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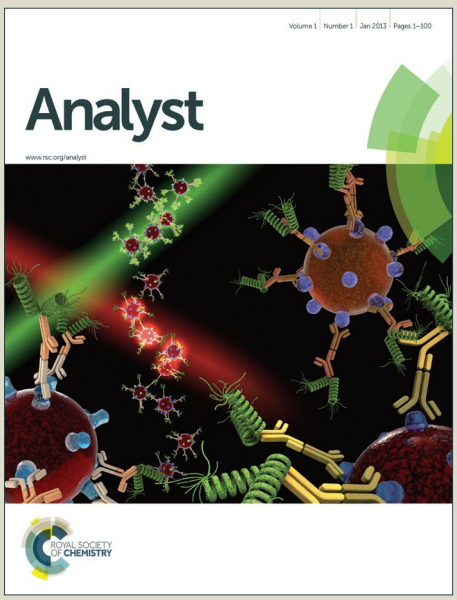

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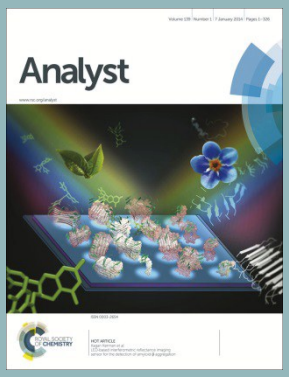

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ROYAL SOCIETY OF CHEMISTRY

Dear editors and referees:

Thank you very much for your careful review and constructive suggestions with regard to my paper (AN-ART-12-2015-002484). We have revised the manuscript according to your suggestions and comments and marked the revised word of the original paper with red styles. Those comments are helpful for us to revise and improve our paper. We have studied comments carefully and tried our best to revise and improve the manuscript according to the referees' good comments. We appreciate for Editors/Referees' warm work earnestly, and hope that the corrections will meet with approval. Please feel free to contact us with any questions and we are looking forward to your consideration. The main corrections in the paper and the responds to the reviewer's comments are as following:

Referee #1:

Thank you for your great comments and suggestions to my paper. We have made some corrections according to your advice:

1. The resolution of all Figures is poor, the authors should improve it.

Response: Thanks for your suggestion. The resolution of the photographs were modified to 600 dpi. At the same time, I've replaced all the pictures in this article. All pictures also were placed into the file "Figures".

2. Page 4, right, line 8, the authors said the LOD was lower than the methods which only used the conformational switching in ATP detection, they should give out the results of "the methods".

Response: Thanks for your suggestion. Zhang et al.[1] constructed a biosensor based on multi-functional Dual-Hairpin DNA structure. First, the target probe conjugated with the adjunct probe in the outer hairpin. In the presence of ATP, the adjunct probe divorced from the Dual-Hairpin structure. And based on this dual-hairpin platform, the detection limit of ATP is 30 nM. I have added the results of "the methods" in the article as following: The LOD obtained by the NEase-assisted target-aptamer complex recycling method was lower than that of the conformational switching approach whose detection limit of ATP was as low as 20 nM .

1 X. He, G. Wang, G. Xu, Y. Zhu, L. Chen, X. Zhang, Langmuir, 2013, 29, 14328-14334.

Referee #2:

Thank you for your great comments and suggestions to my paper. We have made some corrections according to your advice:

1. In the part of "Amplification performance of Nb.BbvCI", the authors claimed that "the

Nb.BbvI-assisted target recycling can amplify the signal". However, the DPV experiment was not sufficient to explain the target recycle and amplification process. The authors are suggested to provide more evidence to address this claim, for example: PAGE gel assay.

Response: Thanks for your suggestion. I have added the agarose gel electrophoresis analysis to further verify the amplification performance of Nb.BbvCI-catalyzed cleavage mechanism. The relevant content was presented as following: To further verify the amplification performance of Nb.BbvCI-catalyzed cleavage mechanism, agarose gel electrophoresis analysis was performed. As shown in Figure 2B, the band of capture DNA (Lane 1) was too weak to be observed. After capture DNA hybridized with target-aptamer complex, a faint band of capture DNA/hairpin DNA (Lane 2) appeared due to the formation of dsDNA structure.However, when incubating capture DNA/hairpin DNA hybrid duplex with Nb.BbvCI, the probe was cleaved to give a band of ~10 bp length and the target-aptamer complex, a brighter band (Lane 3) of ~35 bp closed to Lane 2 was ascribed to the released target-aptamer complex. These results confirmed that the capture DNA/hairpin DNA hybrid duplex was cleaved by Nb.BbvCI. So the released target-aptamer complex can still hybridize with capture DNA which realized signal amplification. The content of the experimental part were also added in the article as following: The capture DNA, capture DNA/hairpin DNA hybrid duplex and capture DNA/hairpin DNA hybrid duplex with Nb.BbvCI were prepared. The relevant reagents were replaced by their corresponding buffer solution. The 4 % agarose gels contained 0.5 μg of ethidium bromide/mL of gel volume and were prepared with 1×TAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.0). 10 μL of each samples and 2 μL of DNA marker were mixed with 1.5 μL of GelDye TM Super Buffer Mix, and loaded into the gels. The gels were run at 85 V for 50 min and visualized under UV light. The relevant picture were listed as following:

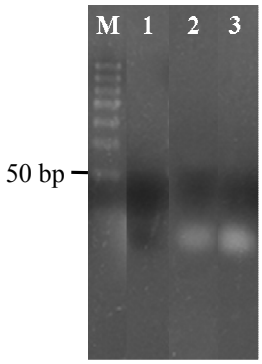

Fig. 1. Agarose gel electrophoresis images of capture DNA and hairpin DNA with Nb.BbvCI. Lane M,

DNA marker (50 bp); Lane 1, 100 μM capture DNA; Lane 2, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP; Lane 3, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP with 50 U Nb.BbvCI. The gels were run for 50 min.

2. The authors optimized the concentration of the capture DNA and calculation time of the assay in the part of "Optimization of experimental conditions". However, there were no evidence of why the authors choose to use "25 nM hairpin DNA and 5 U Nb.BbvCI". More data are needed to support the optimization assays.

Response: Thanks for your suggestion. Yang et al.[2] constructed a general colorimetric detection of thrombin. In this work, the nicking endonuclease (Nb.BbvCI) was used to cut the linker DNA. So the amount of Nb.BbvCI was along with the concentration of the linker DNA. In our work, the concentration of hairpin DNA is 25 nM, so the amount of Nb.BbvCI is enough to cut capture DNA. The concentration of capture DNA in this experiment is chosen as 100 nM. Huang et al.[3] constructed a fluorescence polarization aptasensors for adenosine and thrombin detection. In this work, the concentration of the DNA-2 probe and the AAA probe were 85 nM and 20 nM, respectively. The percentage of them was about 1:4. In my own work, the concentration of capture DNA is 100 nM, so 25 nM is adjusted as the concentration of hairpin DNA. At the same time, I have added the relevant references in the article.

2 J. Li, H.E. Fu, L.J. Wu, A.X. Zheng, G.N. Chen, H.H. Yang, Anal. Chem., 2012, 84, 5309-5315.

3 Y. Huang, X. Liu, L. Zhang, K. Hu, S. Zhao, B. Fang, Z.F. Chen, H. Liang, Biosens. Bioelectron., 2015, 63, 178-184.

Thank you very much for your consideration.

Best wishes.

Yours sincerely,

Shengfu Wang.

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College of Chemistry & Chemical Engineering

DEPARTMENT OF ANALYTICAL CHEMISTRY

Shengfu Wang
Professor and Dean of School of chemistry and chemical engineering, Hubei University

Dear Editor,

I am submitting the enclosed manuscript entitled ***"Nicking endonuclease-assisted recycling of target-aptamer complex for sensitive electrochemical detection of adenosine triphosphate"***, which we wish to be considered for publication in ***Analyst***.

The creative of this work were the target-induced conformation switching and Nb.BbvCI-assisted target-aptamer complex recycling. Until now, although Nb.BbvCI-assisted target recycling have been applied in the detection of ATP based fluorescence polarization assays. In this paper, we designed a target-induced conformation switching biosensor for sensitive detection of ATP based on Nb.BbvCI-assisted target recycling using electrochemical detection for the first time. Hairpin DNA was designed, which could not be cleaved by Nb.BbvCI. In the presence of ATP, the hairpin DNA structure unfolded, forming a G-quadruplex structure with ATP due to the specific recognition between hairpin DNA and ATP. Then the exposed part of target-aptamer complex hybridized with the 3'-terminus of capture DNA to form a specific nicking site for Nb.BbvCI. Through the enzymatic cleavage of MB labeled capture DNA by Nb.BbvCI, resulting in the release of many short DNA fragments labeled MB and ATP-aptamer complex. The complex structure hybridized with the left capture DNA, leading to the continuous cleavage of the capture DNA and release of many short DNA fragments carrying MB, generating signal decrease. We confirm that the manuscript has not been published previously using electrochemical method by any of the authors and also is not under consideration for publication in another journal at the time of submission.

Best Regards.

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College of Chemistry & Chemical Engineering

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Yours sincerely,

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Nicking endonuclease-assisted recycling of target-aptamer complex for sensitive electrochemical detection of adenosine triphosphate

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Tianxing Hu, Wei Wen*, Xiuhua Zhang, Shengfu Wang*

In this paper, an electrochemical biosensor was developed for the detection of adenosine triphosphate (ATP) based on target-induced conformation switching and nicking endonuclease (NEase)-assisted signal amplification. The electrochemical biosensor was constructed by the base pairing and target recognition. After capture DNA hybridized with the gold electrode, a significant current of methylene blue (MB) was obtained by differential pulse voltammetry. In the presence of ATP, the hairpin DNA formed a G-quadruplex structure due to the specific recognition between hairpin DNA and ATP. Then the exposed part of target-aptamer complex hybridized with the 3'-terminus of capture DNA to form a specific nicking site for Nb.BbvCI, which led to the NEase-assisted target-aptamer complex recycling. The released target-aptamer complex hybridized with the left capture DNA. Nb.BbvCI-assisted target-aptamer complex recycling caused the continuous cleavage of capture DNA with MB at its 5'-terminus, resulting in releasing a certain amount of DNA fragment labeled with MB. Then the current value decreased significantly. The reduced current showed a linear range from 10 nM to 1 μM with a limit of detection as low as 3.4 nM. Furthermore, the proposed strategy can be used for other similar substances detection.

Introduction

Adenosine-5'-triphosphate (ATP), is a multifunctional nucleoside that acts as 'molecular currency' of intracellular energy transfer in all living cells.[1] It is a vital substrate in living organisms which is involved in metabolic processes,[2,3] including cell viability and the cell injury.[4,5] For the key role of ATP, change of ATP concentration in cells was related to many diseases,[6,7] such as hypoxia, hypoglycemia, ischemia, parkinson's disease, and some malignant tumors.[8] Therefore, sensitive and reliable detection of ATP is of great importance in biochemical analysis and clinical diagnoses. Different methods have been used for ATP detection, such as ultra performance liquid chromatography,[9] fluorescence,[10] electrochemical impedance spectroscopy (EIS),[11] electrochemiluminescent (ECL).[12,13] However, electrochemical biosensors have attracted great interests, due to their combined advantages of sensitivity, miniaturization capability, minimal power requirement, and low cost, as well as high stability.[14,15]

Aptamers are single-stranded DNA or RNA oligonucleotides, which are synthesized by systematic evolution of ligands by exponential enrichment (SELEX) technology.[16,17] Aptamers can be specifically combined with a variety of analytes, including inorganic ions,[18] nucleosides,[19] peptides, proteins,[20] and even whole virsus or cells. The development of biosensors have attracted substantial research efforts, and a variety of techniques have been utilized to develop biosensors[21,22] for detection of various targets. Hairpin DNA can specific recognize the ATP, forming a stable G-quadruplex structure,[23] then the recycling of target-aptamer complex can utilize to amplify electrochemical signal. The ability of aptamers to bind to different targets with high affinity and specificity makes them promising molecular receptors for bioanalytical application.[24] Now, more sophisticated analytical devices for biosensors or aptamer assays have been reported on the basis of various signal generation principles.[25,26] Among them, the recycling of target-aptamer complex could utilize to amplify electrochemical signal. Herein, nicking endonuclease (NEase) used as biocatalytic amplifiers for sensitive electrochemical detection of ATP.

Nb.BbvCI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA (dsDNA).[27] This function has been used to develop different approaches amplified NEase-based detection platforms which involve NEase-catalyzed cleavage of DNA by target recognition.[28] Some techniques based on nicking endonuclease are applied for developing homogeneous fluorescence or quantum-dots based sensors.[29, 30] However, to the best of our knowledge, such NEase-assisted signal amplification systems have not

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† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

been used for ATP detection in electrochemical biosensor before.

Herein we develop a new strategy for the sensitive and selective detection of target molecules in homogeneous solution based on NEase-assisted signal amplification. Compared with traditional homogeneous biosensors, the detection sensitivity of the developed biosensors is significantly improved by taking advantage of the Nb.BbvCI and target-aptamer complex amplification strategy. Furthermore, the proposed method can be extended to other similar substances detection by changing the sequence of hairpin aptamer.

Experimental

Reagents and materials

Adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP) were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Nb.BbvCI and 10 × CutSmart® Buffer (1 × CutSmart® Buffer: 50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 100 μg.mL^{-1} BSA) were obtained from New England Biolabs. Inc. Human serum, DNA marker, 6-mercapto-1-hexanol (MCH) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (USA). All other reagents were analytical reagent grade. In our work, 10 mM phosphate buffered saline (PBS pH 7.4) was used as supporting electrolyte for testing the electrochemical signals, 20 mM Tris-HCl (pH 7.4) containing 50 mM NaCl, 10 mM Mg^{2+}, 50 mM K^+ was used to prepare aptamer and ATP solutions. All aqueous solutions were prepared with ultrapure water (18.2 MΩ.cm) produced by an Aquapro water purification system.

Oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd. The sequences of these oligonucleotides were as follows:

Capture DNA: 3'-HS-$(CH_2)_6$-TTTTTTTGGAGTCG-MB-5'

Hairpin DNA: 5'-ACCTCAGCACCTGGGGGAGTATTGCGGA GGAAGGT-3'

Apparatus

All electrochemical measurements were carried out with a CHI 660E electrochemical work station (Chenhua, Shanghai). A conventional three-electrode system composed of platinum wire as the auxiliary, saturated calomel electrode (SCE) as reference and a 2-mm-diameter gold electrode (GE) as working electrode. All electrochemical characterizations including cyclic voltammetry (CV), differential pulse voltammetric (DPV) and electrochemical impedance spectroscopic (EIS) measurements were conducted. DPV was scanned from -0.5 to 0 V in PBS (pH 7.4) and the electrochemical signals were recorded.

Agarose gel electrophoresis analysis

The capture DNA, capture DNA/hairpin DNA hybrid duplex and capture DNA/hairpin DNA hybrid duplex with Nb.BbvCI were prepared. The relevant reagents were replaced by their corresponding buffer solution. The 4 % agarose gels contained 0.5 μg of ethidium bromide/mL of gel volume and were prepared with 1×TAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.0). 10 μL of each samples and 2 μL of DNA marker were mixed with 1.5 μL of GelDye TM Super Buffer Mix, and loaded into the gels. The gels were run at 85 V for 50 min and visualized under UV light.

Preparation of the electrochemical biosensor

The bare GE was polished carefully with 0.05 μm of γ-alumina powder and ultrasonically treated with water and ethanol for 5 min in sequence. Prior to modification, the GE was scanned in 0.5 M H_2SO_4 between -0.2 and 1.6 V by CV at a scan rate of 100 mV/s for 10 scans, until a representative cyclic voltammogram of a clean electrode was obtained. After rinsed thoroughly with double distilled water and dried with nitrogen, the GE was incubated with 100 nM capture DNA containing 0.2 mM TCEP for 12 h at room temperature. TCEP is an effective disulfide bond reducing agent. It was used to cut off disulfide bond, preventing the capture DNA from crosslinking, which ensure more capture DNA fixing onto GE. The modified GE was immersed in 1 mM MCH for 1 h to obtain a well-aligned DNA monolayer. Finally, it was rinsed with PBS (pH 7.4) to remove physically adsorbed DNA and MCH. After that, the GE was incubated with 25 nM[31] hairpin DNA containing 5 U Nb.BbvCI[32] and different concentrations of ATP for 1 h at 37□. Due to the formation of double-stranded DNA, Nb.BbvCI could selectively recognized the sequence GGAGT/CG of the complementary double stranded at 5'-terminus of capture DNA (The oblique line indicated the nicking position of Nb.BbvCI). The released target-aptamer complex hybridized with the left capture DNA. Then the recycling of target-aptamer complex leads to the continuous cleavage of the capture DNA, resulting in the signal current of MB decreased. The electrode was rinsed with PBS (pH 7.4) to remove the nonspecific adsorption before DPV scanning.

Results and discussion

Principle of electrochemical biosensor

The proposed electrochemical biosensor involves target-aptamer complex recycling and Nb.BbvCI-assisted signal amplification strategy for ATP detection. As shown in Fig. 1, The MB labeled capture DNA contained the cleavage site of Nb.BbvCI at the 5'-terminus was immobilized on GE surface through Au-S binding.[33] Hairpin DNA was complementary between 5'-terminus and 3'-terminus. In the absence of ATP, a significant current signal of MB was obtained by DPV. In the presence of ATP, hairpin DNA can be specifically combined with ATP and formed a G-quadruplex structure with ATP which resulted in structure-switching of

the hairpin DNA, then the exposed part of hairpin DNA hybridized with capture DNA on it's 5'-terminus to form a specific nicking site for Nb.BbvCI. Through the enzymatic cleavage of MB labeled capture DNA by Nb.BbvCI, many ATP-aptamer complex and DNA fragments labeled MB were released. The complex structure hybridized with the left capture DNA which led to the continuous cleavage of the capture DNA,[34, 35] resulting in the signal current of MB decreased.

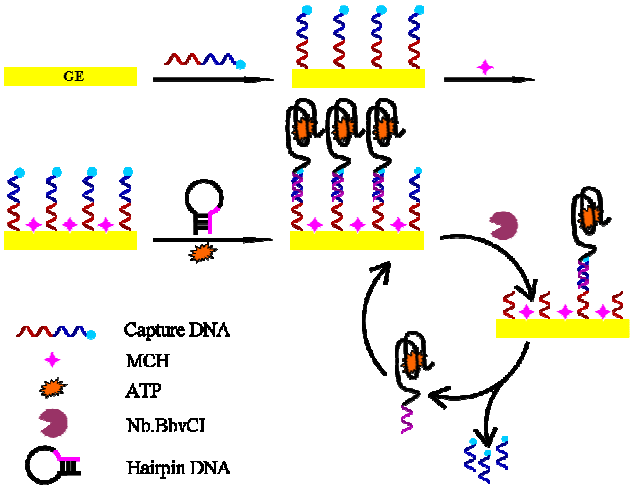

Fig. 1 Principle of the nicking endonuclease-assisted recycling of target-aptamer complex for sensitive electrochemical detection of ATP.

Amplification performance of Nb.BbvCI

To verify the amplification effect of Nb.BbvCI, DPV response of the proposed biosensor in the absence and presence of Nb.BbvCI were compared. As shown in Fig. 2, without ATP and Nb.BbvCI, the DPV signal was significant (curve a). When the hairpin DNA and ATP (600 nM) were added, the hairpin DNA formed a G-quadruplex structure due to the specific recognition between hairpin DNA and ATP. The electrochemical response decreased (curve b) for the reason of the formation of the double-strand DNA between capture DNA and target-aptamer complex. When 5 U Nb.BbvCI was added, peak current of MB decreased (curve c)

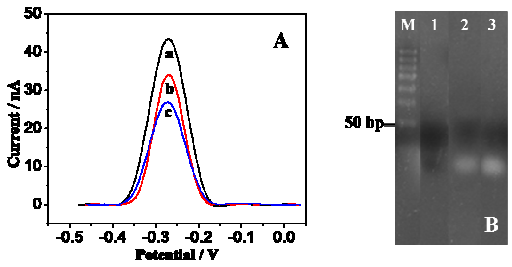

Fig. 2 (A) DPV responses of the electrochemical biosensor after 60 min incubation with: no ATP, no Nb.BbvCI (a), 600 nM ATP, no Nb.BbvCI (b), 600 nM ATP, 5 U Nb.BbvCI (c), (B) Agarose gel electrophoresis images of capture DNA and hairpin DNA with Nb.BbvCI. Lane M, DNA marker (50 bp); Lane 1, 100 μM capture DNA; Lane 2, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP; Lane 3, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP with 50 U Nb.BbvCI. The gels were run for 50 min.

for the Nb.BbvCI-catalyzed target-aptamer complex recycling and digestion the capture DNA on the GE. These results indicated the Nb.BbvCI-assisted target recycling can amplify the signal.

To further verify the amplification performance of Nb.BbvCI-catalyzed cleavage mechanism, agarose gel electrophoresis analysis was performed. As shown in Fig. 2B, the band of capture DNA (Lane 1) was too weak to be observed. After capture DNA hybridized with target-aptamer complex, a faint band of capture DNA/hairpin DNA (Lane 2) appeared due to the formation of dsDNA structure.However, when incubating capture DNA/hairpin DNA hybrid duplex with Nb.BbvCI, the probe was cleaved to give a band of ~10 bp length and the target-aptamer complex, a brighter band (Lane 3) of ~35 bp closed to Lane 2 was ascribed to the released target-aptamer complex. These results confirmed that the capture DNA/hairpin DNA hybrid duplex was cleaved by Nb.BbvCI. So the released target-aptamer complex can still hybridize with capture DNA which realized signal amplification.

Characterization of biosensor fabrication

In order to verify the step-by-step construction of the modified electrode, the stepwise procedure was investigated by EIS and CV measurements. In the terms of EIS, $[Fe(CN)_6]^{3-/4-}$ was utilized as the redox probe and the semicircle diameter was equal to electron transfer resistance, R_{et}. In 0.5 mM $[Fe(CN)_6]^{3-/4-}$, bare GE exhibited an almost straight line (Fig. 3A, curve a), which allowed a fast electron-transfer on the surface of the electrode. After thiolated capture DNA self-assembled onto the electrode surface, the R_{et} increased obviously (curve b), because the non-conductive oligonucleotides on the GE hindererd the electron-transfer. The R_{et} kept increasing (curve c) when the biosensor was blocked by MCH on unoccupied sites, since the MCH also hindered electron transfer. When the electrode immersed with 25 nM hairpin DNA and different concentrations of ATP, the R_{et} was further increased (curve d) due to the steric hindrance of electron transfer after the formation of the dsDNA. When 5 U Nb.BbvCI added, R_{et} decreased, because of the release of target-aptamer complex and partial capture DNA, and the R_{et} greatly decreased (curve e), for the amount of non-conductive oligonucleotides on the electrode significantly decreased through the Nb.BbvCI-assisted target recycling. These results were in a good agreement with those obtained from CV

measurement (Fig. 3B). These results demonstrated the successful fabrication of the biosensor.

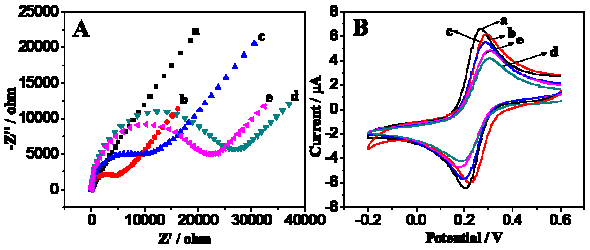

Fig. 3 EIS (A) and CV (B) in 0.5 mM $Fe(CN)_6^{3-/4-}$ at (a) bare GE, (b) capture DNA/GE, (c) MCH/ capture DNA/GE, (d) hairpin DNA/ATP/MCH/capture DNA/GE and (e) Nb.BbvCI/hairpin DNA/ATP/MCH/capture DNA/GE.

Optimization of experimental conditions

In order to acquire a higher sensitively of the electrochemical biosensor, optimal experimental conditions were investigated. The concentration of capture DNA could impact the relative reduced signal. In order to better conduct the experiments, the different concentrations of capture DNA were assembled on the GE surface. From Fig. 4A, the relative reduced signal increased with the decreasing concentration of capture DNA. Finally, it stabilized when the concentration of capture DNA was 100 nM. Thus the optimum concentration of capture DNA was chosen as 100 nM. The effect of the hybridization time between hairpin DNA, target and capture DNA on the electrochemical response was shown in Fig. 4B. The current response increased with the increasing of hybridization time and then approached a constant value after 60 min, so the optimal time was 60 min for the combination of hairpin DNA, target and capture DNA.

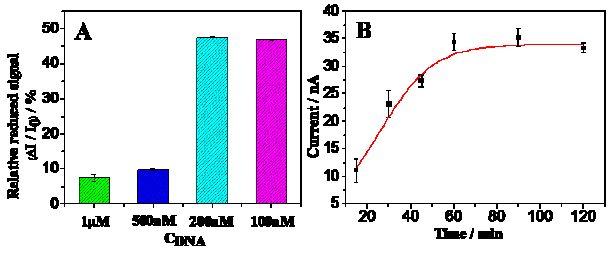

Fig. 4 Effects of (A) the concentration of capture DNA, (B) hybridization time between the biosensor and the mixture containing 25 nM hairpin DNA, 100 nM ATP and 5 U Nb.BbvCI.

Detection of ATP

Different concentrations of ATP were analyzed by the biosensor with DPV measurements in 10 mM pH 7.4 PBS. Under the optimized conditions, capture DNA modified gold

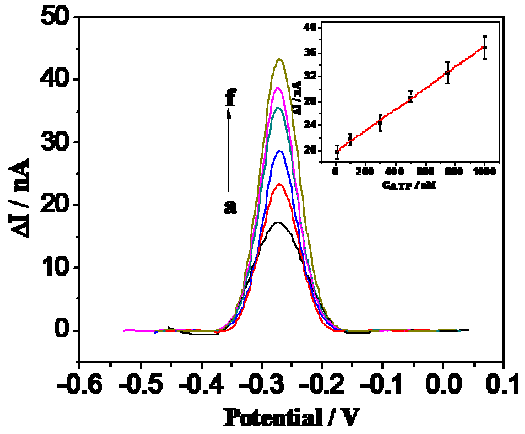

Fig. 5 DPV responses of 10 nM, 100 nM, 300 nM, 500 nM, 750 nM and 1 μM (from a to f) ATP in PBS (pH 7.4). Insert: linear relationship between the decrease peak current and the concentration of ATP. The error bars represent the standard deviation of three measurements.

electrode was incubated in 25 nM ATP aptamer containing 5 U Nb.BbvCI and different concentrations of ATP for 1 h at 37^0C. Then DPV responses of the biosensor were recorded. The reduced current (ΔI) increased proportionally with the increasing concentration of ATP in the range of 10 nM to 1 μM. The linear equation was fitted as ΔI (A) = 1.971E-8 + 1.742E-11c (nM) (R^2 = 0.9954) (Fig. 5). This amplified biosensors allowed the ATP detection with a limit of detection (LOD) as low as 3.4 nM (S/N = 3). The LOD obtained by the NEase-assisted target-aptamer complex recycling method was lower than that of the conformational switching[36, 37] approach whose detection limit of ATP was as low as 20 nM .

Selectivity, reproducibility, and stability of the biosensor for ATP detection

To evaluate the selectivity of the proposed method, several interfering agents were tested, including GTP, CTP, UTP. The concentration of ATP was 0.1 μM, while the concentrations of GTP, CTP, UTP were 1 μM. As shown in Fig. 6, ATP produced a much stronger current response, while three interfering agents led to inconspicuous signal change, indicating that the fabricated biosensor was very specific to ATP. The reproducibility of the biosensor was investigated by the following methods: five equally prepared electrodes were used to detect ATP (0.1 μM) under the optimum experiment conditions. The relative standard deviation (RSD) of the DPV current was 6.9%, which showed the proposed biosensor had good reproducibility for ATP detection. In order to test the stability of the proposed biosensor, the biosensors were stored in a refrigerator for one week, 89% of the current response for ATP (600 nM as an example)

remained, indicated that the proposed method possessed an acceptable stability.

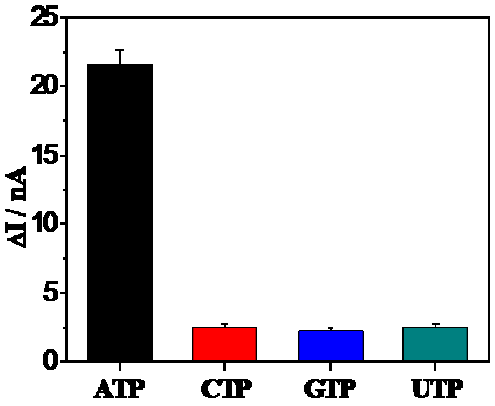

Fig. 6 The selectivity of the proposed electrochemical biosensor: ATP (0.1 μM), CTP (1 μM), GTP (1 μM) and UTP (1 μM), respectively. Error bars are obtained based on three independent measurements.

Analytical application of the proposed biosensor

To monitor the reliability of the proposed biosensor, recovery experiments were performed by adding various concentrations of ATP in human serum samples by the standard addition method. In the test, 30 μL of healthy human serum was diluted to 1.0 mL with Tris-HCl (pH 7.4). Then, different concentration of ATP (80, 150, 600 nM) were prepared by diluted human serum samples. The detection value was the average of three results by testing three times. As shown in Table 1, the recovery and and the RSD were ranging from 96.3% to 106.8% and from 1.9% to 4.5%, respectively, which indicated that the proposed biosensor was available for ATP detection in real clinical samples.

Table 1. Determination of ATP added in human serum (n=3) with the electrochemical biosensor

Serum sample	Added, nM	Found, nM	Recovery, %	RSD, %
1	80	85.4	106.8	2.4
2	150	144.4	96.3	1.9
3	600	592.1	98.7	4.5

Conclusions

In summary, we developed a simple electrochemical approach for convenient detection of ATP based on target-induced conformation switching and NEase-assisted signal amplification. The presence of target ATP leads to conformational changes of the hairpin structure and forms catalytic cleavage sites for Nb.BbvCI to recycle ATP-aptamer complex to achieve signal amplification. Under the optimal conditions, the biosensor showed high sensitivity, good stability, acceptable reproducibility, wide detection range and a low detection limit. The employment of nicking endonuclease improved the sensitivity of the proposed biosensor. Compared to some amplified detections for ATP based on micro/nanofabrications, the proposed method has better stability and which is cheap and easy to operate. The proposed biosensor exhibited excellent analytical performance for electrochemical detection of ATP, providing a promising applications in sensitive and selective detection of ATP and other similar substances.

Acknowledgements

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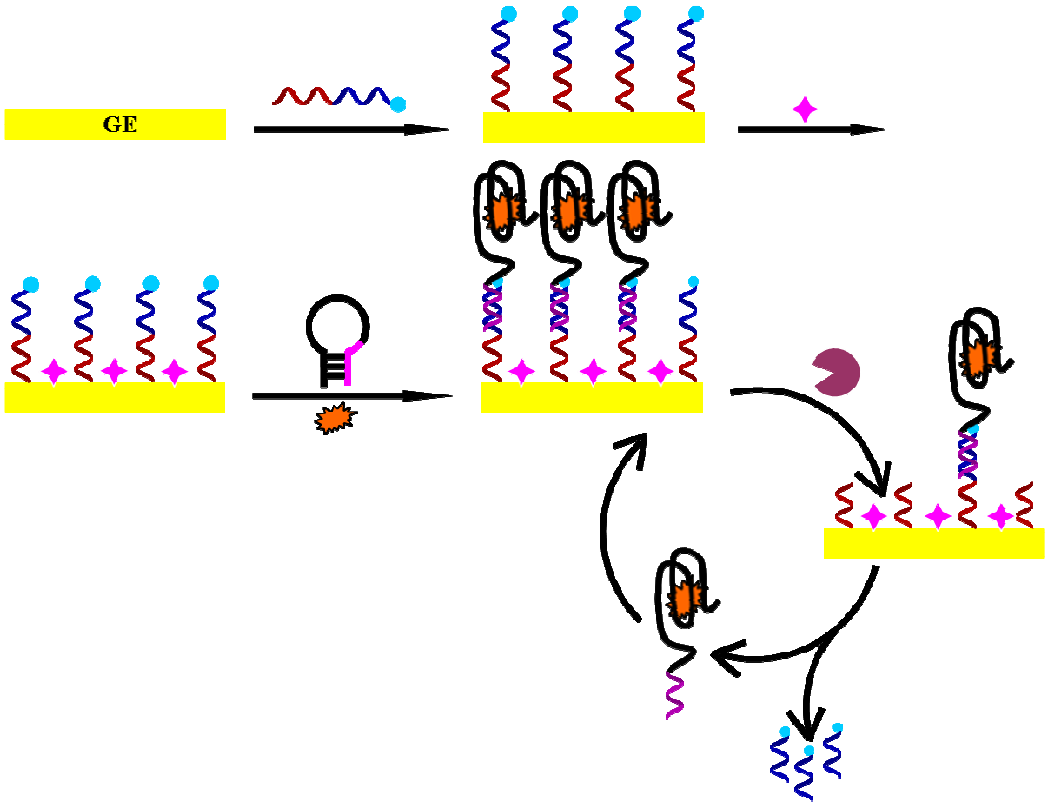

Figures and Tables

Fig. 1 Principle of the nicking endonuclease-assisted recycling of target-aptamer complex for sensitive electrochemical detection of ATP.

Fig. 2 (A) DPV responses of the electrochemical biosensor after 60 min incubation with: no ATP, no Nb.BbvCI (a), 600 nM ATP, no Nb.BbvCI (b), 600 nM ATP, 5 U Nb.BbvCI (c); (B) Agarose gel electrophoresis images of capture DNA and hairpin DNA with Nb.BbvCI. Lane M, DNA marker (50 bp); Lane 1, 100 μM capture DNA; Lane 2, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP; Lane 3, 100 μM capture DNA, 25 μM hairpin DNA and 50 mM ATP with 50 U Nb.BbvCI. The gels were run for 50 min.

Fig. 3 EIS (A) and CV (B) in 0.5 mM $Fe(CN)_6^{3-/4-}$ at (a) bare GE, (b) capture DNA/GE, (c) MCH/ capture DNA/GE, (d) hairpin DNA/ATP/MCH/capture DNA/GE and (e) Nb.BbvCI/hairpin DNA/ATP/MCH/capture DNA/GE.

Fig. 4 Effects of (A) the concentration of capture DNA, (B) hybridization time between the biosensor and the mixture containing 25 nM hairpin DNA, 100 nM ATP and 5 U Nb.BbvCI.

Fig. 5 DPV responses of 10 nM, 100 nM, 300 nM, 500 nM, 750 nM and 1 μM (from a to f) ATP in PBS (pH 7.4). Insert: linear relationship between the decrease peak current and the concentration of ATP. The error bars represent the standard deviation of three measurements.

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Table 1. Determination of ATP added in human serum (n=3) with the electrochemical biosensor.

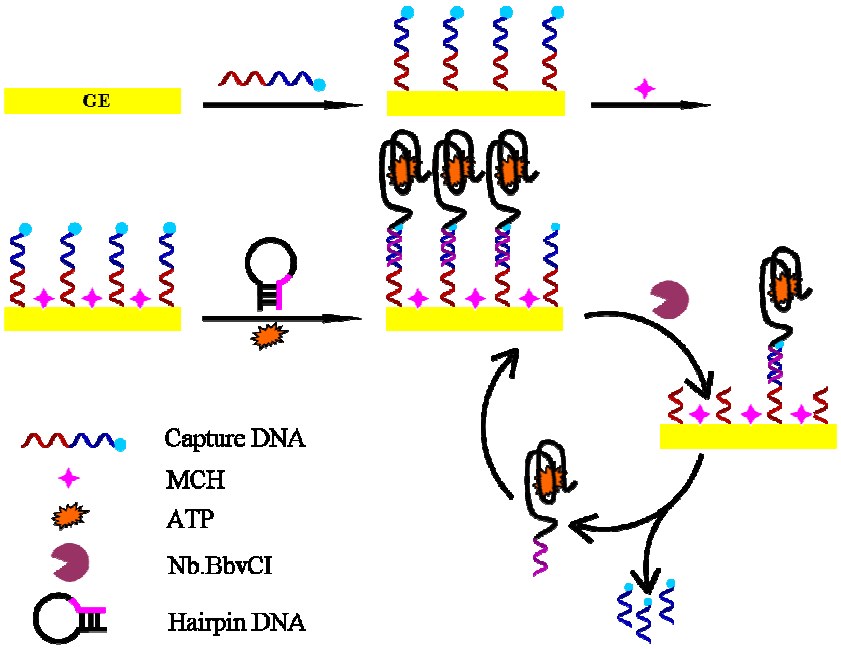

Fig. 1

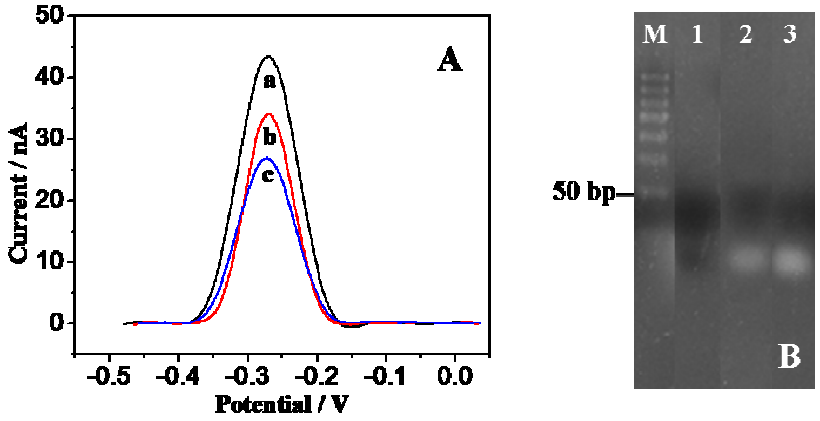

Fig. 2

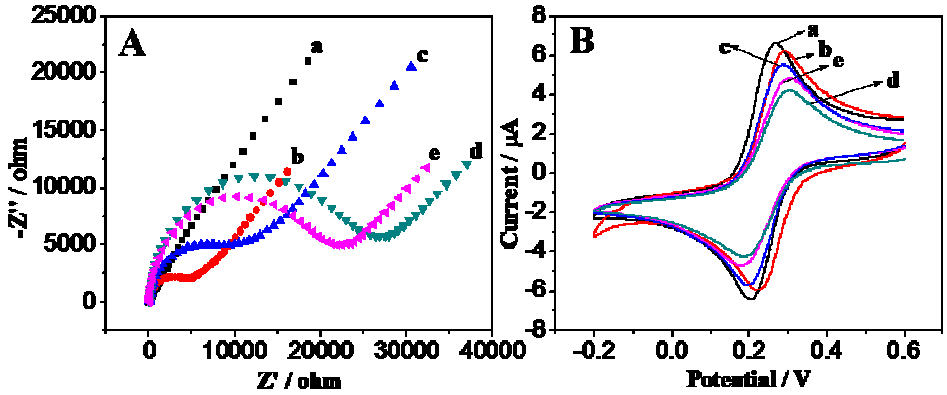

Fig. 3

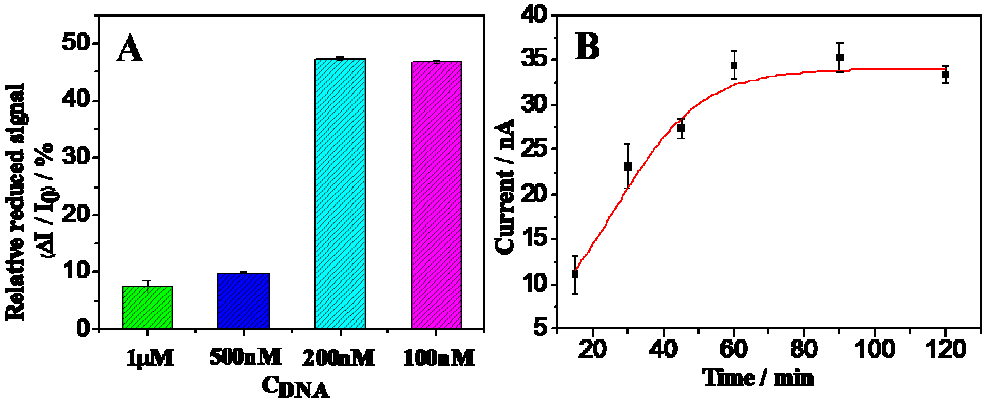

Fig. 4

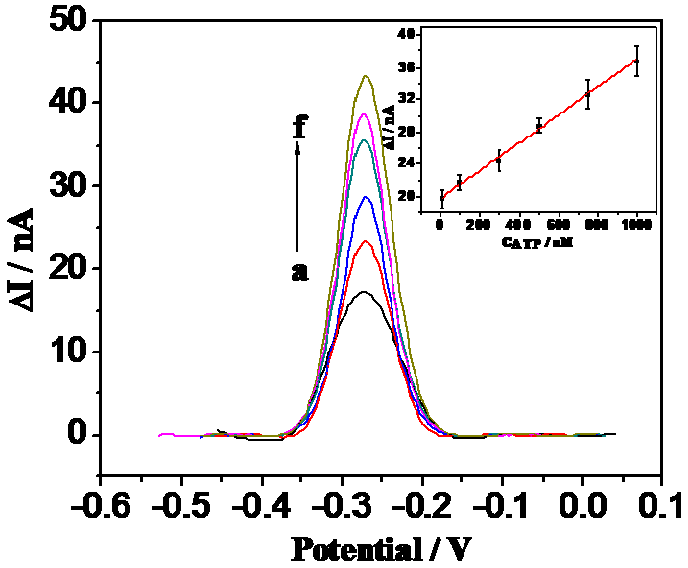

Fig. 5

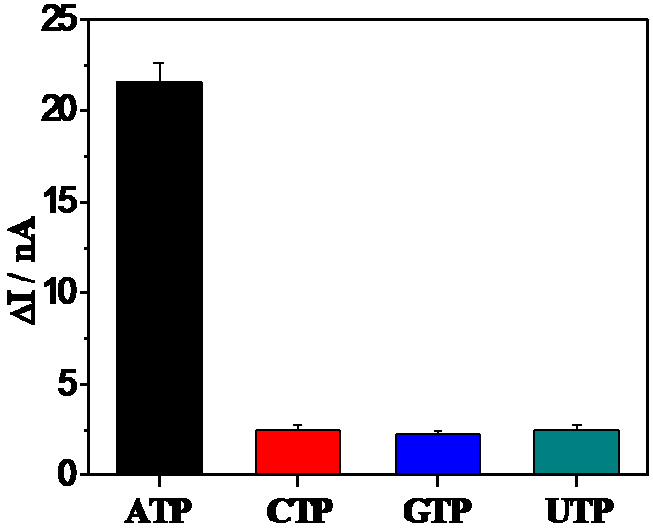

Fig. 6

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