analytical chemistry

Article

pubs.acs.org/ac

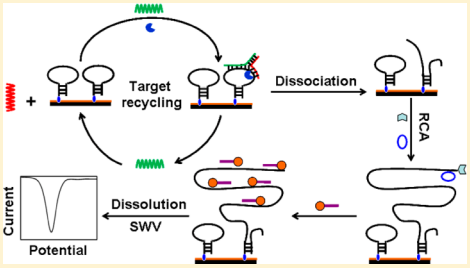
Ultrasensitive Electrochemical Detection of Nucleic Acids by Template Enhanced Hybridization Followed with Rolling Circle Amplification

Hanxu Ji,[†] Feng Yan,[‡] Jianping Lei,[†] and Huangxian Ju*,[†]

[†]State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, P.R. China

[‡]Jiangsu Institute of Cancer Prevention and Cure, Nanjing 210009, P.R. China

ABSTRACT: An ultrasensitive protocol for electrochemical detection of DNA is designed with quantum dots (QDs) as a signal tag by combining the template enhanced hybridization process (TEHP) and rolling circle amplification (RCA). Upon the recognition of the molecular beacon (MB) to target DNA, the MB hybridizes with assistants and target DNA to form a ternary "Y-junction". The target DNA can be dissociated from the structure under the reaction of nicking endonuclease to initiate the next hybridization process. The template enhanced MB fragments further act as the primers of the RCA reaction to produce thousands of repeated oligonucleotide sequences, which can bind with oligonucleotide functionalized QDs. The attached signal tags can be easily read out by square-wave voltammetry after dissolving with acid. Because of the cascade signal amplification and the specific TEHP and RCA reaction, this newly designed protocol provides an ultrasensitive electrochemical detection of DNA down to the attomolar level (11 aM) with a linear range of 6 orders of magnitude (from 1×10^{-17} to 1×10^{-11} M) and can discriminate mismatched DNA from perfect matched target DNA with high selectivity. The high sensitivity and specificity make this method a great potential for early diagnosis in gene-related diseases.



Sequence-specific analysis of nucleic acid with extremely low abundance is essential to genetic research of diseases, clinical diagnosis, and clinical therapy. A number of techniques such as molecular biology,[1] electrochemistry,[2−4] spectroscopy,[5−9] and colorimetry[10−13] have been applied to achieve sensitive detection of nucleic acids. Especially, signal amplification strategies have been introduced into these detection tools to achieve the ultrasensitive detection of DNA at a low level, even in single molecule detection.[14,15] Besides the common polymerase chain reaction, the signal amplification can generally be achieved by two ways: amplifying the signal by large loading of signal labels such as enzymes[16,17] or nanomaterials[18,19] and enhancing the intermediate product by target cycling. The latter means that a single target can be amplified to cyclically produce many hybridization events. Typically, the target DNA cycling methods via nuclease, e.g., endonuclease,[20−24] polymerase,[25−27] and exonuclease[28,29] have been widely employed to realize the trace level detection of target DNA with the specific recognition of sequences.

Recently, nicking endonuclease has been of increasing interest for applications in biological detection due to its strong recognition for DNA sequences.[20−24] For example, a homogeneous colorimetric DNA detection has been developed by nicking endonuclease assisted amplification for 10^3-fold improvement of selectivity in distinguishing a single-base mismatch oligonucleotide.[20] However, nicking endonuclease is sequence-specific, which limits the range of detectable target DNA sequences. To address this problem, a Y-shaped junction structure consisting of three complementary oligonucleotide branches has been introduced to overcome the disadvantage of limited nicking endonuclease recognition.[30,31] The Y-shaped structure can be cleaved in the presence of nicking endonuclease to release the target DNA and signal labels such as fluorescent molecules[30,32,33] and an electrochemical active probe.[34] The released target DNA then initiated the next hybridization process, leading to a so-called "template enhanced hybridization process" (TEHP) for isothermal amplification of the cleaved nucleic acids, which possess the same amount as the released signal labels.[30−34] This work further combined the TEHP with rolling circle amplification (RCA) for prolonging the cleaved molecular beacon (MB) fragments. Upon a binding process with oligonucleotide functionalized signal labels, the combination of two signal amplification ways led to the attachment of a mass of signal labels on the prolonged MB fragments, leading to an extremely sensitive strategy for detection of target DNA with low abundance.

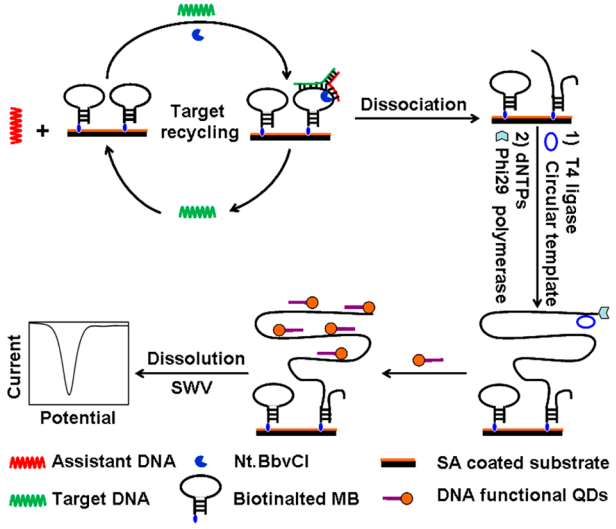
As an advanced DNA amplification technique, RCA can achieve a great signal amplification via production of thousands of repeated sequences. This technique possesses the significant advantages such as mild reaction conditions, speediness, high efficiency, good sensitivity, and specificity.[35] Thus, it has widely been employed for the analysis of proteins and nucleic acids by

Received: June 4, 2012
Accepted: July 23, 2012
Published: July 23, 2012

   dx.doi.org/10.1021/ac3015356 | *Anal. Chem.* 2012, 84, 7166−7171

coupling with electrochemistry,[36−39] chemiluminescence,[40,41] surface-enhanced Raman,[42] and colorimetry.[43,44] On the basis of our previous work,[37] this work immobilized MB on a substrate by a highly specific biotin-streptavidin (SA) system to recognize the target DNA and produce a Y-shaped junction structure for performing the TEHP (Scheme 1). The TEHP

Scheme 1. Schematic Representation of the Cascade Signal Amplification Strategy for DNA Detection



could cleave a large number of immobilized MB with the initiation by a few target DNA molecules. Every cleaved MB fragment was then used as the primer to trigger the RCA reaction. Using oligonucleotide functionalized quantum dot (QDs) as a model of a signal tag to bind the repeated oligonucleotide sequences, an extremely sensitive method for electrochemical detection of low abundance DNA was thus proposed by stripping voltammetric analysis. This protocol could detect DNA down to the attomolar level with a linear range of 6 orders of magnitude and could discriminate mismatched DNA from perfectly matched target DNA. This strategy of cascade signal amplification could be extended to other analytical techniques by changing the signal tag. This method provided a versatile tool in detecting nucleic acids with extremely low abundance in bioanalysis and clinical biomedicine.

EXPERIMENTAL SECTION

Materials and Reagents. The SA-coated microplates were purchased from Greiner bio-one (Germany). Tris-(hydroxymethyl) aminomethane (Tris) and polyethylene glycol sorbitan monolaurate (Tween-20) were purchased from Sigma-Aldrich Inc. Mercaptoacetic acid and cadmium chloride hemipentahydrate ($CdCl_2·2.5H_2O$) were purchased from Alfa Aesar China, Ltd. Phi29 DNA polymerase, RCA buffer, and dNTP were obtained from Fermentas (Lithuania). T4 DNA ligase and ligase buffer were obtained from Promega. Hybridization buffer (pH 7.4) contained Tris (10 mM), ethylenediaminetetraacetic acid (EDTA) (1 mM), and NaCl (100 mM). Rinsing buffer (pH 7.4) contained Tris (10 mM), EDTA (1 mM), NaCl (100 mM), and Tween-20 (0.1%). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore, Billerica, MA) was used in all runs. Nicking endonuclease (Nt.BbvCI) and NEB buffer 4 were purchased from New England Biolabs (Ipswich, MA) and used without further purification. All other reagents were of analytical grade without further purification. The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography: (a) target DNA, 5′-CCGAAGAGACCTTTCCGTTCGAC-3′; (b) assistant DNA, 5′-GCTGAGGAAAAAAGGTCTCTTCGG-3′; (c) MB, 5′-biotin-TTTTTTTCTGTGTCATTAGACCGTC-GAACGGTCCTCAGCAATGAAAGACACAG-3′; (d) padlock probe, 5′-p-GACGGTCTAAACCCAACCCGCCC-TACCCAAAACCCAACCCGCC CTACCCAAAACC-CAACCCGCCCTACCCAAGGACCGTTC-3′; (e) DNA for functionalization of QDs, 5′-SH-$(CH_2)_6$-GGGTAGGGCGGGTTGGG-3′; (f) control probe, 5′-SH-$(CH_2)_6$-GGACCGTTCGACGGTCTAATGACACAG-3′.

Apparatus. Electrochemical measurements were performed on a CHI 430A electrochemical workstation (CH Instruments Inc.) with conventional three-electrode system consisting of mercury film modified glassy carbon electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire as the counter electrode. The UV−vis absorption and photoluminescence spectra were recorded with a UV-3600 UV−vis-near-infrared (NIR) spectrophotometer (Shimadzu, Japan) and a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., U.K.).

Preparation of DNA Functionalized CdTe QDs. The synthesis of water-soluble mercaptopropionic acid (MPA)-capped CdTe QDs referred to the method reported previously.[37] The obtained QD solution was subjected to ultrafiltration using a Vivaspin concentrator (Sartorius, 10 000 MW) at 10 000*g* for 10 min to remove excessive MPA. The upper phase was washed twice with water and diluted to a certain concentration with water. The resulting CdTe QDs solution could be stable for 3 months, and the concentration and the size of QDs were calculated with the UV−vis absorption. Then MPA-capped CdTe QDs (20 nmol) were mixed with 100 nmol of thiolated DNA probe in 500 μL of 50 mM, pH 7.4 Tris-HCl buffer and left overnight under shaking and free of light. The resulting mixture was ultrafiltrated using the Vivaspin concentrator (10 000 MW) at 10 000*g* for 10 min at 4 °C to remove the nonconjugated DNA. The obtained conjugates were washed thrice with 50 mM Tris-HCl buffer by ultrafiltration to obtain the CdTe QD-DNA probe, which was kept at 4 °C prior to use.

Immobilization of MB on Substrate. The wells of the SA-coated plates (growth area, 0.35 cm^2; total volume, 323 μL) with high capacity were used as the substrate. After the well was washed twice with 100 μL of ultrapure water, the MB was conjugated to the substrate by adding 5 μL of biotin labeled MB solution (1.0×10^{-5} mol/L) and 45 μL of hybridization buffer into the well and incubating for 2 h at room temperature. The solution was then removed. In order to remove the unconjugated DNA on the substrate, the well was washed three times with 100 μL of rinsing buffer and two times with 100 μL of ultrapure water, respectively.

Template Enhanced Hybridization. The TEHP was performed in 50 μL of reaction solution which contained 1× NEB buffer 4, 5 nM assistant DNA, 1 U Nt.BbvCI, and varying concentrations of target DNA. After incubation at 37 °C for 30 min, the well was washed three times with 100 μL of rinsing buffer and two times with 100 μL of ultrapure water, respectively.

dx.doi.org/10.1021/ac3015356 | *Anal. Chem.* 2012, 84, 7166−7171

Rolling Circle Amplification and QD Tagging. A volume of 50 μL of solution containing 1× ligase buffer, 5 nM padlock probe, 1 U T4 ligase was hybridized with the MB fragments produced in TEHP on the substrate at 37 °C for 1 h. The introduction of the T4 ligase made the 5′ and 3′ termini of the padlock probe link together to form a circular template. The excess circular template was removed by washing three times with 100 μL of rinsing buffer and twice with 100 μL of ultrapure water, respectively. The RCA reaction was initiated by addition of 1 U of phi29 DNA polymerase and 10 mM dNTP in 50 μL of RCA buffer and incubated for 1 h at 37 °C.

After the wells were carefully washed thrice with 100 μL of rinsing buffer and twice with 100 μL of ultrapure water, 100 μL of 20 nM QD-DNA probe was added to each well and hybridized at 37 °C for 30 min. After the microplates were carefully washed thrice with 100 μL of rinsing buffer to remove the excess QD-DNA probe, 100 μL of 0.1 M HNO_3 was added to each well to dissolve the tagged QDs under sonication. The resulting Cd^{2+} solutions were used for electrochemical detection.

Electrochemical Detection. The obtained solutions of Cd^{2+} were mixed with 900 μL of 0.2 M, pH 4.6 HAc-NaAc buffer to perform square-wave voltammetric (SWV) analysis using a mercury film coated glassy carbon electrode. The mercury film coated working electrode was prepared by 4 cycles of alternate deposition at −1.0 V for 40 s and scan from −0.9 to −0.3 at 0.1 V s^{-1} in 0.2 M, pH 4.6 HAc-NaAc buffer containing 40 μg mL^{-1} Hg^{2+} under N_2 atmosphere. The SWV detection was carried out by electrodepositing cadmium at −1.1 V for 6 min and stripping from −0.9 to −0.3 V under N_2 atmosphere using a square wave with 25 mV amplitude, 4 mV potential step, and 25 Hz frequency.

RESULTS AND DISCUSSION

Design of Strategy. In order to realize the ultrasensitive detection of target DNA, the cascade signal amplification was performed with the nicking endonuclease assisted TEHP, RCA, QDs tagging, and stripping voltammetric analysis. As shown in Scheme 1, the biotinylated MB was first attached to the SA-coated 96-plates via the biotin-SA affinity binding. After washing out the excess biotinylated MB, a mixture of target DNA, assistant DNA, and nicking endonuclease Nt.BbvCI was added on the MB modified substrate for incubation at 37 °C. The target DNA, assistant DNA, and MB hybridized to form a stable "Y" junction structure. In this structure, the double-stranded DNA consisted of assistant DNA and MB provided the specific nucleotide sequence for recognition and cleaving by nicking endonuclease Nt.BbvCI. After the nicking endonuclease cleaved the specific nucleotide sequence on MB, the target DNA and assistant DNA spontaneously dissociated from the Y structure at the surface of the substrate. Therefore, amplification is accomplished by hybridization of the dissociated assistant DNA and target DNA with another MB to continue the strand-scission cycle, which meant one target DNA resulted in the cleavage of many MBs to form MB fragments. On the contrary, in the absence of target DNA, although assistant DNA has some matched bases with the MB, they could not form a stable double-stranded structure due to the short matched nucleotide bases.

After the circular template was formed by incubating the padlock probe and T4 ligase on the MB fragment captured substrate, the RCA reaction was initiated by adding Phi29 DNA polymerase and dNTPs. The RCA product contained hundreds of tandem-repeat sequences for the attachment of a large number of CdTe QD-DNA probes. Lastly, the cadmic component of the resulting QD-tagged RCA product was dissolved with HNO_3 and quantified by the SWV method. This strategy could present a target DNA recognition event to a large number of quantum dot tags for greatly enhancing the signal. Moreover, the QDs contained a large number of detection reporters, and the stripping voltammetric analysis further enhanced the sensitivity of the proposed protocol. The cascade signal amplification strategy provided a new possibility for ultrasensitive detection of DNA.

Characterization of QD-DNA Probe. The fluorescence spectrum and UV−vis absorption of QDs were examined to prove the successful synthesis of QDs and the QD-DNA probe. From the UV−vis absorption spectrum of the MPA-capped CdTe QDs solution (Figure 1, curve a), the size of QDs was

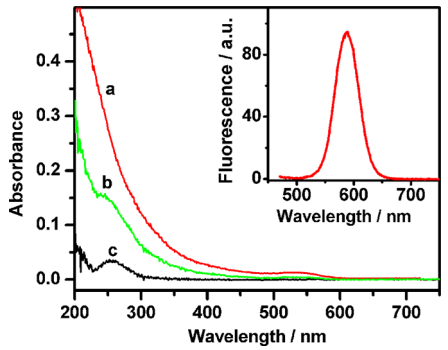


Figure 1. Absorption spectra of MPA-capped CdTe QDs (a), DNA functionalized QDs (b), and pure DNA (c). Inset: PL emission spectrum of MPA-capped CdTe QDs.

calculated to be 2.8 nm with a narrow distribution.[45] This result was also verified by the photoluminescence (PL) spectrum with a half-wave width less than 46 nm (Figure 1, inset). For tagging QDs to the tandem-repeat sequences of the RCA product, the CdTe QDs were further functionalized with a thiolated oligonucleotide probe, which possessed a complementary sequence with RCA products. The obtained QD-DNA probe was confirmed by comparison of absorption spectra of QDs, the DNA functionalized QDs, and pure DNA (Figure 1). The pure QDs only showed an absorption peak around 539 nm (curve a). A new absorption peak of the DNA functional QDs appeared at 260 nm (curve b), which should be contributed to the adsorption of DNA (curve c). This result indicated the successful functionalization of QDs with the oligonucleotide probe via Cd−S chemistry between the thiolatled oligonucleotide probe and the MPA-capped CdTe QDs.

Signal Amplification of RCA and TEHP. To prove the signal amplification of the RCA strategy, the DNA functionalized QDs were directly hybridized with the cleaved MB fragments for comparison. In absence of RCA, the strategy integrating TEHP with functionalized QDs only showed a detection limit of about 10 fM for target DNA (Figure 2). In contrast, after RCA, the electrochemical response to target DNA at the same concentration was significantly higher than that without RCA. After subtracting the response of the blank solution, the peak currents obtained with the RCA method for 100 and 10 fM of target DNA were about 10.9 and 18.0 times higher than those without RCA, respectively. Moreover, the signal for 10 fM of target DNA was even higher than that for 10 pM target DNA without RCA, showing the remarkable

dx.doi.org/10.1021/ac3015356 | Anal. Chem. 2012, 84, 7166−7171

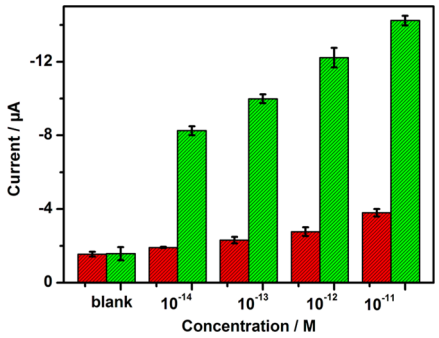


Figure 2. Stripping voltammetric signals of DNA functionalized QDs without RCA (red) and with RCA (green) for detection of target DNA.

amplification performance of RCA. A more large scale QD-tagged RCA product could be generated at lower target DNA concentration due to the excess substrate and decreasing steric inhibition. Thus, the amplification efficiency increased with the decreasing of target DNA concentration, which greatly improved the sensitivity of the designed strategy for detection of low abundance DNA. The stripping voltametric measurements showed low background response, which could be attributed to the highly specific recognition of the immobilized MB to the target DNA and the specific TEHP and RCA.

Further, an experiment was investigated to confirm the signal amplification of the TEHP. As shown in Figure 3, the stripping

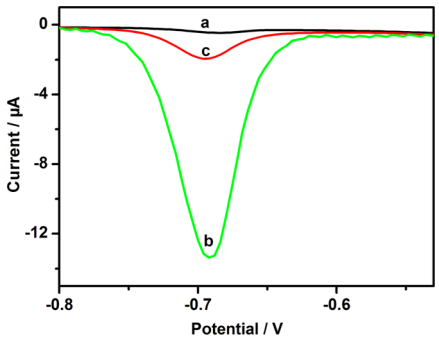


Figure 3. Stripping voltammetric curves of cadmic cation responding to 1 pM target DNA without (a) and with (b) nicking endonuclease and in the absence of target DNA (c).

voltammogram without nicking endonuclease did not show an obvious electrochemical response of the cadmic cation reporter (curve a). This indicated that the "Y" structure could not dissociate in the absence of nicking endonuclease, thus the RCA reaction could not happen. On the contrary, in the presence of target DNA, assistant DNA, and nicking endonuclease, the electrochemical response of the cadmic cation reporter enhanced by 29.1 times in comparison to the signal in the absence of nicking endonuclease (curve b) indicates the high amplification efficiency of TEHP. In addition, the electrochemical response in the absence of target DNA was very low due to the lack of stable cleaving sites, because the reaction temperature of 37 °C was higher than the melting temperature of the hybridization product of assistant DNA and the MB (curve c). These results showed that the TEHP was significant for improving the electrochemical signal and obtaining the high signal-to-noise ratio.

Time Dependence of Cascade Signal Amplification. The SWV signal was dependent on the amount of cleaved MB fragments, which could be bound with the RCA circular template. As shown in Figure 4A, with the increasing reaction

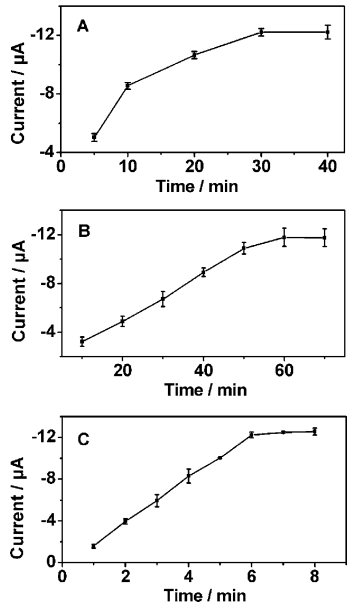


Figure 4. Effects of (A) time of TEHP, (B) RCA reaction time, and (C) predeposition time of the cadmic cation on stripping voltammetric signals responding to 1 pM target DNA.

time, more cleaved MB fragments could be formed in TEHP, which produced more RCA circular templates and led to rapidly increased peak current. The peak current trended to a constant value at 30 min, indicating the saturated cleavage of the immobilized MB due to the decreased activity of nicking endonuclease. Although a long time of TEHP was expected to generate more complementary cleaved MB fragments for the RCA circular templates, considering the convenience and the sensitivity, 30 min was selected as the optimal time for the TEHP.

The effect of RCA reaction time on the electrochemical signal was also investigated. As shown in Figure 4B, the peak current increased rapidly with the augment of the reaction duration since more repeated sequences could be formed and hybridize with the QDs-DNA probe. The maximum peak current was observed when the RCA reaction was maintained at about 60 min. Afterward, the peak decreased slightly possibly due to the entanglemant of RCA products with each other,[36] which hindered the hybridization with the QDs-DNA probe. Therefore, 60 min was chosen as the reaction duration for RCA.

Upon the dissolution of CdTe QDs with HNO_3, the dissolved cadmic cation reporter could be conveniently detected by SWV. A distinct stripping voltammetric peak of cadmic cation reporter could be observed around −0.70 V in 0.2 M, pH 4.6 HAc-NaAc buffer. To achieve a sensitive and stable electrochemical signal, the predeposition time was optimized. As shown in Figure 4C, with the increasing predeposition time, the SWV response increased and reached a plateau at 6 min. Thus, 6 min was selected as the optimal time for all the electrochemical measurements.

Stripping Voltammetric Analysis of Target DNA. In view of the outstanding ability for signal amplification, the

dx.doi.org/10.1021/ac3015356 | Anal. Chem. 2012, 84, 7166−7171

dynamic range of the designed method for target DNA was examined (Figure 5). The stripping peak current of the cadmic

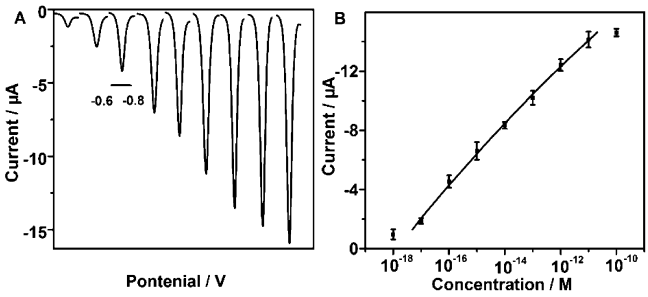


Figure 5. (A) Stripping voltammetric curves of cadmic cation responding to 10^{-18}, 10^{-17}, 10^{-16}, 10^{-15}, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, and 10^{-10} M target DNA (left to right). (B) Quantitative dynamic range.

cation was proportional to the logarithm value of the target DNA concentration over a 6-decade range from 10 aM to 10 pM with a linear correlation coefficient of 0.995. The corresponding detection limit was calculated to be 11.0 aM by evaluating 3 times the average standard deviation of the blank response, which corresponded to 0.55 zmol of DNA target in 50 μL of solution. The detection limit of this method was much lower than the combination of RCA with surface enhanced Raman (10 pM),[42] colorimetric detection of RCA with gold nanoparticle aggregates (70 fM),[43] and competitive with that of the PCR technique.[1] Thus the results identified that this signal amplification method was efficient for ultrasensitive electrochemical detection of DNA hybridization.

The specificity of the proposed detection method was investigated by exposing the immobilized MB to four kinds of DNA sequences, including perfect complementary target, single-base mismatched oligonucleotide, three-base of mismatched oligonucleotide, and noncomplementary oligonucleotide at the same concentration (1 pM). The perfect complementary target showed a signal of 3.8-fold, 5.7-fold, and 6.6-fold of single-base mismatched oligonucleotide, three-base mismatched oligonucleotide, and noncomplementary oligonucleotide, respectively (Figure 6). This method exhibited good performance to discriminate perfect complementary target and the base mismatched targets and great potential for single nucleotide polymorphism analysis.

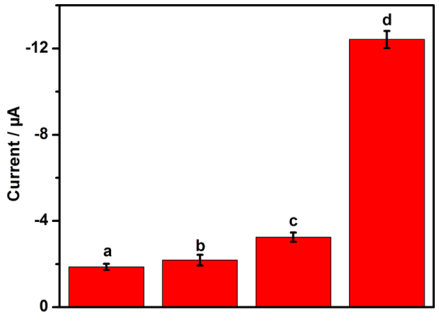


Figure 6. Stripping voltammetric peak currents at 1 pM of noncomplementary oligonucleotide (a), single-base mismatched oligonucleotide (b), three-base of mismatched oligonucleotide (c), and perfect complementary target (d).

CONCLUSIONS

This work has designed an electrochemical platform for ultrasensitive DNA detection based on the combination of two typical signal amplification ways. TEHP can produce a large number of cleaved MB fragments with the initiation of a few target DNA molecules, which then act as the primers of the RCA to initiate the reaction of the RCA for realizing the dual signal amplification as reported previously.[46] The amount of target DNA can be easily read out through the dissolved cadmic cation with SWV measurement. By integrating molecular biological technology, nanobiotechnology, and electrochemical detection, this novel cascade signal amplification strategy can detect target DNA down to the attomolar level with high selectivity to differentiate mismatched DNA. The high sensitivity and selectivity of this strategy might be attributed to three factors: (1) the TEHP and QD-tagged RCA products offer an enormous ratio of metal component to target DNA and dramatically increase the sensitivity; (2) the specific cleaving site of the "Y-junction" provides excellent selectivity toward sequence mismatch DNA, showing great potential for single nucleotide polymorphism analysis; (3) the specific TEHP and RCA reaction provides low background. This primary research opens new horizons for integrating different disciplines. Moreover, this strategy of cascade signal amplification is suitable for combining with other analytical techniques by changing the signal tag. This method provides a versatile tool in detecting nucleic acids for bioanalytical and clinical biomedicinal applications.

AUTHOR INFORMATION

Corresponding Author

*Phone/Fax: +86-25-83593593. E-mail: hxju@nju.edu.cn.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the National Basic Research Program of China (Grant 2010CB732400), the National Natural Science Foundation of China (Grants 21121091, 21135002, and 20835006), and the Program for Leading Medical Talents from the Department of Health of Jiangsu Province.

REFERENCES

(1) Kuang, H.; Zhao, S. G.; Chen, W.; Ma, W.; Yong, Q. Q.; Xu, L. G.; Wang, L. B.; Xu, C. L. *Biosens. Bioelectron.* **2011**, *29*, 2495−2499.
(2) Hsieh, K.; White, R. J.; Ferguson, B. S.; Plaxco, K. W.; Xiao, Y.; Soh, H. T. *Angew. Chem., Int. Ed.* **2011**, *50*, 11176−11180.
(3) Xiao, Y.; Lou, X. H.; Uzawa, T.; Plakos, K. J. I.; Plaxco, K. W.; Soh, H. T. *J. Am. Chem. Soc.* **2009**, *131*, 15311−15316.
(4) Zhang, X. L.; Li, L. L.; Li, L.; Chen, J.; Zou, G. Z.; Si, Z. K.; Jin, W. R. *Anal. Chem.* **2009**, *81*, 1826−1832.
(5) Dong, H. F.; Gao, W. C.; Yan, F.; Ji, H. X.; Ju, H. X. *Anal. Chem.* **2010**, *82*, 5511−5517.
(6) Gerasimova, Y. V.; Peck, S.; Kolpashchikov, D. M. *Chem. Commun.* **2010**, *46*, 8761−8763.
(7) Wang, F.; Johann, E.; Teller, C.; Willner, I. *Angew. Chem., Int. Ed.* **2011**, *50*, 295−299.
(8) Zuo, X. L.; Xia, F.; Xiao, Y.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, *132*, 1816−1818.
(9) Cheng, Y. N.; Tim, S.; Pol, V. D.; Liesbet, L.; Wang, M.; Chen, H. Z.; Gustaaf, B. *Anal. Chem.* **2011**, *83*, 1307−1314.
(10) Ji, H. X.; Dong, H. F.; Yan, F.; Lei, J. P.; Ding, L.; Gao, W. C.; Ju, H. X. *Chem.—Eur. J.* **2011**, *17*, 11344−11349.
(11) Liu, J. W.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 90−93.

(12) Xia, F.; Zuo, X. L.; Yang, R. Q.; Xiao, Y.; Kang, D.; Vallée-Bélisle, A.; Gong, X.; Yuen, J. D.; Hsu, B. B. Y.; Heeger, A. J.; Plaxco, K. W. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 10837−10841.
(13) Zheng, X. X.; Liu, Q.; Jing, C.; Li, Y.; Li, D.; Luo, W. J.; Wen, Y. Q.; He, Y.; Huang, Q.; Long, Y. T.; Fan, C. H. *Angew. Chem., Int. Ed.* **2011**, *50*, 11994−11998.
(14) Comstock, M. J.; Ha, T.; Chemla, Y. R. *Nat. Methods* **2011**, *8*, 335−340.
(15) Sorgenfrei, S.; Chiu, C. Y.; Gonzalez, R. L., Jr.; Yu, Y.-J.; Kim, P.; Nuckolls, C.; Shepard, K. L. *Nat. Nanotechnol.* **2011**, *6*, 126−132.
(16) Gao, Z. Q.; Siti, R.; Lay, H. L. *Adv. Mater.* **2007**, *19*, 602−606.
(17) Gao, W. C.; Dong, H. F.; Lei, J. P.; Ji, H. X.; Ju, H. X. *Chem. Commun.* **2011**, *47*, 5220−5222.
(18) Numnuam, A.; Chumbimuni-Torres, K. Y.; Xiang, Y.; Bash, R.; Thavarungkul, P.; Kanatharana, P.; Pretsch, E.; Wang, J.; Bakker, E. *J. Am. Chem. Soc.* **2008**, *130*, 410−411.
(19) Liao., W. C.; Ho, A. H. *Anal. Chem.* **2009**, *831*, 2470−2476.
(20) Xu, W.; Xue, X. J.; Li, T. H.; Zeng, H. W.; Liu, X. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 6849−6852.
(21) Bi, S.; Zhang, J. L.; Zhang, S. S. *Chem. Commun.* **2010**, *46*, 5509−5511.
(22) Li, J. J.; Chu, Y. Z.; Lee, B. Y. H.; Xie, X. L. S. *Nucleic Acids Res.* **2008**, *36*, e36.
(23) García, T.; Revenga-Parra, M.; Sobrino, B.; Carracedo, A.; Alonso, C.; Lorenzo, E.; Pariente, F. *Biosens. Bioelectron.* **2011**, *29*, 40−45.
(24) Zhu, X. L.; Zhao, J.; Wu, Y.; Shen, Z. M.; Li, G. X. *Anal. Chem.* **2011**, *83*, 4085−4089.
(25) Guo, Q. P.; Yang, X. H.; Wang, K. M.; Tan, W. H.; Li, W.; Tang, H. X.; Li, H. M. *Nucleic Acids Res.* **2009**, *37*, e20.
(26) Connolly, A. R.; Trau, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 2720−2723.
(27) Ren, R.; Leng, C. C.; Zhang, S. S. *Chem. Commun.* **2010**, *46*, 5758−5760.
(28) Su, J.; Zhang, H. J.; Jiang, B. Y.; Zheng, H. Z.; Chai, Y. Q.; Yuan, R.; Xiang, Y. *Biosens. Bioelectron.* **2011**, *29*, 184−188.
(29) Yang, C. Y. J.; Cui, L.; Huang, J. H.; Yan, L.; Lin, X. Y.; Wang, C. M.; Zhang, W. Y.; Kang, H. Z. *Biosens. Bioelectron.* **2011**, *27*, 119−124.
(30) Nakayama, S.; Yan, L.; Sintim, H. O. *J. Am. Chem. Soc.* **2008**, *130*, 12560−12561.
(31) Zhang, J.; Wu, X. Y.; Chen, P. P.; Lin, N. T.; Chen, J. H.; Chen, G. N.; Fu, F. F. *Chem. Commun.* **2010**, *46*, 6986−6988.
(32) Yan, L.; Nakayama, S.; Yitbarek, S.; Greenfield, I.; Sintim, H. O. *Chem. Commun.* **2011**, *47*, 200−202.
(33) Kong, R. M.; Zhang, X. B.; Zhang, L. L.; Huang, Y.; Lu, D. Q.; Tan, W. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2011**, *83*, 14−17.
(34) Wang, Q.; Yang, L. J.; Yang, X. H.; Wang, K. M.; He, L. L.; Zhu, J. Q.; Su, T. Y. *Chem. Commun.* **2012**, *48*, 2982−2984.
(35) Liu, D. Y.; Daubendiek, S. L.; Zillman, M. A.; Ryan, K.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 1587−1594.
(36) Zhang, S. B.; Wu, Z. S.; Shen, G. L.; Yu, R. Q. *Biosens. Bioelectron.* **2009**, *24*, 3201−3207.
(37) Cheng, W.; Yan, F.; Ding, L.; Ju, H. X.; Yin, Y. B. *Anal. Chem.* **2010**, *82*, 3337−3342.
(38) Su, H. L.; Yuan, R.; Chai, Y. Q.; Mao, L.; Zhu, Y. *Biosens. Bioelectron.* **2011**, *26*, 4601−4604.
(39) Wu, Z. S.; Zhou, H.; Zhang, S. B.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2010**, *82*, 2282−2289.
(40) Cheglakov, Z.; Weizmann, Y.; Basnar, B.; Willner, I. *Org. Biomol. Chem.* **2007**, *5*, 223−225.
(41) Bi, S.; Li, L.; Zhang, S. S. *Anal. Chem.* **2010**, *82*, 9447−9454.
(42) Hu, J.; Zhang, C. Y. *Anal. Chem.* **2010**, *82*, 8991−8997.
(43) Li, J. S.; Deng, T.; Chu, X.; Yang, R. H.; Jiang, J. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2010**, *82*, 2811−2816.
(44) Ali, M. M.; Li, Y. F. *Angew. Chem., Int. Ed.* **2009**, *48*, 2512−2515.
(45) Yu, W. W.; Qu, L. H.; Guo, W. Z; Peng, X. G. *Chem. Mater.* **2003**, *15*, 2854−2860.
(46) Shen, Z. L.; Nakayama, S.; Semancik, S.; Sintim, H. O. *Chem. Commun.* **2012**, *48*, 7580−7582.