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Label-Free and Ultrasensitive Electrochemical Detection of Nucleic Acids Based on Autocatalytic and Exonuclease III-Assisted Target Recycling Strategy

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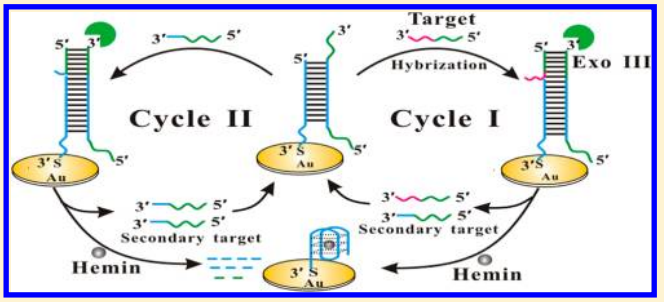
Supporting Information

ABSTRACT: In this work, a very simple, label-free, isothermal, and ultrasensitive electrochemical DNA biosensor has been developed on the basis of an autocatalytic and exonuclease III (Exo III)-assisted target recycling amplification strategy. A duplex DNA probe constructed by the hybridization of a quadruplex-forming oligomer with a molecular beacon is ingeniously designed and assembled on the electrode as recognition element. Upon sensing of the analyte nucleic acid, the strand of molecular beacon in the duplex DNA probe could be stepwise removed by Exo III accompanied by the releasing of target DNA and autonomous generation of new secondary target DNA fragment for the successive hybridization and cleavage process. Simultaneously, numerous quadruplex-forming oligomers are liberated and folded into G-quadruplex–hemin complexes with the help of K^+ and hemin on the electrode surface to give a remarkable electrochemical response. Because of this autocatalytic target recycling amplification and the specifically catalyzed formation of G-quadruplex–hemin complexes, this newly designed protocol provides an ultrasensitive electrochemical detection of DNA down to the 10 fM level, can discriminate mismatched DNA from perfectly matched target DNA, and holds a great potential for early diagnosis in gene-related diseases. It further could be developed as a universal protocol for the detection of various DNA sequences and may be extended for the detection of aptamer-binding molecules.



The development of DNA biosensors capable of amplified detection of DNA is being greatly motivated by its potential applications in molecular diagnostics, forensic investigations, genetics therapy, and biomedical development.[1−3] Till now, a number of techniques such as molecular biology,[4] electrochemistry,[5−7] spectroscopy,[8−10] and colorimetry[11−14] have been utilized toward the detection of nucleic acids. Especially, signal amplification strategies are often combined in the fabrication of DNA biosensor to improve detection sensitivity.[15,16] They can be generally categorized into two distinct types: postamplification strategy toward the signal produced by hybridization event and target recycling strategy. Examples of postamplification strategy include the use of enzymes,[17,18] metal nanoparticles,[19,20] DNA concatamer,[21] and DNA biobarcode[22] as amplifying labels. However, these methods usually involve multiple assay steps and require the addition of many exogenous reagents. The target DNA recycling methods via nuclease are especially attractive for the trace level detection of target DNA owing to its specific recognition of sequences.[23−27] Very recently, a simple and rapid exonuclease III (Exo III)-aided target recycling method has been proposed for amplified DNA detection.[28] Unlike nicking endonucleases,[25,29] Exo III is a sequence-independent enzyme which does not require a specific recognition site and can catalyze the stepwise removal of mononucleotides from 3′ terminus of double-stranded DNA in the case of substrate with a blunt or recessed 3′-terminus. It shows limited activity on single-stranded DNA or duplex DNAs with a protruding 3′ end. Thus, Exo III provides a more versatile platform for amplification detection of DNA.[30−33]

In order to further satisfy the requirements for the profiling low abundance of target DNA, the DNA-based machine has become an attractive tool for DNA detection that holds great promise to substitute polymerase chain reaction (PCR) due to its easy operation, isothermal reaction, and high sensitivity.[34−36] For example, the autonomous replication–scission–displacement process based on polymerase and nicking endonuclease was reported as an effective amplified replication system for the detection of DNA or RNA.[37−39] The ion-dependent DNAzyme was also utilized as a biocatalyst for the isothermal and autocatalytic detection of DNA.[40] A DNA nanomachine based

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on Exo III-assisted cascaded recycling amplification has also been designed for the label-free sensitive detection of DNA.[41] However, these DNA machine sensors are mainly operated by fluorescence signal transduction means and often confronted with relatively complex design procedures. By comparison, electrochemical methods are particularly attractive for DNA detection due to its superior features of simple and portable instrumentation, rapid response, and low cost.[5−7] Thus, the design of a convenient, isothermal, and autonomous amplification system for the label-free and sensitive electrochemical detection of nucleic acid is high desirable.

Herein, we developed a simple, isothermal, and label-free electrochemical DNA machine biosensor based on the ingenious combination of Exo III-assisted autocatalytic target recycling amplification and induced formation of G-quadruplex on the electrode surface. A DNA strand designed as quadruplex-forming oligomer was engineered to form inactive hybrids by binding with a molecular beacon and assembled on the electrode as a duplex DNA electrochemical probe for target DNA detection. This strategy of autocatalytic target recycling amplification could be used to detect DNA down to the 10 fM level and could discriminate mismatched DNA from perfectly matched target DNA. This method provides a versatile tool in detecting nucleic acids with low abundance in bioanalysis and clinical biomedicine.

EXPERIMENTAL SECTION

Reagents. The HPLC-purified oligonucleotide sequences are purchased from Sangon Biotech. Co., Ltd. (Shang hai, China) and listed below: the quadruplex-forming oligomer (S1), 5′-CACTGGGTTGGGCGGGATGGGTTTTTT-$(CH_2)_6$-SH-3′; the molecular beacon (S2), 5′-GGAAG-ACTCATGTTATCCCGCCCAACCCAGTGGAGTCTTCC-3′; the blocker DNA (S3), 5′-TTATCCCGCCCAACCCA-GTGGAGTCTTCC-3′; the 15-base target DNA (TD), 5′-GGAAGACTCTTGTCT-3′; one-base mismatched DNA sequence, 5′-GGAACACTCTTGTCT-3′; two-bases mismatched DNA sequence, 5′-GGAATGCTCTTGTCT-3′; non-complementary target DNA sequence, 5′-ACCGACGGTTTG-TCT-3′. Exonuclease III and 4-(2-hydroxyethyl)piperazine-1 ethanesulfonic acid sodium salt (HEPES) were also purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Hexaammineruthenium(III) chloride (RuHex), 6-mercaptohexanol, and hemin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and used without further purification. A hemin stock solution was prepared by dissolving 2 mg of hemin in 3 mL of DMSO and stored in the dark at −20 °C. All DNA sequences were diluted in 10 mM phosphate buffer (PB) (pH 7.4) to give stock solutions of 10 μM. All solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA) with resistivity of 18.2 MΩ cm.

Electrode Pretreatment. The gold electrode was cleaned by immersion in a freshly prepared piranha solution (a 3:1 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 20 min, followed by a thorough rinse with ultrapure water. *(CAUTION: "Piranha" solution reacts violently with organic materials; it must be handled with extreme care).* Then, the electrode was polished on a microcloth (Shanghai Chenhua Inc., China) with 50 nm alumina slurry to obtain a mirror surface, followed by sonication in acetone and ultrapure water for 5 min each, to remove residual alumina powder. The well-polished electrode was then subjected to electrochemical pretreatment by cycling the potential between −0.2 and 1.5 V in H_2SO_4 (0.5 M) at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was obtained, and then, the cleaned electrode was allowed to be dried at room temperature.

Immobilization of Duplex DNA Probe on Au Surface. The DNA duplex probe was first prepared by mixing the same concentration of S1 and S2 or S3 sequences in 10 mM PB solution (pH 7.4, 0.1 M NaCl). The DNA sequences were first heated to 90 °C for 5 min and then allowed to cool to room temperature for at least 2 h before use. The cleaned gold electrode was incubated into the above DNA duplex probe solution for 12 h at room temperature and then thoroughly rinsed with ultrapure water and dried under a stream of nitrogen gas. The electrode was subsequently immersed in 1 mM MCH solution for 1 h to remove the nonspecific DNA adsorption. Then, the electrode surface was rinsed thoroughly and dried in nitrogen.

Exo III-Assisted Autocatalytic Target Recycling. The DNA duplex probe modified gold electrode was then incubated into 70 μL of 10 mM Tris−HCl solution (pH 8.0, 50 mM NaCl, 10 mM $MgCl_2$) containing 1 unit/μL Exo III and different concentrations of target DNA for 120 min at 37 °C. Then, the resulting electrode was thoroughly rinsed and incubated with 0.2 mM hemin in 10 mM HEPES buffer (pH 8.0, 50 mM KCl, 1% DMSO) for 30 min to induce the liberated S1 on the electrode to fold into a G-quadruplex−hemin complex.

Electrochemical Measurement and Apparatus. Electrochemical measurements were conducted on CHI 832B and 660B electrochemical analyzers (CH Instruments) with a conventional three-electrode system comprising a gold working electrode, a platinum wire auxiliary electrode, and a saturated calomel reference electrode (SCE). The differential pulse voltammogram (DPV) was recorded in 20 mM HEPES buffer (pH 8.0, 20 mM KCl) with the potential window from −0.15 to −0.5 V. The electrochemical impedance spectroscopy (EIS) experiments were measured in 1 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.5 M KCl with the frequency range from 0.1 Hz to 10 kHz. The chronocoulometry measurements were taken over a range of 0 to −0.35 to 0 V at a pulse period of 250 ms with the RuHex as an indicator. Before measurements, the electrolyte solution should be thoroughly purged with high purity nitrogen for about 30 min, to avoid the interference from the reduction of oxygen.

RESULTS AND DISCUSSION

Design Principle of the Sensor. The new electrochemical strategy for ultrasensitive nucleic acid detection consists primarily of an inactive duplex DNA hybrid probe and Exo III-assisted autocatalytic target recycling module, which induced the catalyzed formation of G-quadruplex−hemin complexes on the electrode surface for the generation of amplified electrochemical signal (Scheme 1). The inactive hybrid probe was first prepared in the solution by hybridizing a DNA strand designed as a quadruplex-forming oligomer (denoted as S1) with a traditional molecular beacon (denoted as S2). The molecular beacon possess a loop region II that is complementary to S1, a target binding stem region I that is complementary to part of the sequence of the target DNA, and a stem region III in which part of the sequence is complementary to the sequence of region I. The duplex DNA probe was immobilized on the gold

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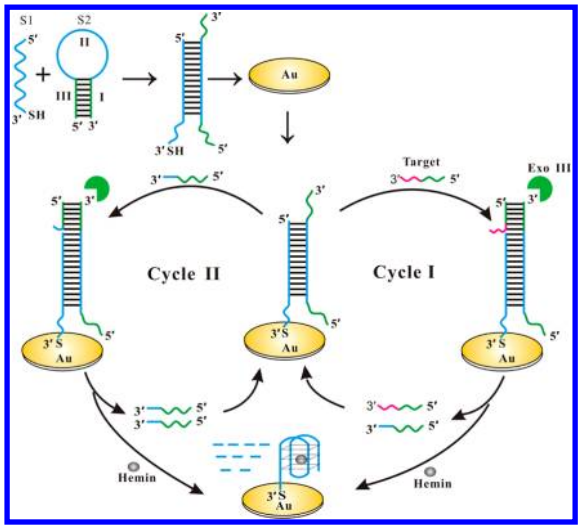
Scheme 1. Schematic Illustration of Exo III-Assisted Autocatalytic Target DNA Recycling Strategy for DNA Assay



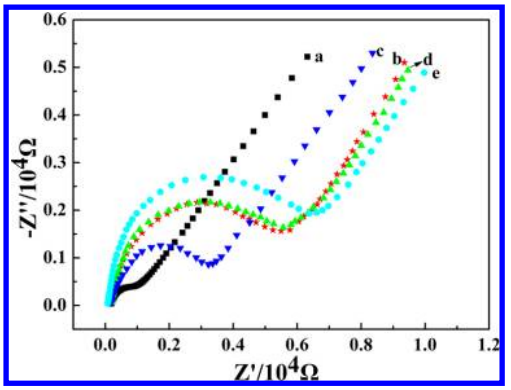


Figure 1. Nyquist diagrams for the electrochemical impedance measurements of the gold electrode at different modification stages. Curve (a) is for the bare gold electrode, while curves (b), (c), (d), and (e) are the cases after the duplex DNA probe immobilization, further treatment by target DNA and Exo III, control experiment using noncomplementary target DNA and Exo III, and the formation of G-quadruplex–hemin complex, respectively.

electrode by a strong Au–S interaction. The S1 is prevented from forming a hemin-containing active conformation owing to the hybridization of S1 with the region II within the strand of molecular beacon. This duplex DNA probe is ingeniously designed with the protruding 3′ end and cannot be digested by Exo III which specifically cleaves duplex DNA from blunt or recessed 3′ termini. When the probe is challenged with target DNA, the recognition of target DNA with the dangling ssDNA at the 3′ end of S2 by a template enhanced hybridization process leads the probe to have a blunt 3′-terminus.[42] Exo III then can catalyze the stepwise removal of mononucleotides from this terminus, releasing the target DNA and ultimately liberating the quadruplex-forming oligomer. Thus, a G-quadruplex–hemin complex can be formed on the electrode surface with the help of K^+ and hemin to give an electrochemical response.[43−48] Furthermore, the released target DNA is free to bind to another 3′-protruding terminus of duplex probe to trigger a new cleavage process, which constitutes the Cycle I in Scheme 1. At the same time, the DNA fragment III (the region III of S2) is also released during each cleavage process, which can be used as a secondary target DNA to activate the successive cleavage process. The secondary target DNA has a longer base sequence than that of region I. After its recognition with duplex DNA probe, the Exo III can also only catalyze the cleavage of duplex DNA probe, releasing again the DNA fragment III as secondary target DNA for the next cleavage cycle and liberating the quadruplex-forming oligomer for amplified electrochemical response. This constituted the Cycle II in Scheme 1. On the basis of the autonomous cleavage in cycle I and II, the target DNA trigger can be exponentially produced. Therefore, the current autocatalytic target recycling strategy by a simple Exo III cleavage process is hopeful to offer an ultrahigh sensitivity for the assay of nucleic acid.

Exo III-Assisted Autocatalytic Target DNA Recycling Amplification. The stepwise modification process of the gold electrode has been first confirmed by EIS measurements. Figure 1 shows the nyquist plots of EIS for the electrode modification at different stages. Obviously, the bare gold electrode exhibits a very small semicircle domain with charge transfer resistance (Rct) value of about 868 Ω (Figure 1a). Nevertheless, after immobilization of duplex DNA probe on the electrode, a big semicircle with Rct value of about 5947 Ω can be observed (Figure 1b), indicating an increase of the charge transfer resistance, which can be ascribed to the repellence of negatively charged redox species, $Fe(CN)_6^{3-/4-}$, from approaching electrode surface by negative-charged phosphate skeletons of duplex DNA probe. After further treatment with target DNA in the presence of Exo III, the diameter of the semicircle decreases dramatically with Rct value of about 3518 Ω (Figure 1c) since the DNA duplex has been transformed into the single-stranded oligomer accompanied by the decrease of negative charge of the electrode interface for the enhanced interfacial charge transfer, confirming the feasibility of the designed Exo III-assisted target recycling strategy. It should be noted that almost no change is observed for the diameter of the semicircle in the case of noncomplementary target DNA as an alternative or only Exo III indicates that almost no cleavage process occurs (Figure 1d). Furthermore, when the electrode is treated with K^+ and hemin, the Rct value is observed with a further increase to be about 6997 Ω compared with that of duplex DNA probe assembled electrode (Figure 1e). This may be explained that the formed G-quadruplex–hemin complexes make a negatively charged interface on the electrode surface to pack more orderly and densely, repelling the negatively charged redox species more strongly.[44]

To further verify the feasibility of designed Exo III-assisted autocatalytic target DNA recycling strategy, differential pulse voltammograms (DPV) obtained upon analyzing the target DNA in the presence of Exo III and those obtained in a series of control experiments are depicted in Figure 2. As shown in Figure 2a, a well-defined DPV peak at around −0.35 V can be observed in the presence of target DNA and Exo III. This peak can be contributed to the electrochemical reduction of the hemin incorporated in G-quadruplex.[43−47] However, in the absence of target DNA or Exo III, only small DPV peaks can be observed (Figure 2b,c), which was almost the same value with that of duplex DNA probe modified electrode treated only by K^+ and hemin (Figure 2d). This strongly indicated that only target DNA can trigger the cleavage process by Exo III to release the quadruplex-forming oligomer for the amplified electrochemical response. The small peak for the assembled duplex DNA hybrid probe can be attributed to the nonspecific adsorption of hemin and possible formation of G-quadruplex–

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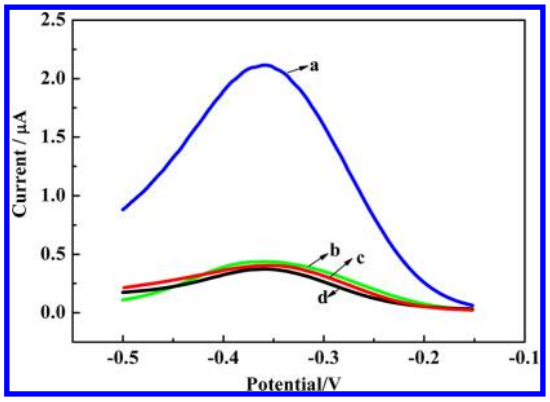

Figure 2. Differential pulse voltammograms obtained upon analyzing 1 pM target DNA in the presence of 1 unit/μL Exo III (curve a). Curves b to d are for the control experiments which are performed with no target DNA (b), no Exo III (c), and no target DNA and Exo III (d).

hemin complexes by small amounts of unhybridized quadruplex-forming oligomers on the electrode surface.[44]

To prove the signal amplification effect by Exo III-assisted autocatalytic target recycling strategy, another duplex DNA probe was also designed (Figure 3A) for comparison. This duplex DNA probe contains the same quadruplex-forming oligomer and a different blocker DNA strand (S3). The only difference for this blocker DNA with advised molecular beacon (S2) is the lack of fragment III. Upon the recognition of target DNA with this duplex DNA probe, Exo III could stepwise cleave the blocker DNA strand to release target DNA but no new secondary target DNA is generated. Thus, only target DNA could be recycled to trigger the successive cleavage process. It could be denoted as a typical target recycling strategy. It could be seen from Figure 3B that this strategy only showed a detection limit of about 1 pM for target DNA. In contrast, the electrochemical response to target DNA by autocatalytic target recycling strategy at the same concentration was significantly higher than that by this typical target recycling strategy. After subtracting the background response of the blank solution, the peak currents with the autocatalytic target recycling method for 1 pM target DNA were about 8 times higher than those by the typical target recycling strategy, showing the remarkable amplification performance of Exo III-assisted autocatalytic target recycling strategy.

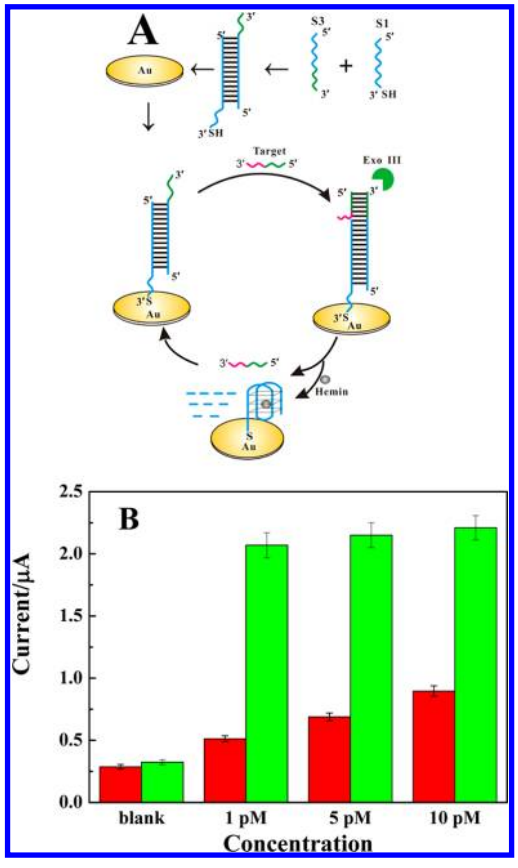

Figure 3. (A) Schematic illustration of Exo III-assisted typical target recycling amplification strategy for DNA assay. (B) DPV peak currents corresponding to autocatalytic target recycling amplification strategy (green) and typical target recycling amplification strategy (red) for detection of target DNA. The Exo III concentration is 1 unit/μL, and the incubation time is 120 min. Error bars represent standard deviations of measurements (n = 3).

Optimization of Exo III-Assisted Autocatalytic Target Recycling Strategy. The assembly density of duplex DNA probe on the electrode surface would have an important effect on the performance of electrochemical DNA biosensor.[49] The immobilization concentration of duplex DNA probe was first examined on the basis of the electrochemical response toward target DNA detection. The duplex DNA probe was prepared with the same concentrations of S1 and S2. It could be found that the immobilization concentration of duplex DNA probe of 5×10^{-7} M could achieve the better electrochemical response than that at other concentrations (Figure S-1, Supporting Information). The higher immobilization concentration of duplex DNA probe can increase the assembly density on the electrode surface, but it may restrict the hybridization efficiency of target DNA to the duplex DNA probe owing to the steric hindrance effect. Also, the densely packed duplex DNA probe will not be favorable for the effective folding of quadruplex-forming oligomer S1. These two factors may contribute to the relatively low electrochemical response at high concentration of duplex DNA probe. Thus, the concentration of 5×10^{-7} M duplex DNA probe was chosen as the optimized immobilization concentration for the following DNA detection. The surface coverage of assembled duplex probe under the optimum condition had also been determined on the basis of Tarlov's method by chronocoulometry.[50] It was estimated to be 4.21 ($\pm$0.16) $\times 10^{-12}$ mole cm^{-2} based on three independent experiments.

To further verify the signal amplification mechanism of Exo III-assisted autocatalytic target recycling, a time-course experiment was conducted. The duplex DNA probe modified electrode was incubated into a mixture containing 1 pM target DNA and 1 units/μL Exo III in Tris–HCl buffer (pH 8.0, 50 mM NaCl, 10 mM $MgCl_2$) at 37 °C for a certain period of time ranging from 20 to 180 min. After that, the electrodes were treated by K^+ and hemin (0.2 mM) and the electrochemical signal was measured and plotted against the time of incubation. As shown in Figure S-2 (Supporting Information), the electrochemical signal increased with a longer time of incubation before reaching saturation after 120 min. The continuously increasing signal indicates that the designed Exo III-assisted autocatalytic target recycling was indeed taking place, while the signal saturation at 120 min suggests the quadruplex-forming oligomers were almost liberated from the duplex DNA probe for the formation of G-quadruplex–hemin complex.

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Detection Performance of the Assay. The sensitivity of the fabricated electrochemical DNA biosensor based on Exo III-assisted autocatalytic target recycling strategy was investigated using target DNA with different concentrations. As shown in Figure 4A, the DPV peak current increased when the concentration of target DNA was raised from 0 to 10 pM, indicating that the liberation of the quadruplex-forming oligomer on the electrode is highly dependent on the concentration of target DNA. This confirms the working principle that the target DNA is hybridized with the duplex probe that triggers the Exo III digestion. Figure 4B shows the DPV peak current of G-quadruplex–hemin complex as a function of the concentration of target DNA. Under the optimal conditions, the average DPV peak currents showed a good linear correlation with the concentrations of the target DNA ranging from 10 to 500 fM. Thus, the directly measured detection limit toward target DNA is as low as 10 fM, providing superior detection sensitivity compared with those reported electrochemical methods that involve in the use of nucleases (Table S-1, Supporting Information). The results demonstrated that this signal amplification method was efficient for ultrasensitive electrochemical detection of DNA hybridization.

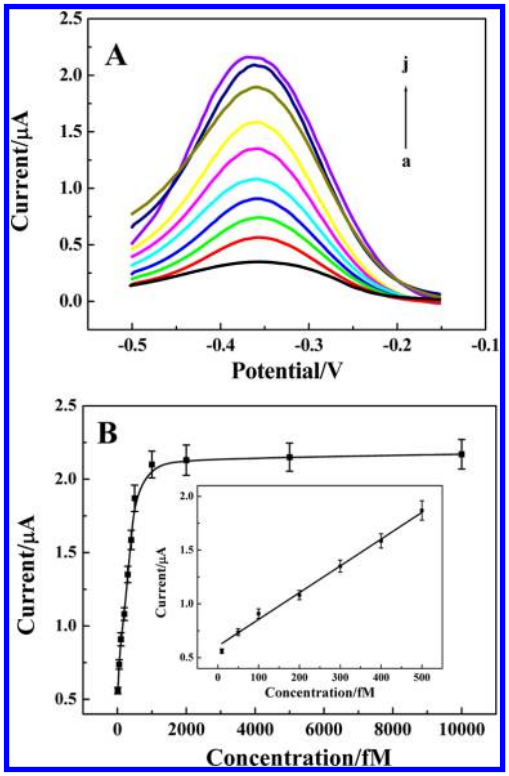

Figure 4. (A) Differential pulse voltammograms corresponding to the analysis of different concentrations of target DNA. The concentrations of target DNA for the curves (a) to (j) are: (a) 0 M, (b) 10 fM, (c) 50 fM, (d) 100 fM, (e) 200 fM, (f) 300 fM, (g) 400 fM, (h) 500 fM, (i) 1 pM, and (j) 10 pM. (B) Calibration curve corresponding to the DPV peak currents measured at −0.35 V for variable concentration of target DNA. Error bars represent standard deviations of measurements (n = 3). Inset shows the linear relationship between the DPV peak current and the target DNA concentration.

The specificity of the proposed detection method was further investigated by exposing the immobilized duplex DNA probe to four kinds of DNA sequences, including perfect complementary target DNA, single-base mismatched DNA, two-bases mismatched DNA, and noncomplementary DNA at the same concentration of 1 pM. As shown in Figure 5, the DPV peak current for one-base mismatched DNA and two-bases mismatched DNA was only about 32.3% and 19.4% of that for perfect target at the same concentration, respectively. The noncomplementary target DNA shows almost the same response with the blank solution. This high specificity could be attributed to the impaired hybridization ability of mismatched DNA with duplex probe and the limited Exo III digestion property for the mismatched DNA duplex. Thus, this method exhibited a good performance to discriminate perfect complementary target and the mismatched targets and great potential for single nucleotide polymorphism analysis.

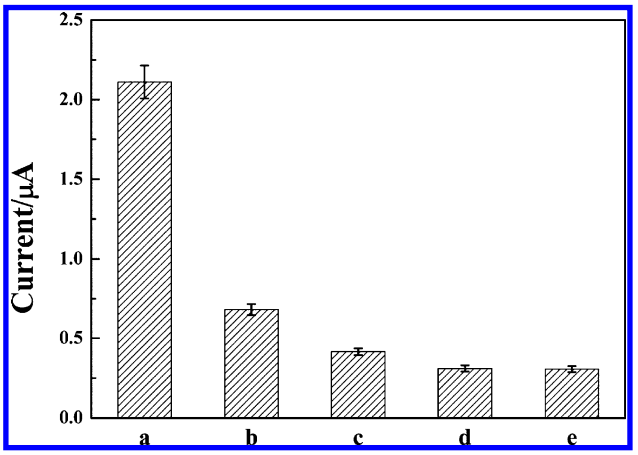

Figure 5. DPV peak currents at 1 pM of complementary target DNA (a), single-base mismatched DNA (b), two-bases mismatched DNA (c), noncomplementary target DNA (d), and the absence of DNA (e). Error bars represent standard deviations of measurements (n = 3).

The proposed Exo III-assisted autocatalytic target recycling strategy can also be developed as a versatile approach for the sensing of any DNA sequences. For this purpose, an additional nucleic acid hairpin structure that recognizes the target DNA was introduced into the system (Figure S-3A, Supporting Information). The loop domain consists of the recognition sequence of the target DNA, whereas the stem region contains the conserved sequence complementary to the overhangs at 3′ end of DNA duplex probe on the electrode. In the presence of the target DNA, the hairpin opens to form a double-stranded nucleic acid with overhangs at the 3′ ends, which can hybridize with the DNA duplex probe to trigger the selective Exo III digestion of the 3′ end of DNA duplex probe, resulting in the release of the opened hairpin duplex and generation of new target triggers for the next hybridization and cleavage process. The quadruplex-forming oligomers on the electrode are accordingly liberated to fold into the G-quadruplex–hemin complexes for the amplified detection of target DNA. The calibration curve corresponding to the DPV peak current increase upon analyzing different concentrations of the target DNA is shown in Figure S-3B, Supporting Information. As the concentration of target DNA is higher, the DPV peak current is intensified. This is consistent with the higher content of the opened hairpin structure that enhances the digestion of S2 on the DNA duplex probe by Exo III, leading to higher released amounts of quadruplex-forming oligomers (S1) for increased electrochemical responses, while the introduction of an additional hairpin-like nucleic acid for the versatile approach

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toward DNA sequence likely limits the target−probe hybridization rate and further target accessibility to the duplex probe modified electrode, which was presumed to contribute to the observed change in detection performance.

The stability for the duplex probe modified electrode has also been checked. Three independent experiments demonstrated that the duplex probe modified electrode could retain about 94% of its initial response toward target DNA after its storage in the refrigerator at 4 °C over 1 week, showing a relatively robust stability of the duplex probe modified electrode.

CONCLUSIONS

We have developed a simple, label-free electrochemical platform for ultrasensitive DNA detection using an ingeniously designed DNA duplex probe and a simple Exo III-assisted autocatalytic cleavage process. The DNA duplex probe is constructed by the hybridization of a quadruplex-forming oligomer with a traditional molecular beacon and used as the DNA recognition element and electrochemical signal carrier. Upon its recognition to target DNA, the autocatalytic target DNA recycling, including the recycling of target DNA itself and concomitant generation of new secondary target DNA triggers, can be easily achieved by a simple Exo III assisted cleavage process, accompanied by the formation of numerous G-quadruplex−hemin complexes on the electrode for the remarkable amplified electrochemical responses toward target DNA. The current protocol is really simple and avoids complex procedures for the achievement of target DNA recycling amplification. Moreover, it does not require any modification of DNA probes. It can achieve the ultrasensitive electrochemical detection of DNA down to the 10 fM level and hold a great potential for early diagnosis in gene-related diseases. The current strategy may be extended for the detection of aptamer-binding molecules and combined with other detection tools. More importantly, it opens a promising approach to develop an autocatalytic target DNA recycling strategy based on Exo III for the fabrication of ultrasensitive biosensor.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lubin, A. A.; Plaxco, K. W. *Acc. Chem. Res.* **2010**, *43*, 496−505.
(2) Li, D.; Song, S. P.; Fan, C. H. *Acc. Chem. Res.* **2010**, *43*, 631−641.
(3) Wang, F.; Elbaz, J.; Orbach, R.; Magen, N.; Willner, I. *J. Am. Chem. Soc.* **2011**, *133*, 17149−17151.
(4) 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.
(5) Hsieh, K.; White, R. J.; Ferguson, B. S.; Plaxco, K. W.; Xiao, Y.; Soh, H. T. *Angew. Chem., Int. Ed.* **2011**, *50*, 11176−11180.
(6) 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.
(7) 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.
(8) Dong, H. F.; Gao, W. C.; Yan, F.; Ji, H. X.; Ju, H. X. *Anal. Chem.* **2010**, *82*, 5511−5517.
(9) Gerasimova, Y. V.; Peck, S.; Kolpashchikov, D. M. *Chem. Commun.* **2010**, *46*, 8761−8763.
(10) Cheng, Y. N.; Tim, S.; Pol, V. D.; Liesbet, L.; Wang, M.; Chen, H. Z.; Gustaaf, B. *Anal. Chem.* **2011**, *83*, 1307−1314.
(11) 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.
(12) Liu, J. W.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 90−93.
(13) 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.
(14) 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.
(15) Comstock, M. J.; Ha, T.; Chemla, Y. R. *Nat. Methods* **2011**, *8*, 335−340.
(16) 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.
(17) Patolsky, F.; Lichtenstein, A.; Willner, I. *Nat. Biotechnol.* **2001**, *19*, 253−257.
(18) Liu, G.; Wan, Y.; Gau, V.; Zhang, J.; Wang, L.; Song, S.; Fan, C. *J. Am. Chem. Soc.* **2008**, *130*, 6820−6825.
(19) 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.
(20) Liao, W. C.; Ho, A. H. *Anal. Chem.* **2009**, *831*, 2470−2476.
(21) Chen, X.; Hong, C. Y.; Lin, Y. H.; Chen, J. H.; Chen, G. N.; Yang, H. H. *Anal. Chem.* **2012**, *84*, 8277−8283.
(22) Li, H.; Sun, Z.; Zhong, W.; Hao, N.; Xu, D.; Chen, H. Y. *Anal. Chem.* **2010**, *82*, 5477−5483.
(23) Xu, W.; Xue, X. J.; Li, T. H.; Zeng, H. W.; Liu, X. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 6849−6852.
(24) Ji, H.; Yan, F.; Lei, J.; Ju, H. *Anal. Chem.* **2012**, *84*, 7166−7171.
(25) Li, J. J.; Chu, Y. Z.; Lee, B. Y. H.; Xie, X. L. S. *Nucleic Acids Res.* **2008**, *36*, e36.
(26) García, T.; Revenga-Parra, M.; Sobrino, B.; Carracedo, A.; Alonso, C.; Lorenzo, E.; Pariente, F. *Biosens. Bioelectron.* **2011**, *29*, 40−45.
(27) Connolly, A. R.; Trau, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 2720−2723.
(28) Zuo, X.; Xia, F.; Xiao, Y.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, *132*, 1816−1818.
(29) Kiesling, T.; Cox, K.; Davidson, E. A.; Dretchen, K.; Grater, G.; Hibbard, S.; Lasken, R. S.; Leshin, J.; Skowronski, E.; Danielsen, M. *Nucleic Acids Res.* **2007**, *35*, No. e117.
(30) Freeman, R.; Liu, X.; Willner, I. *Nano Lett.* **2011**, *11*, 4456−4461.
(31) Hsieh, K.; Xiao, Y.; Soh, T. *Langmuir* **2010**, *26*, 10392−10396.
(32) Xuan, F.; Luo, X.; Hsing, I. M. *Anal. Chem.* **2012**, *84*, 5216−5220.
(33) Lee, H. J.; Li, Y.; Wark, A. W.; Corn, R. M. *Anal. Chem.* **2005**, *77*, 5096−5100.
(34) Teller, C.; Willner, I. *Curr. Opin. Biotechnol.* **2010**, *21*, 376−391.
(35) Ren, J.; Wang, J.; Han, L.; Wang, E.; Wang, J. *Chem. Commun.* **2011**, *47*, 10563−10565.
(36) Weizmann, Y.; Cheglakov, Z.; Willner, I. *J. Am. Chem. Soc.* **2008**, *130*, 17224−17225.

(37) Weizmann, Y.; Beissenhirtz, M. K.; Cheglakov, Z.; Nowarski, R.; Kotler, M.; Willner, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 7384−7388.
(38) Zhang, C. Y.; Wang, G. L. *Anal. Chem.* **2012**, *84*, 7037−7042.
(39) Jia, H.; Li, Z.; Liu, C.; Cheng, Y. *Angew. Chem., Int. Ed.* **2010**, *49*, 5498−5501.
(40) Wang, F.; Elbaz, J.; Teller, C.; Willner, I. *Angew. Chem., Int. Ed.* **2011**, *50*, 295−299.
(41) Bi, S.; Li, L.; Cui, Y. *Chem. Commun.* **2012**, *48*, 1018−1020.
(42) Zhao, C.; Wu, L.; Ren, J.; Qu, X. *Chem. Commun.* **2011**, *47*, 5461−5463.
(43) Zhang, K.; Zhu, X.; Wang, J.; Xu, L.; Li, G. *Anal. Chem.* **2010**, *82*, 3207−3211.
(44) Yang, N.; Cao, Y.; Han, P.; Zhu, X.; Sun, L.; Li, G. *Anal. Chem.* **2012**, *84*, 2492−2497.
(45) Pelossof, G.; Tel-Vered, R.; Willner, I. *Anal. Chem.* **2012**, *84*, 3703−3709.
(46) Tang, J.; Hou, L.; Tang, D.; Zhang, B.; Zhou, J.; Chen, G. *Chem. Commun.* **2012**, *48*, 8180−8182.
(47) Pelossof, G.; Tel-Vered, R.; Elbaz, J.; Willner, I. *Anal. Chem.* **2010**, *82*, 4396−4402.
(48) Shimron, S.; Wang, F.; Orbach, R.; Willner, I. *Anal. Chem.* **2012**, *84*, 1042−1048.
(49) Zhang, J.; Song, S.; Zhang, L.; Wang, L.; Wu, H.; Pan, D.; Fan, C. *J. Am. Chem. Soc.* **2006**, *128*, 8575−8580.
(50) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670−4677.