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A Simple Electrochemical Biosensor for Rapid Detection of MicroRNA Based on Base Stacking Technology and Enzyme Amplification

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MicroRNAs (miRNA) are pivotal regulators of a wide range of cellular processes and have been identified as promising cancer biomarkers due to their stable presence in serum. In this study, we have developed a simple and rapid electrochemical biosensing strategy for detection of miRNA based on base stacking hybridization technology and enzyme amplification. In the presence of target miRNA-122, miRNA-122 can hybridize with the capture probes immobilized on the electrode surface, then the biotinylated detection probes hybridize with the remaining part of miRNA-122. Consequently, strong base stacking effect can make the target sequence, detection probes and capture probes have a good and stable combination. Finally, the streptavidin-alkaline phosphatase (ST-AP) binding with biotinylated detection probes catalyzes the hydrolysis of the substrate α-naphthyl phosphate (α-NP). The electrochemical biosensor could detect miRNA down to 0.4 pM with a linear range from 1 pM to 100 nM, and exhibited good specificity and reproducibility. This developed sensing strategy may be readily expanded to other miRNA detection and provide a promising tool for the basic research and clinical application

Keywords: MicroRNA; Base-stacking; Electrochemical biosensor; Enzyme amplification.

1. INTRODUCTION

MicroRNAs, an important class of endogenous non-coding single-strand RNAs, regulate gene expression interacting with a specific mRNA, either inducing its degradation or blocking the

translation process [1]. MicroRNAs are processed from stem-loop-containing precursors mediated by a series of enzyme complexes [2， 3]. These small miRNA molecules which are widely found in plants, humans, and animals play fundamental roles in gene silencing and cell growth, development, and differentiation [4, 5]. And they also perform key functions in neuronal asymmetry, apoptosis, insulin secretion, and different metabolic reactions [6-9]. Moreover, recent studies have indicated that the changes of miRNA levels are observed in many human diseases, especially for cancers [10]. From these findings, miRNAs are aberrantly expressed in many human diseases and promising novel biomarkers for early clinical diagnosis [11]. However, giving the fact that the mature miRNAs are short in length, homologous in sequences (i.e., let-7 family), and low abundance in total RNA samples, the simple and rapid detection of miRNAs remains a challenging task [12]. Therefore, there is an urgent need to explore novel strategy for reliable and simple detection for miRNAs.

To date, many methods have been developed for miRNA detection, including northern blotting, real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and microarrays. Northern blotting, regarded as the "gold standard" for miRNA detection, has been proved to be a robust and widely used technique. However, it has time consuming and limited sensitivity [13]. QRT-PCR based approaches are a prominent tool for miRNA quantitative analysis due to their high sensitivity and specificity. But the approaches need thermal cycling, careful primer design, and special equipment, which restrict their extensive application [14, 15]. Thought the microassays make multiple miRNA analysis feasible, the kits provided are expensive and microassays for miRNA detection require the equipped laboratory with specialized and well-trained biologists [16]. Nowadays, various new strategies have been developed to improve the detection sensitivity and selectivity, such as surface plasmon resonance (SPR), surface plasmon resonance imaging (SPRi), surface enhanced raman spectroscopy, bioluminescence, fluorescence, and electrochemistry [17-23]. Among the above-mentioned methods, electrochemical biosensor has been becoming the most promising approach for miRNAs detection owing to its advantages, such as low-cost, small-size, simplicity of construction, ease of use, high sensitivity and selectivity [24, 25].

Base stacking is the interaction (Van der Waals force) that exists in the aromatic rings of the bases between two consecutive nucleotides, which has been exhaustively investigated by Lane's team and Yakovchuk's team [26, 27]. It was found that stacking force identified as another significant non-covalent interaction can provide a stable double helix structure for DNA and RNA hybridization [28]. Base stacking supplies an additional stability to the hybridization in two or more oligonucleotides in a longer complementary strand of a continuous series, in addition to the base pairing of the complementary strand. Short nucleotide chain with its double-stranded complementary DNA forms a end to end structure that two or more juxtaposed terminal nucleotides hybridize to a longer complementary DNA (or RNA) single-strand. And the formed structure improves the hybridization effect, which builds a fairly stable thermodynamically favored complex sandwich structure. The base stacking effect is composited only by the incision not the gap among these adjacent short nucleotides. Thus, based on base stacking hybridization technology, various methods have been developed for specifically detecting short length DNAs and RNAs [29-31].

In this study, a new strategy has been developed for simple and rapid detection of miRNAs by coupling base stacking technology with enzymatic amplification. In the presence of target miRNA-122,

miRNA-122 can hybridize with the capture probes immobilized on the electrode surface, then the biotinylated detection probes hybridize with the remaining part of miRNA-122. Consequently, the ternary complex is obtained through stacking interactions. Finally, the streptavidin-alkaline phosphatase (ST-AP) binding with biotinylated detection probes catalyzes the hydrolysis of the substrate α-naphthyl phosphate (α-NP). The established biosensor displays an excellent analytical performance toward miRNA detection and may present a powerful and convenient tool for biomedical research and clinic diagnostic application.

2. EXPERIMENTAL

2.1. Materials and reagents

Streptavidin-alkaline phosphatase (ST-AP), 6-mercapto-1-hexanol (MCH), α-naphthyl phosphate (α-NP), bovine serum albumin (BSA), and polyethylene glycol sorbitan monolaurate (Tween-20) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Diethy pyrocarbonate (DEPC) was from Solarbio (Beijing, China). All other reagents were of analytical grade. All DNA oligonucleotides and miRNAs were synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China) and TaKaRa Biotechnology Co., Ltd (Dalian, China), respectively [32]. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays. All other reagents were of analytical grade.

The buffer solutions employed in the experiment were as follows. MiRNA hybridization buffer contained 45 mM NaCl, 3 mM EDTA, 30 mM $Na_2HPO_4 \cdot 12H_2O$ (pH 8.0). The Tris-HCl buffer as washing buffer contained 50 mM Tris, 6 mM $MgCl_2$ and 10 mM NaCl (pH 7.5). In order to protect miRNA from RNase degradation, all buffer solutions and water were treated with DEPC by mixing 0.1 % of DEPC with the solution, and the mixed solution was shaken overnight prior to autoclaving.

All miRNA and DNA oligonucleotides were purified by HPLC, and their sequences are listed below. All the miRNA and DNA oligonucleotides were dissolved in water, and diluted in miRNA hybridization buffer before to use.

MiRNA122：5’-UGG AGU GUG ACA AUG GUG UUU G-3’
Capture probe：5’-TTG TCA CAC TCC ATT TTT TTT TTT T-SH-3’
Detection probe：5’-Biotin-TTT TTT TTT CAA ACA CCA-3’
Detection probe 2：5’-Biotin-TTT TTT TTT CAA ACA CC-3’
Non-complement sequence：5’-TGC ATC GGC AAC CCA-3’

2.2. Apparatus

The electrochemical measurements were performed on a CHI660D electrochemical analyzer (Shanghai Chenhua Instruments Co. Ltd., China) with a three-electrode system, where a 3-mm diameter gold electrode (GE) was used as working electrode, platinum wires as auxiliary electrode, Ag/AgCl electrode as reference electrode. Low-speed desktop centrifuge (Heraeus, Germany),

constant temperature water bath box (Ruihua, China), KQ 3200DB ultrasound (Kunshan, China), milli Q water meter (Millipore, USA) were used in the work.

2.3. Preparation of electrochemical biosensor

The gold electrode was polished to a mirror using 0.3 and 0.05 μm alumina slurry respectively and ultrasonically treated in deionized water. Then, the electrode was soaked in piranha solution (H_2SO_4:H_2O_2=3:1) for 10 min, followed by rinsed thoroughly with ultrapure water to eliminate other substances. After drying in the room temperature, 10 μL aliquot of 1.0 μM thiolated capture probes was dropped onto the prepared electrode surface and incubated at 4 ℃ overnight. After washed with pH 7.5 Tris-HCl buffer, the electrode was immersed into 1 mM MCH solution for 1 h to obtain well-aligned DNA monolayer. Finally, the electrode was further treated with 1 % BSA in DEPC water 30 min to avoid nonspecific adsorption of DNA and enzyme on the electrode surface, and then thoroughly rinsed with the washing buffer and used for following operation.

2.4. Target miRNA detection protocol

Prior to use, the prepared electrochemical biosensor was further washed with washing buffer. 10 μL of mixture that contained 500 nM biotin modified detection probes and target miRNAs diluted at variable concentrations was then dropped on the prepared electrode surface, incubated at 4 ℃ for 60 min to form the sandwich structure, and rinsed thoroughly with diethanolamine (DEA) buffer (0.1 M diethanolamine, 1 mM $MgCl_2$, 100 mM KCl, pH 9.6). Subsequently, 10 μL of DEA buffer containing 1.25 μg mL^{-1} ST-AP and 10 mg mL^{-1} BSA was dropped onto the biosensor surface and incubated for 30 min at 4 ℃. The biosensor was washed with DEA buffer containing 0.05 % Tween-20. The differential pulse voltammetry (DPV) were performed in DEA buffer containing 1mg mL^{-1} of α-naphthyl phosphate with potential scan from -0.1 V to 0.6 V, modulation time of 0.05 s, interval time of 0.017 s, step potential of 5 mV and modulation amplitude of 70 mV.

3. RESULTS AND DISCUSSION

3.1. Design of the electrochemical biosensor

The principle of the electrochemical biosensor for detection of miRNA is illustrated in Fig 1. Coupling the base stacking technology with enzyme amplification, detection of miRNA is performed in two simple steps. First, the thiolated DNA probes are covalently immobilized on the electrode, which have a sequence complementary to the part sequence of target miRNA. Then the mixture of the miRNA and detection probes are dropped on the sensor surface. As the detection probes specifically bind with another part sequence of target miRNA to form a stable hybridization, the formed sandwich structure significantly can increase the sensitivity of the assay due to the strong base stacking effect. According to the bridging role of the streptavidin-biotin system, the biotinylated detection probes combine with ST-AP, which catalyzes the conversion of substrate α-NP to an electroactive product for

producing an amplified electrochemical signal [33, 34]. Base on the base stacking strategy, a simple and rapid electrochemical method has been successfully developed for sensitive and highly specific detection of target miRNA.

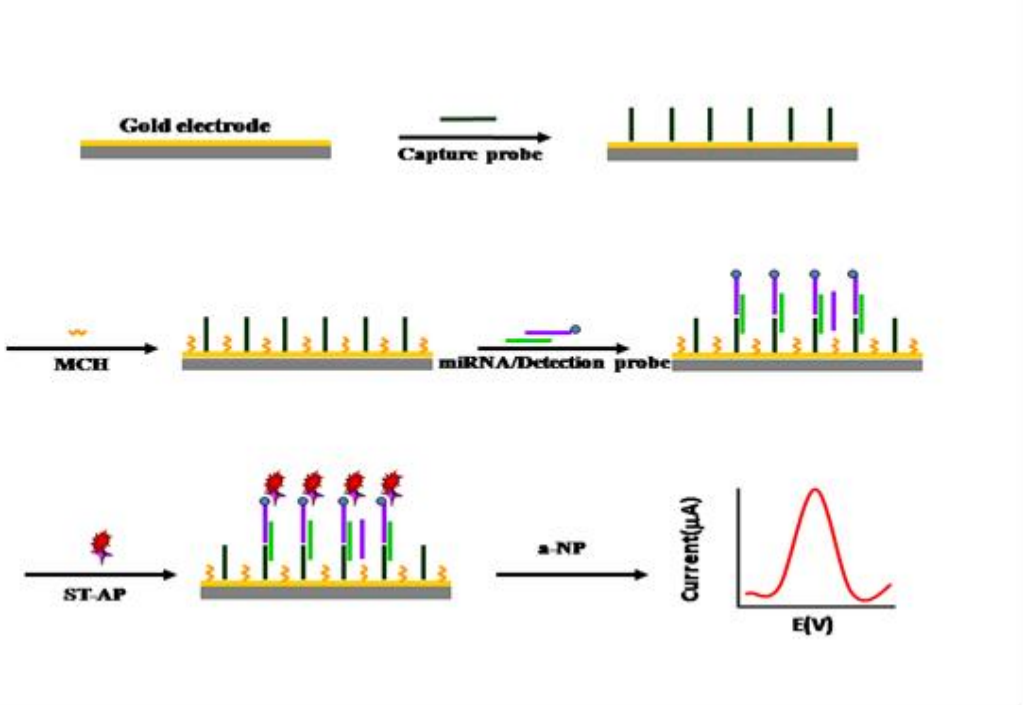

Figure 1. Schematic illustration of the electrochemical biosensor for microRNA detection based on base stacking technology and enzyme amplification.

3.2. Verification of the hybrid models

There are some associations between the occurrence of the base stacking interaction and the hybridization of these nucleotides in the assay. Herein, to explore the base stacking effect on signal amplification, two different hybridization models were used to test using electrochemical and surface plasmon response (SPR) approaches. As shown in the Fig 2. The model 1 stood for that the target and the detection probes were mixed and linked in the liquid environment, and then dropped on the sensor surface for hybridizing with the capture probes. The model 2 represented the target hybridized with the capture probe first, then the detection probe complemented to the other part of the target sequence in the solid-liquid interface. Two concentrations (100 nM and 10 nM) of the target were selected for the experiment respectively. The experimental results show that the model 1 produced a higher signal than the model 2, indicating that the target and the detection probe has a better interaction in the model 1. The reason is attributed to multiple factors, such as inhomogeneity, steric, the specific sites and so on, influence the hybridization of the target and the detection probe in the solid-liquid interface of the electrode surface. While in the liquid environment, those factors can be avoided or neglected so that the target and the detection probe have the higher affinity and the stronger binding force.

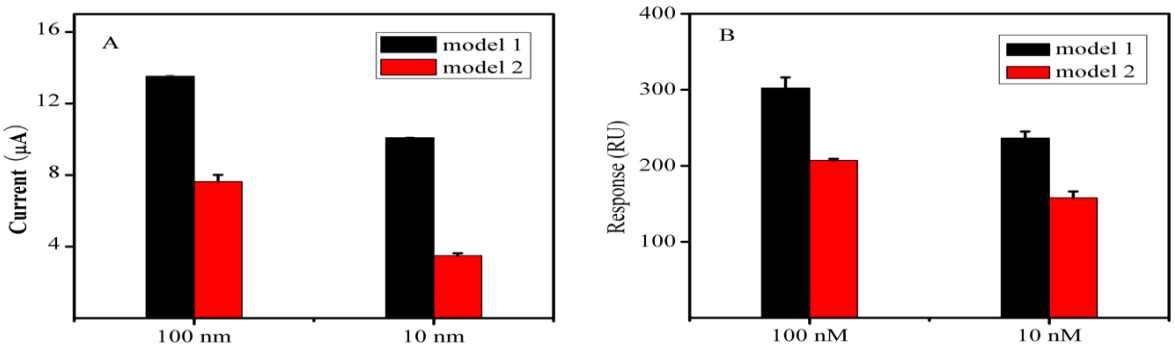

Figure 2. The responses vs different target RNA concentrations at the two models: black column stands for the model 1, the target hybridizes with detection probe first and red column means the model 2, the target hybridizes with capture probe first.

3.3. Verification of the base stacking

Base stacking hybridization is that short oligonucleotides with its double-stranded complementary DNA form end to end structure. The base stacking effect is composited only by the incision not the gap among these adjacent short nucleotides (Fig 3). Under this condition, the bonding force between bases becomes stronger so that it can provide sensitive and specific detection [35-38]. Therefore, we designed detection probe 2 lack of a base compared with the detection probe, so that a gap in the base stacking effect exists when the adjacent short nucleotide hybridizes with the target. The base stacking effect was investigated by measuring the electrochemical and SPR signals. 100 nM target miRNA and 500 nM detection probe were adopted to detect the signals. In the Fig 4A and 4B, the electrochemical detection results showed that the detection probe (column a) completely and closely linking to the end had nearly one time growth in the DPV signal compared with the detection probe 2 (column b). The results were in good agreement with those results obtained from SPR method (column c, d). The above results further prove that the higher signal can be got and the better detection effect is achieved when the base stacking effect exists.

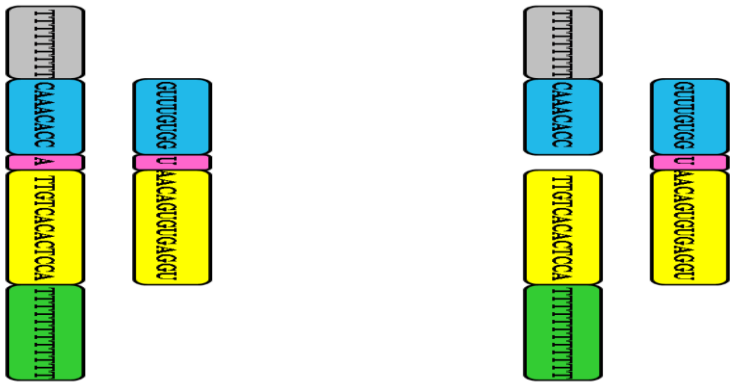

Figure 3. Schematic illustration of the base stacking effect.

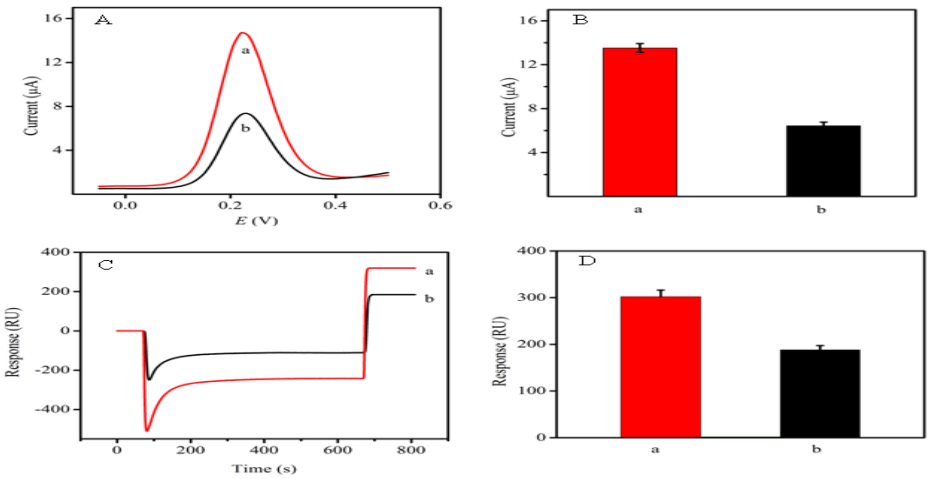

Figure 4. Base stacking reaction: (A) and (B) electrochemical response, (C) with (D) SPR response. (a) The target miRNA concentration of 100 nM and (b) at the 10 nM target miRNA.

3.4. Characterization of electrode surface modification

Electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) were employed to monitor the interface properties of the modified gold electrode surface during stepwise biosensor assembly. Fig 5A shows the Nyquist plots of 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.4 M KCl at different modified electrodes, and the semicircle diameter of EIS curse equals to the electron transferring resistance (R_{et}). The bare gold electrode exhibited an almost straight line (curve a), which was characteristic of a mass diffusion limiting step of the electron transfer process. The immobilization of the thiolated-modified capture probes on the bare gold electrode surface resulted in an increase (curve b), because the single-stranded DNA was negative charge and its phosphate backbone hampered the electron transfer between $Fe(CN)_6^{3-/4-}$ solution and electrode [39, 40].

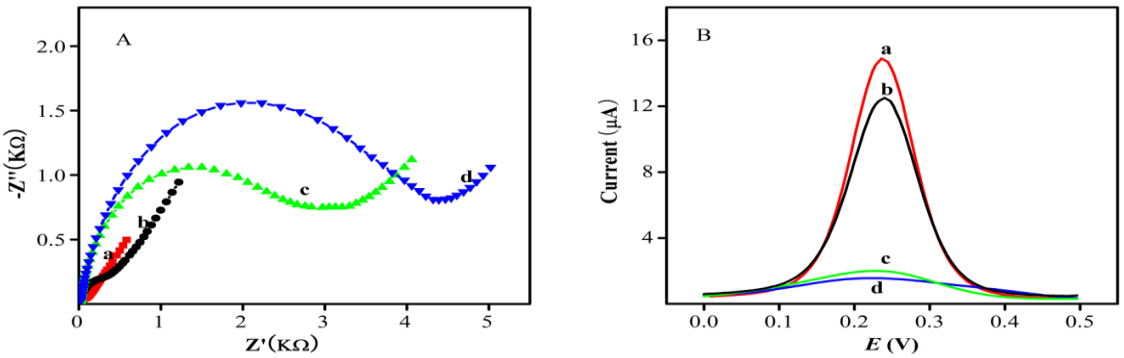

Figure 5. EIS (A) and SWV (B) of bare electrode (a), capture probe modified electrode (b), MCH/BSA modified electrode (c) and electrode after base stacking reaction (d) in 0.5 mM $Fe(CN)_6^{3-/4-}$containing 0.4 M KCl.

The results demonstrated that the probes were successfully modified on electrode surface. When the MCH and BSA were self-assembled onto the gold electrode, the remaining blank sites were blocked so that the electrode surface substance increased and the R_{et} increased remarkably (curve c). Subsequently, the R_{et} further enhanced to a larger value after capture probes hybridized with the added mixture of the miRNA and detection probes (curve d) due to the increasing negative charge, indicating successful combination of capture probes, target and detection probes. The above results were in a good agreement with those obtained from SWV (Fig 5B), in which the peak currents decreased upon the assembly and hybridization processes. Both experiment results proved the successful modifications and reactions as described in the principle scheme.

3.5. Optimization of experimental conditions

The performance of the electrochemical analysis is related to different parameters in the measuring system. The reaction process can be affected mainly by the α-NP concentration and the ST-AP concentration. The DPV peak current of the biosensor in DEA buffer increased with the concentration of α-NP from 0.25 to 2.0 mg mL^{-1}, and then maintained the maximum value at higher concentrations. Therefore, the optimal α-NP concentration for DPV detection was 1.0 mg mL^{-1} (Fig 6A) [41].

The effect of ST-AP concentration obviously influenced the signal (Fig 6B) responses of the biosensor. With the increasing concentration of ST-AP, the DPV response increased rapidly up to 0.75 μg mL^{-1} and then trended to a stable value. Thus, 0.75 μg mL^{-1} was the optimal concentration to obtain high ratio of signal to background [42].

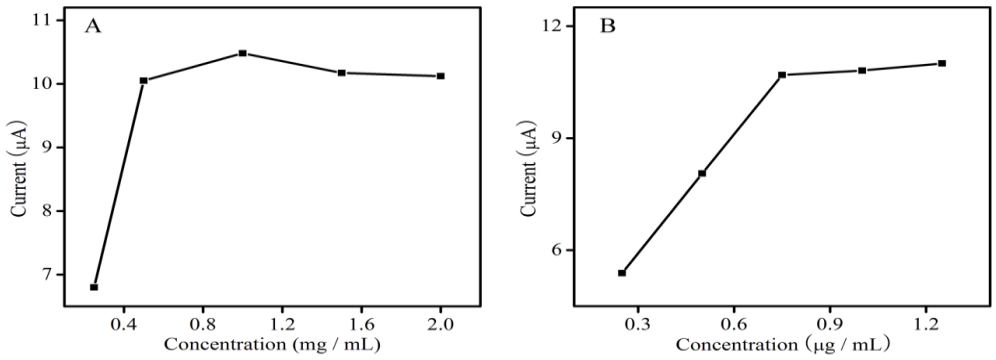

Figure 6. Dependences of DPV peak current on concentration of α-NP (A) and ST-AP concentration (B). When one parameter changed the others were under its optimal condition.

3.6. Analytical performance of miRNA sensor

Under the optimal experimental conditions, the analytical performance of the designed electrochemical biosensor was investigated. These experiments were carried out by adding different concentrations of target miRNA to the developed biosensor. The DPV peak currents rose significantly with the increasing concentrations of target miRNA (Fig 7A). A good linear relationship between the

DPV currents and the logarithm of miRNA concentration was achieved in range of 1 pM to 100 nM （Fig 7B）. The resulting linear equation was ip (μA) =7.8 + 2.3 log C with a correlation coefficient of 0.9978, and the limit of detection (LOD) was calculated to be 0.4 pM at a signal to noise of 3. In addition, the reproducibility of the developed sensor was further investigated by measuring the target miRNA at 100 pM and 50 nM with five replicates. The coefficients of variation for both concentrations were 2.2% and 4.5%, respectively. Thus, the established biosensor displayed high sensitivity and acceptable reproducibility, which could be applied to quantification of miRNA with low detection concentration [43].

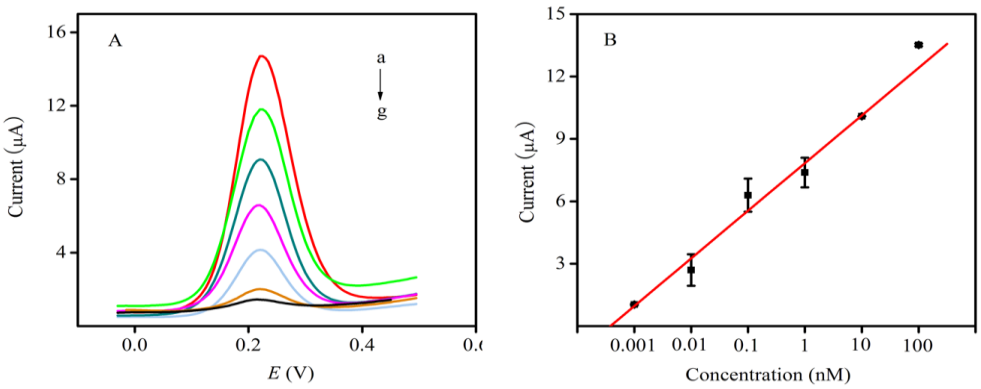

Figure 7. (A) DPV curves of the biosensor obtained with target miRNA concentrations of 0, 1, 10, 100 pM, 1, 10 and 100 nM (from a to g). (B) Plot of DPV peak current vs. target miRNA concentration. Error bars show the standard deviation of three experiments.

3.7. Specificity of the strategy

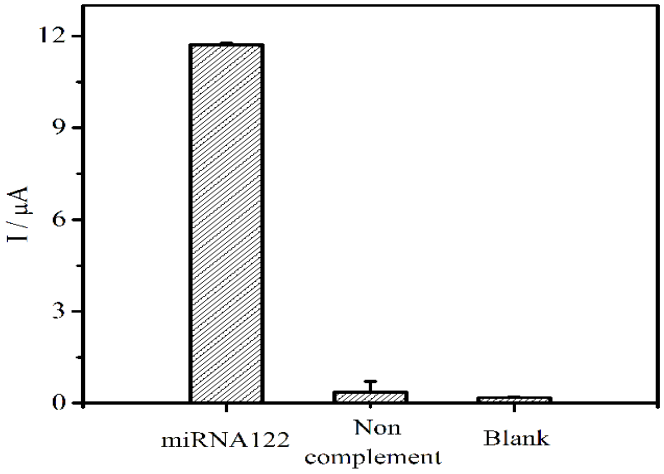

Figure 8. Specificity of the method evaluated by the DPV peak currents of miR-122, no complement miRNA and blank. The concentration of each miR-122 and non complement miRNA is 100 nM. Error bars show the standard deviation of three experiments.

To evaluate the specificity of the biosensor, 100 nM non-complementary, which may interfere with the detection of target miRNA, and 100 nM target miRNA were examined, respectively. As shown in Fig 8. The DPV signals produced by fully complementary target was much greater than that by the non-complementary target. At the same time, the signal produced by non-complementary target was close to the blank signal. The error bars represented average standard errors for three measurements. The experimental results demonstrated that the electrochemical sensor demonstrated specific response to target miRNA-122.

4. CONCLUSIONS

In summary, an electrochemical biosensor has been developed for miRNA detection based on base stacking and enzyme amplification. Meanwhile, the high catalytic activity of ST-AP toward hydrolysis of α-NP can increase the sensitivity of the biosensor. With a competitive assay format, the fabricated electrochemical biosensor for miRNA detection shows good analytical performance with a wide linear range, excellent specificity, and good reproducibility. In addition, the effect of base stacking on amplification has been verified using electrochemical and SPR approach. Thus, this biosensing method may provide a pragmatic tool for the basic research and clinical application in the future.

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References

1. Y. Zhao, D. Srivastava, *Trends in biochemical sciences*, 32(2007)189.
2. R. Stefl, F.C. Oberstrass, J.L. Hood, M. Jourdan, M. Zimmermann, L. Skrisovska, C. Maris, L. Peng, C. Hofr, R.B. Emeson, F.H.T. Allain, *Cell*, 143(2010)225.
3. D.P. Bartel, *Cell*, 116(2004)281.
4. D.P. Bartel, *Cell*, 136(2009)215.
5. K.K.H. Farh, A. Grimson, C. Jan, B.P. Lewis, W.K. Johnston, L.P. Lim, C.B. Burge, D. P. Bartel, *Science*, 310(2005)1817.
6. N. Liu, M. Landreh, K.J. Cao, M. Abe, G.J. Hendriks, J.R. Kennerdell, Y.Q. Zhu, L.S. Wang, N.Y. M. Bonini, *Nature*, 482(2012)519.
7. H.F. Dong, J. Zhang, H.X. Ju, H.T. Lu, S.Y. Wang, S. Jin, K.H. Hao, H.W. Du, X.J. Zhang, *Anal. Chem.* 84 (2012)4587.
8. H.F. Dong, S. Jin, H.X. Ju, K.H. Hao, L.P. Xu, H.T. Lu, X.J. Zhang, *Anal. Chem*. 84 (2012)8670.
9. J.Y. Liao, J.Q. Yin, J.C. Yue, *Journal of Sensors*, 2009 (2009)1.
10. R. Baffa, M. Fassan, S. Volinia, B. O'Hara, C.G. Liu, J.P. Palazzo, M. Gardiman, M. Rugge, L.G. Gomella, C.M. Croce, A. Rosenberg, *J. Pathol*. 219 (2009)214.
11. A.W. Wark, H.J. Lee, R.M. Corn, *Angew. Chem. Int. edit. 120(2008)654.*
12. K.A. Cissell, S. Shrestha, S.K. Deo, *Anal. Chem*. 79(2007)4754.
13. É. Várallyay, J. Burgyán, Z. Havelda, *Methods*, 43 (2007) 140.

14. C. Chen, D.A. Ridzon, A.J. Broomer, Z.H. Zhou, D.H. Lee, J.T. Nguyen, M. Barbisin, N. L. Xu, V.R. Mahuvakar, M.R. Andersen, K.Q. Lao, K.J. Livak, K.J. Guegler, *Nucleic Acids Res*. 33 (2005) e179.
15. Z.Q. Yu, Y. Zhu, Y.X. Zhang, J. Li, Q. Fang, J.Z. Xi, B. Yao, *Talanta*, 85(2011) 1760.
16. D.M. Duan, K.X. Zheng, Y. Shen, R. Cao, L. Jiang, Z.X. Lu, X.Y. Yan, J. Li, *Nucleic Acids Res*. 39 (2011) e154.
17. D.C. Zhang, Y.R. Yan, W. Cheng, W. Zhang, Y.H. Li, H.X. Ju,S.J. Ding, *Microchim. Acta*, 180(2013)397.
18. Y.T. Zhou, Q. Huang, J.M. Gao, J.X. Lu, X.Z. Shen, C.H. Fan, *Nucleic Acids Res*. 38(2010)e156.
19. K.A. Cissell, Y. Rahimi , S. Shrestha, E.A. Hunt, S.K. Deo, *Anal. Chem*. 80(2008)2319.
20. H.X. Jia, Z.P. Li, C.H. Liu, Y.Q. Cheng, *Angew. Chem. Int. Ed*. 49(2010)5498.
21. J.D. Driskell, A.G. Seto, L.P. Jones, S. Jokela, R.A. Dluhy, Y.P. Zhao, R.A. Tripp, *Biosens. Bioelectron.* 24(2008):917.
22. Z. Gao, Y. Peng, *Biosens. Bioelectron*. 26(2011)3768.
23. H.S. Yin, Y.L. Zhou, H.X. Zhang, X.M. Meng, S.Y. Ai, *Biosens. Bioelectron*. 33(2012):247.
24. G. Ziyatdinova, J. Galandova, J. Labuda, *Int. J. Electrochem. Sci*. 3 (2008) 223.
25. O.A. Arotiba, P.G. Baker, B.B. Mamba, E.I. Iwuoha, *Int. J. Electrochem. Sci*. 6 (2011) 673
26. P. Yakovchuk, E. Protozanova, M.D. Frank-Kamenetskii, *Nucleic Acids Res*. 34 (2006) 564.
27. R. Luo, H.S.R. Gilson, M.J. Potter, M.K. Gilson, *Biophysical Journal*, 80(2001)140.
28. J. Sponer, K.E. Riley, P. Hobza, *Physical Chemistry Chemical Physics*, 10(2008)2595.
29. H. Arata, H. Komatsu, A. Han, K. Hosokawa, M. Maeda, *Analyst*, 137(2012)3234.
30. L. Jiang, D.M. Duan, Y. Shen, J. Li, *Biosens. Bioelectron*. 34 (2012) 291.
31. Z.X. Lu, D.M. Duan,R. Cao, L.M. Zhang, K.X. Zheng, J. Li, *Chem. Com*mun. 47(2011)7452.
32. W.M. Zheng, T.E. Chasea, L. He, *Analytical Methods*, 6(2014)2399.
33. M. Labid, S.M. Ghobadloo, N. Khan, D.M. Kolpashchikov, M.V. Berezovski, *Anal. Chem.* 85(2013) 9422.
34. F. Dong, R. Luo, H. Chen, W. Zhang, S.J. Ding, *Int. J. Electrochem. Sci.*, 9 (2014) 6924 - 6935
35. C.C. Chang, S.C. Wei, T.H. Wu, C.H. Lee, C.W. Lin, *Biosens. Bioelectron*. 42 (2013) 119.
36. Y.X. Zhao, L. Qi, F. Chen, Y. Zhao, C.H. Fan, *Biosens. Bioelectron*. 41 (2013) 764.
37. D. Duan, K.X. Zheng, Y. Shen, R. Cao, L. Jiang, Z.X. Lu, X.Y. Yan, J. Li, *Nucleic Acids Res*. 39 (2011) 22.
38. W.P. Liu, X.M. Zhou, D. Xing, *Biosens. Bioelectron*. 58 (2014) 388.
39. N. Xia, X.J. Wang, D.H. Deng, G.F. Wang, H.Y. Zhai, S.J. Li, *Int. J. Electrochem. Sci.*, 8 (2013) 9714 – 9722
40. Q. Li, W. Cheng, D.C. Zhang, T.X. Yu, Y.B. Yin, H.X. Ju, S.J. Ding, *Int. J. Electrochem. Sci.*, 7 (2012) 844 - 856
41. T.X. Yu, J.B. Li, Q.F. Liu, W. Cheng, D.C. Zhang, H.X. Ju, S.J. Ding, *Int. J. Electrochem. Sci.*, 7 (2012) 8533 – 8542.
42. R. Luo, W. Zhang, W. Cheng, D. Zhao, Y.H. Li, X.J. Li, F. Dong, S.J. Ding, *Int. J. Electrochem. Sci.*, 8 (2013) 3186 – 3196
43. Z.M. Cai, Y.L. Song, Y.F. Wu, Z. Zhu, C.Y. James Yang, X. Chen, 41(2013) 783, *Biosens. Bioelectron.*