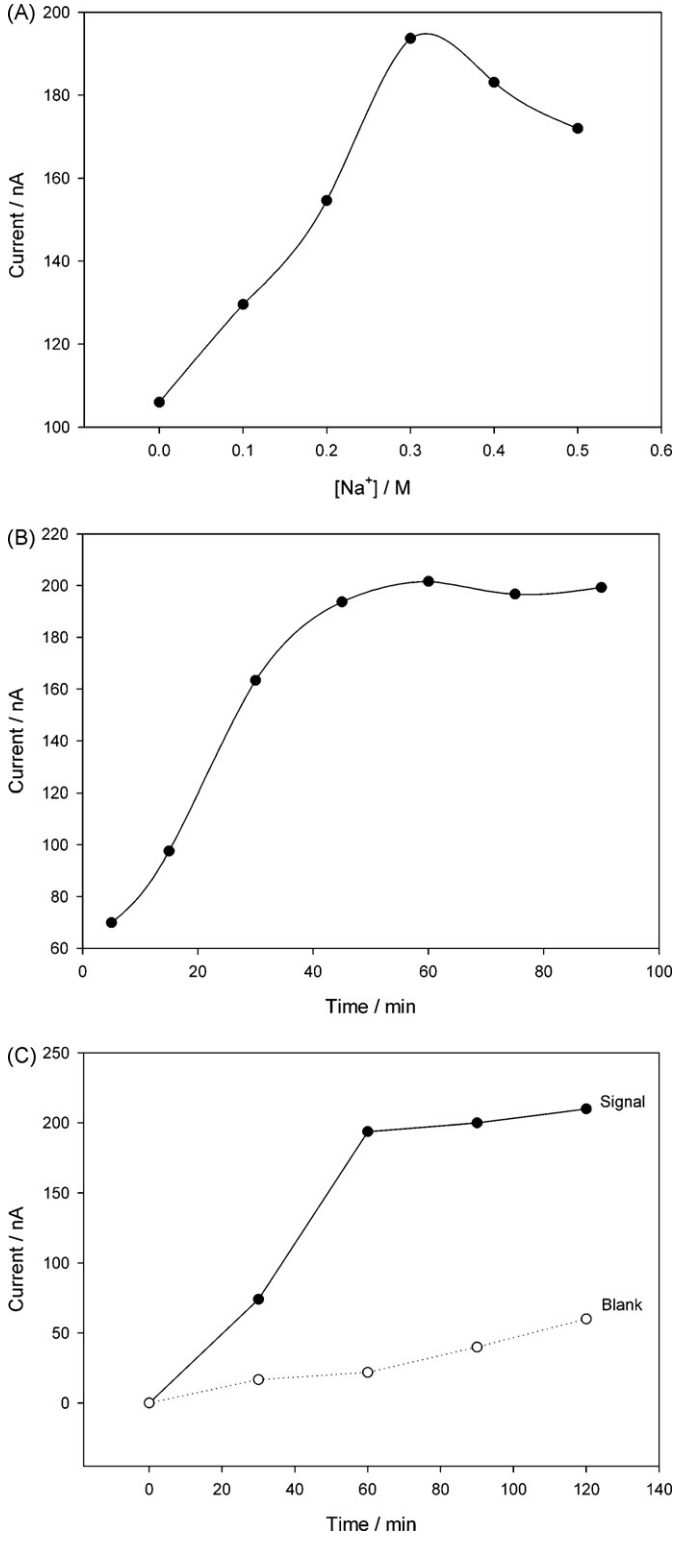


Fig. 4. Optimization of experimental conditions: (A) effect of the Na^+ concentration (from 0 M to 0.5 M) for 100 nM adenosine detection. (B) Effect of incubation time of the cleavage reaction for 100 nM adenosine detection. (C) Effect of the capturing time from 0 h to 2 h for 100 nM adenosine (solid line) detection and blank (dashed line).

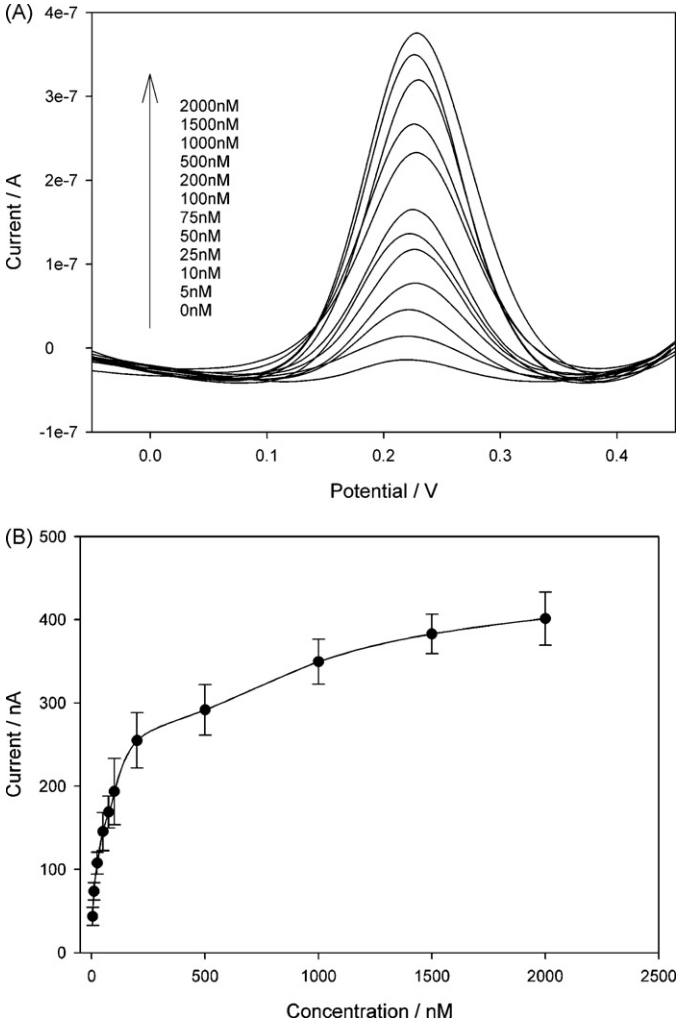


Fig. 5. Analytical performance of the electrochemical DNA biosensor: (A) differential pulse voltammetry response curves at various adenosine concentrations, from 5 nM to 2000 nM. (B) The plots of peak currents versus the adenosine concentrations.

background increased significantly as the capturing time increased up to 1 h, showing improved hybridization performance. A slight signal enhancement could be observed after 1 h. However, the background continued to increase with the capturing time. The longer the capturing time, the more hairpin substrates might be disturbed and the stronger background signal was generated. In order to obtain higher signal and lower background, 1 h was chosen as the optimum capturing time.

3.4. Target detection of the electrochemical sensor

DPV is a pulse technique that allows much higher sensitivity than conventional sweep techniques when detecting very low concentration of a redox probe. This is achieved by applying a small voltage pulse superimposed on the linear voltage sweep and sampling the differential current at a short time after the pulse. As shown in Fig. 5(A), the DPV method was used for the target analyte with different concentrations. Fig. 5(B) shows that the peak current increased along with the increasing target adenosine concentration ranging from 5 nM to 2000 nM. The peak current of the sensor exhibited a linear correlation to the logarithm of the target concentration. The linear equation was $y = 141.7146 \log x - 78.7772$ (y and x stand for the peak current (nA) and the target concentration (nM) respectively) with a correlation coefficient of 0.994, and the detection limit was 5 nM (S/N = 3). The wide linear range

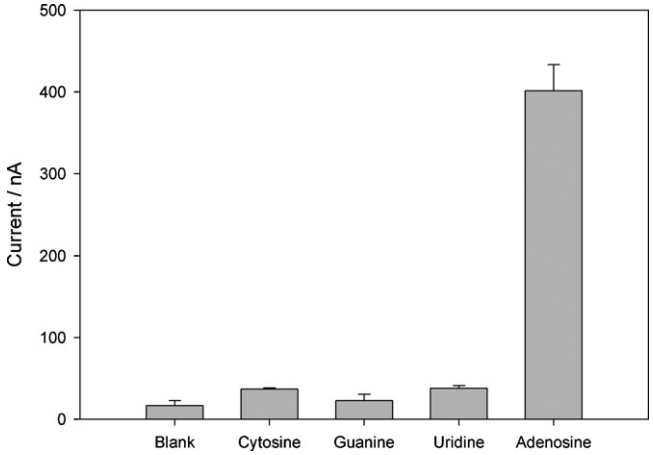


Fig. 6. Bar chart of the differential pulse votalmmetry responses of the blank and 2000 nM adenosine, 1 mM cytosine, 1 mM guanine, 1 mM uridine.

and high sensitivity could be attributed to multiple cleavages of copies of hairpin substrate by the DNAzyme, which generated significantly increased amount of signal probes. Compared with other optical DNAzyme sensors for the detection of adenosine [32,34], the proposed method was superior in sensitivity and wide linear range.

To investigate the specificity of the aptazyme, two types of control aptazymes were tested. The sequences of the two oligonucleotides employed were: 5′-TGGAAGGAGGCGTTATGAGGGGGTCCATTCGTCTGTTTGATTAAAAAACGGACGAATGGACCCAG-3′ and 5′-TGGATAATAACGTTATGAGGGGGTCCATTCGTCTGTTTGATTCCGAGCCGGACGAATGGACCCAG-3′. Key mutations (underlined) in the catalytic core of the 8-17 DNAzyme were applied to inactivate the DNAzyme in the first control sequence. Likewise, key mutations (underlined) in the aptamer sequence were applied to inactivate the aptamer in the second control sequence. The average peak currents of the two control aptazymes after three repetitive detections of 100 nM adenosine were 16.73 nA and 21.86 nA, respectively, which might be attributed to the slight nonspecific absorption of ferrocene and other background signals. This result indicated that the proposed sensor provided a simple yet general approach for rationally designing aptazyme-based biosensors.

3.5. Selectivity of the electrochemical sensor

Four types of nucleotides including adenosine (A), cytosine (C), guanine (G) and uridine (U) were chosen to study the selectivity. Fig. 6 shows the caparison of the peak currents of these four nucleotides and the background, with a concentration of 1 mM for C, G, U and 2000 nM for A. Among the four types of nucleotides, the peak current of A was 401.4 nA, much higher than those of other nucleotides whose peak currents were 36.85 nA (C), 22.81 nA (G), and 37.91 nA (U) respectively, with the same order of magnitude to the current of the blank background (21.63 nA). A significant current difference demonstrated that the sensor was able to discriminate adenosine as the target from its analogues with sufficient specificity.

3.6. Analysis of serum sample

To evaluate the sensor's ability to detect small molecule in complex samples, we investigated the stability of the sensing system in the presence of human blood serum. Human blood samples were obtained from healthy volunteers. The serum 10-fold diluted with the buffer (50 mM Tris-acetate, 0.3 M NaCl, pH 8.2) was used to prepare the adenosine solution and other experimental conditions were the same as described in Section 2. The results in Table 2 indicated that the developed sensing system could be still work in the complex serum matrix and it might be promising for the determination of adenosine in clinical examination.

Table 2
Recovery of the proposed biosensor ($n = 5$).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD[a] (%)
1	10	9.2	91.2–94.3	3.1
2	100	106	95.2–107.1	7.5
3	1000	1080	98.7–108.2	6.2

[a] Relative standard derivation.

4. Conclusion

In the present work, a facile yet effective aptazyme method for the electrochemical detection of adenosine based on target-induced conformational change has been demonstrated. The strategy depends on target-induced displacement of aptamer to generate active DNAzyme structure upon the recognition of adenosine. The activation of the DNAzyme, in turn, activates multiple cleavages of copies of hairpin substrate and lots of signal probes were generated which could hybridize with the capture probe immobilized on the gold electrode. Using ferrocene as the signaling moiety, as low as 5 nM adenosine can be detected and discriminated from its analogues, indicating high sensitivity and high selectivity. The proposed method takes advantages of both the convenience of homogeneous reaction and the sensitivity of electrochemical detection, which is expected to hold potential for the construction of aptazyme-based biosensors in the detection of various proteins and small molecules.

Acknowledgements

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References

[1] R.R. Breaker, G.F. Joyce, Chem. Biol. 1 (1994) 223–229.
[2] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818–822.
[3] J. Liu, Z. Cao, Y. Lu, Chem. Rev. 109 (2009) 1948–1998.
[4] N.K. Navani, Y. Li, Curr. Opin. Chem. Biol. 10 (2006) 272–281.
[5] J.F. Lee, G.M. Stovall, A.D. Ellington, Curr. Opin. Chem. Biol. 10 (2006) 282–289.
[6] J. Li, Y. Lu, J. Am. Chem. Soc. 122 (2000) 10466–10467.
[7] B. Cuenoud, J.W. Szostak, Nature 375 (1995) 611–614.
[8] S.W. Santoro, G.F. Joyce, K. Sakthivel, S. Gramatikova, C.F. Barbas, J Am. Chem. Soc. 122 (2000) 2433–2439.
[9] S.H.J. Mei, Z. Liu, J.D. Brennan, Y. Li, J. Am. Chem. Soc. 125 (2003) 412–420.
[10] Y. Wang, S.K. Silverman, J. Am. Chem. Soc. 125 (2003) 6880–6881.
[11] J. Liu, A.K. Brown, X. Meng, D.M. Cropek, J.D. Istok, D.B. Watson, Y. Lu, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 2056–2061.
[12] R.R. Breaker, Nature 432 (2004) 838–845.
[13] J.C. Achenbach, W. Chiuman, R.P. Cruz, Y. Li, Curr. Pharm. Biotechnol. 5 (2004) 321–336.
[14] S.K. Silverman, Nucleic Acids Res. 33 (2005) 6151–6163.
[15] P. Travascio, A.J. Bennet, D.Y. Wang, D. Sen, Chem. Biol. 6 (1999) 779–787.
[16] M. Famulok, G. Mayer, M. Blind, Acc. Chem. Res. 33 (2000) 591–599.
[17] D.S. Wilson, J.W. Szostak, Annu. Rev. Biochem. 68 (1999) 611–647.
[18] D. Shangguan, Y. Li, Z. Tang, Z.C. Cao, H.W. Chen, P. Mallikaratchy, K. Sefah, C.J. Yang, W. Tan, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 11838–11843.
[19] C. Deng, J. Chen, L. Nie, Z. Nie, S. Yao, Anal. Chem. 81 (2009) 9972–9978.
[20] X. Zuo, Y. Xiao, K.W. Plaxco, J. Am. Chem. Soc. 131 (2009) 6944–6945.
[21] Y.L. Zhang, Y. Huang, J.H. Jiang, G.L. Shen, R.Q. Yu, J. Am. Chem. Soc. 129 (2007) 15448–15449.
[22] Z. Tang, P. Mallikaratchy, R. Yang, Y. Kim, Z. Zhu, H. Wang, W. Tan, J. Am. Chem. Soc. 130 (2008) 11268–11269.
[23] O. Neumann, D. Zhang, F. Tam, S. Lal, P. Wittung-Stafshede, N.J. Halas, Anal. Chem. 81 (2009) 10002–10006.
[24] Z.G. Wang, O.I. Wilner, I. Willner, Nano Lett. 9 (2009) 4098–4102.
[25] R.R. Breaker, Curr. Opin. Biotechnol. 13 (2002) 31–39.

[26] G.A. Soukup, R.R. Breaker, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 3584–3589.
[27] W. Chiuman, Y. Li, PLoS ONE 2 (2007) e1224.
[28] Y. Shen, W. Chiuman, J.D. Brennan, Y. Li, ChemBioChem 7 (2006) 1343–1348.
[29] S.H. Mei, Z. Liu, J.D. Brennan, Y. Li, J. Am. Chem. Soc. 125 (2003) 412–420.
[30] M. Levy, A.D. Ellington, Chem. Biol. 9 (2002) 417–426.
[31] K. Schlosser, Y. Li, Chem. Biol. 16 (2009) 311–322.
[32] D.Y. Wang, B.Y. Lai, D. Sen, J. Mol. Biol. 318 (2002) 33–43.
[33] J.C. Achenbach, R. Nutiu, Y. Li, Anal. Chim. Acta 534 (2005) 41–51.
[34] M.M. Ali, Y. Li, Angew. Chem. Int. Ed. 48 (2009) 1–5.
[35] C. Teller, S. Shimron, I. Willner, Anal. Chem. 81 (2009) 9114–9119.
[36] J. Elbaz, M. Moshe, B. Shlyahovsky, I. Willner, Chem. Eur. J. 15 (2009) 3411–3418.
[37] D. Li, B. Shlyahovsky, J. Elbaz, I. Willner, J. Am. Chem. Soc. 129 (2007) 5804–5805.
[38] J. Liu, Y. Lu, Anal. Chem. 76 (2004) 1627–1632.
[39] X. Zuo, S. Song, J. Zhang, D. Pan, L. Wang, C. Fan, J. Am. Chem. Soc. 129 (2007) 1042–1043.
[40] E.E. Ferapontova, E.M. Olsen, K.V. Gothelf, J. Am. Chem. Soc. 130 (2008) 4256–4258.
[41] C. Fan, K.W. Plaxco, A.J. Heeger, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 9134–9137.
[42] F.O. Laforge, T. Kakiuchi, F. Shigematsu, M.V. Mirkin, J. Am. Chem. Soc. 126 (2004) 15380–15381.
[43] Y. Tian, C. Mao, Talanta 67 (2005) 532–537.
[44] R.P. Fahlman, D.J. Sen, J. Am. Chem. Soc. 124 (2002) 4610–4616.
[45] A.K. Brown, J. Li, C.M.-B. Pavot, Y. Lu, Biochemistry 42 (2003) 7152–7161.
[46] M. Zayats, Y. Huang, R. Gill, C. Ma, I. Willner, J. Am. Chem. Soc. 128 (2006) 13666–13667.
[47] Z. Tang, K. Wang, W. Tan, J. Li, L. Liu, Q. Guo, X. Meng, C. Ma, S. Huang, Nucleic Acid Res. 31 (2003) e148.