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ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 2013

Impact Factor: 5.64 · DOI: 10.1021/ac402728d · Source: PubMed

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G-Quadruplex DNAzyme-Based Chemiluminescence Biosensing Strategy for Ultrasensitive DNA Detection: Combination of Exonuclease III-Assisted Signal Amplification and Carbon Nanotubes-Assisted Background Reducing

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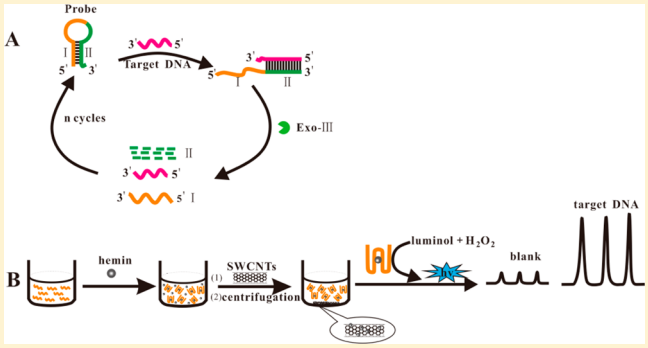
Supporting Information

ABSTRACT: Detection of ultralow concentration of specific nucleic acid sequences is important in early diagnosis of gene-related diseases and biodefense application. Herein, we report an amplified chemiluminescence (CL) biosensing platform for ultrasensitive DNA detection. It is based on the exonuclease III-assisted target recycling amplification and catalytic effect of G-quadruplex–hemin DNAzyme to stimulate the generation of CL in the presence of H_2O_2 and luminol. Moreover, the typical problem of high background induced by excess hemin itself can be effectively addressed through the absorbing of superfluous hemin on the surface of single-walled carbon nanotubes and then removing though centrifugation. Therefore, our proposed biosensing exhibited a high sensitivity toward target DNA with a detection limit of 12 fM, which was about 100-fold lower than that of the DNAzyme-based CL sensor for DNA detection without Exo III-assisted amplification. This sensing platform provides a label-free and cost-effective approach for sensitive detection of DNA.

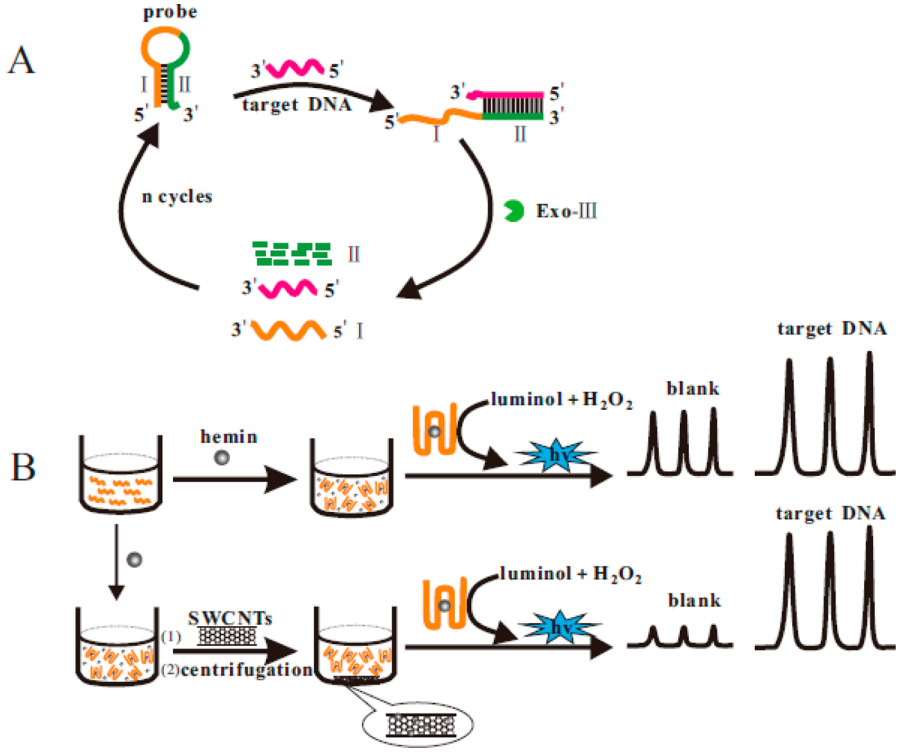


The ability to detect and sense ultralow concentrations of specific DNA sequences by using simple and low-cost assays is important in clinical diagnostics, mutation detection, and biodefense applications.[1] DNA chips with fluorescent detection have been well-known as conventional large-scale gene analysis tools, and the polymerase chain reaction coupled with molecular fluorophore assays offer high sensitivity of detection. However, these chip-based methodologies make use of solid phase interface DNA hybridization, and are heterogeneous methods.[2] Hybridization to sterically constrained probes on solid surfaces is slow. The heterogeneous detection usually needs a long dwell time owing to several cycles of consecutive hybridization and washing steps. Molecular beacon (MB) has rapidly become a mainstay for the detection of DNA because it rapidly and specifically reports the presence of given nucleic acid sequence in homogeneous solution.[3,4] While the MB-based detection system is one of the most successful separation-free probes,[4,5] there remains some challenging problems. Up until now, most of the reported MB biosensors for DNA have been based on fluorescence detection. MB needs to be labeled with a donor fluorophore and a quencher, and the double labeling would result in some problems such as high cost, low yield, and singly labeled impurities.[6] Therefore, the fabrication of label-free, low cost, and simple MB biosensors has become highly focused.

Deoxyribozymes or catalytic DNA (DNAzymes) are one member of the artificial nucleic acid family, and can catalyze some chemical reactions. Compared with traditional protein enzymes, DNAzymes with higher thermal stability are easier to synthesize and modify. Moreover, the flexibility of encoding the recognition region into DNAzyme sequences makes DNAzyme an ideal candidate for developing a biosensing platform.[7,8] An important development in the DNAzyme field is the discovery of the G-quadruplex-based DNAzyme formed by the G-quadruplex and hemin.[9] The G-quadruplex-based DNAzyme displays peroxidase-like activity, and can catalyze H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),[10] luminol,[11,12] or thiamine,[13] accompanied by a colored product, chemiluminescent emission, or fluorescent emission. Thus, the G-quadruplex-based DNAzyme provides the colorimetric, chemiluminescent, or fluorescent sensing platform.[14] Among them, chemilumescence (CL) detection has recently attracted an increasing interest due to its remarkable properties (such as high sensitivity, wide calibration range, and simple instrumentation),[11,12,15−20] and the G-quadruplex-based DNAzyme sensing system with CL

Received: August 27, 2013
Accepted: November 5, 2013
Published: November 5, 2013

Scheme 1. Schematic Illustration of the Sensing Principle for DNA Detection: (A) The Exo III-Assisted Target Recycling Amplification Process and (B) the SWCNTs-Assisted Reducing Background Signal



detection has been applied to DNA assay.[11,16,21] Willner and co-workers[21] reported a G-quadruplex-based DNAzyme CL approach for target DNA detection. In the strategy, the capture DNA, which is complementary to part of the target DNA, is attached to the Au surface. The probe DNA consists of two domains: one is responsible for catalytic activity (G-quadruplex) and the other can bind to the complementary region of the target DNA. First, the target DNA is captured by the capture DNA on the Au surface, and then hybridizes with the probe DNA. Finally, the addition of CL regent (luminol–H_2O_2) into the system leads to the generation of a chemiluminescence emission. The DNA was analyzed with a detection limit of 1.0 nM.[21] In 2012, Yan and co-workers[16] developed the homogeneous G-quadruplex-based DNAzyme CL sensing system for DNA, and the detection limit was 0.3 nM. However, the sensitivity of the methods is strictly limited because of the 1:1 hybridization ratio. Signal amplification strategy will significantly improve the sensitivity of the analytic method.[22]

G-quadruplex–hemin DNAzyme has been widely used to design chemical sensing, but high background signal is one serious challenge in the G-quadruplex–hemin DNAzyme sensing platform.[23,24] Hemin is the cofactor of G-quadruplex–hemin DNAzyme, and hemin itself has catalytic ability toward the H_2O_2-mediated oxidation of ABTS or luminol. An excess of hemin is generally added into the system in order to ensure the full formation of G-quadruplex–hemin DNAzyme. The excess of hemin undoubtedly results in the high background signal, which will seriously affect the detection sensitivity. Thus, to overcome the problem of high background is of great significance to further improve the detection sensitivity of the G-quadruplex–hemin DNAzyme sensing platform. Our recent work[25] showed that single-walled carbon nanotubes (SWCNTs) can effectively decrease the background signal of the G-quadruplex–hemin DNAzyme sensing platform. On the basis of the strong affinity of SWCNTs with hemin, superfluous hemin is arrested on the surface of SWCNTs and then is removed though centrifugation.[25] Compared with the reported ABTS–H_2O_2 colorimetric system in the absence of SWCNTs, the sensitivity for analyzing K^+ was improved by about 10-fold in the presence of SWCNTs.[25]

Herein, we develop a simple and highly sensitive G-quadruplex DNAzyme-based CL biosensing strategy for detection of DNA. Exonuclease III (Exo III) and SWCNTs are introduced into the system to improve the sensitivity of this biosensing. Exo III can catalyze the stepwise removal of mononucleotides from 3′-hydroxyl termini of duplex DNA, which is not active on 3′-overhang end of double-stranded DNA or single-stranded DNA.[26,27] Exo III has been employed as the cleavage enzyme in the target recycling-oriented amplification for sensitive detection.[26,27] Contrary to the nicking endonuclease-based target recycling amplification which requires a specific target sequence,[28] Exo III-assisted target recycling amplification does not require any specific recognition sequence, making it an ideal candidate for universal detection platforms.[26−30] As illustrated in Scheme 1A, we designed one kind of unlabeled stem-loop DNA MB, which consisted of the G-quadruplex–hemin DNAzyme sequence (I) and the target DNA recognition domain (II). In the presence of target DNA, MB is opened by hybridizing with target DNA to form a DNA duplex with a blunt 3′-termini. In this case, Exo III can preferentially bind to the duplex region and stepwise hydrolyze the mononucleotides from the blunt 3′-termini in the direction of 3′-to-5′, which can trigger the target recycling and release more G-quadruplex DNAzyme sequences. After execution of the above work, the released G-quadruplex DNAzyme sequences can self-assemble with hemin to form catalytic DNAzyme units (as shown in Scheme 1B), which can

dx.doi.org/10.1021/ac402728d | Anal. Chem. 2013, 85, 11494−11500

catalyze the luminol–H_2O_2 reaction to produce CL emission. Furthermore, free hemin could be strongly adsorbed on the surface of SWCNTs, whereas the complex of hemin and G-quadruplex (G-quadruplex–hemin DNAzyme) could not be adsorbed. Thus, we employ this different property of free hemin and the G-quadruplex–hemin DNAzyme to efficiently and conveniently reduce the background of the G-quadruplex–hemin DNAzyme sensing platform. In this DNA detection system, the strategy of combination of Exo III-assisted signal amplification and carbon nanotubes-assisted background reducing is introduced to significantly improve the sensitivity, and the detection limit was estimated to be 12 fM (3σ).

EXPERIMENTAL SECTION

Materials and Apparatus. All oligonucleotides were HPLC-Purified and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of oligonucleotides were listed in Table S1 (Supporting Information). The oligonucleotide stock solutions (20 μM) were prepared in Tris–HCl buffer (140 mM NaCl, 20 mM $MgCl_2$, 20 mM KCl, pH 7.4) and diluted to the desired concentration with the same Tris–HCl buffer. Each Oligonucleotide was heated to 90 °C for 10 min, and slowly cooled to room temperature before use to unwind the single-stranded oligonucleotide. Exo III was also purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Hemin and luminol were obtained from Sigma (St. Louis, MO, USA). The single-walled carbon nanotubes (SWCNT) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Hemin stock solution (5 mM) was prepared by dissolving hemin in a water–dimethyl sulfoxide (DMSO) mixture (volume ratio, H_2O/DMSO = 90/10), and stored in the dark at −18 °C. Luminol stock solution (25 mM) was prepared by dissolving 4.43 g of luminol in 20 mL of NaOH (0.10 M) and then diluting to 1 L with water. Luminol solution was stored in the dark for one week prior to use. The working H_2O_2 solutions were prepared fresh daily from 30% (w/w) H_2O_2. Unless otherwise indicated, all reagents and solvents were purchased in their highest available purity and used without further purification. Millipore Milli-Q water (18 MΩ cm^{-1}) was used in all experiments.

The CL intensity was measured and recorded with a model IFFM-D Chemiluminescence Analyzer (Xi'an Ruimai Electronic Sci. Tech. Co. Ltd., Xi'an, China). Circular dichroism (CD) spectra were measured on a Chirascan CD spectrophotometer (Applied Photophysics, Leatherhead, UK). An HH-1electric-heated thermostatic water bath (Beijing Kewen Instrumental Factory, Beijing, China) was used to control the reaction temperature at 0.1 °C intervals.

Pretreatment of SWCNTs. The SWCNTs were pretreated according to the literature.[31] Briefly, the SWCNTs were treated by refluxing in 4.0 M HNO_3 for 24 h, then were filtered with a 220 nm Millipore membrane and washed with water. Subsequently, the 3:1 concentrated H_2SO_4:HNO_3 mixture was realized as the oxidizing acid in this cutting operation. The SWCNTs were sonicated for 8 h in an ice bath. They were washed with water to obtain a neutral lotion, and then filtered with a 220 nm Millipore membrane. Finally, they were dried under vacuum at 60 °C overnight. The pretreated SWCNTs were water-dispersible due to the presence of suspended hydroxyl (−OH) and carboxylic (−COOH) groups at the surface of the sidewall. The transmission electron microscopy (TEM) results showed that the outer diameter of the SWCNTs was ca. 4 nm. The stock solution of carboxyl-modified-SWCNTs (0.8 mg/mL) was obtained by sonicating for 3 h in H_2O.

Procedures for DNA Assay. The detailed procedure for DNA detection was as follows. First, the Exo III-assisted target recycling amplification reaction was performed by mixing 50 μL of hairpin probe (0.1 μM), 50 μL of target DNA (varying concentrations), and 10 μL of Exo III (2.5 U/μL), followed by incubating at 25 °C for 1 h. Then the 50 μL hemin (2.0 μM) solution was added into the above solution, and the mixture was incubated for 1 h (25 °C) to form the G-quadruplex–hemin DNAzyme. Finally, 200 μL of 35 μg/mL SWCNTs was added into the G-quadruplex–hemin solution, which was then incubated for 30 min. Finally, the mixture was centrifugated (18 329 × g) for 10 min to remove superfluous hemin.

CL Measurements. The CL measurements was performed in the Tris–HCl buffer (pH 9.0) by mixing 100 μL of the above prepared G-quadruplex–hemin DNAzyme solution and 100 μL of H_2O_2 (1.0 mM) solution. Then, 20 μL of the resulting solution was decanted to the quartz cuvette (used as CL reactor), and the CL reaction was triggered by injecting 100 μL of luminol (0.4 mM). The CL signal was measured and recorded with the IFFL-D Chemiluminescence Analyzer. The concentration of the target DNA was quantified by the CL peak intensity.

RESULTS AND DISCUSSION

Principle of G-quadruplex DNAzyme-Based CL Biosensing of DNA. This CL biosensing of DNA could be divided into two stages: Exo III-assisted signal amplification and carbon nanotubes-assisted background reducing. As illustrated in Scheme 1A, we designed the unlabeled MB probe, and the probe contains two domains, which are identified as I and II according to their different functions. Region I (yellow) is the G-quadruplex–hemin DNAzyme sequence, and region II (green) is the target DNA recognition domain. In the MB probe, region I (DNAzyme sequence) is partially caged in the duplex structure of the stem by hybridization with region II. As a result, the G-rich segment (region I) is prohibited to bind hemin, and the G-quadruplex–hemin DNAzyme is inactive. Upon hybridization with target DNA, the MB probe is opened to form a DNA duplex with a blunt 3′-termini. It should be noted that after the hybridization, there is still a 4-nt single-stranded sequence in the target at its 3′-termini to resist the cleavage by Exo III because the resistance degree of Exo III on single-stranded DNA at the 3′-protruding termini is dependent on the length of the extension, with extensions 4-nt or longer being essentially resistant to cleavage.[32] In this case, Exo III can preferentially bond to the duplex region and stepwise hydrolyze the mononucleotides from the blunt 3′-terminus in the direction of 3′-to-5′. After the duplex is fully consumed due to the inability of the Exo III to accept single-stranded DNA (ssDNA) as a substrate, the intact target is released, and then it hybridizes with a new MB probe and initiates the next round of cleavage. In this way, one target DNA is able to open multiple probes, resulting in production of multiple G-quadruplex DNAzyme sequences. Upon completion of the Exo III-assisted target recycling, the released G-quadruplex DNAzyme sequences can self-assemble with hemin to form catalytic DNAzyme units (as shown in Scheme 1B), which can catalyze the luminol–H_2O_2 reaction to produce CL emission. The number of catalytic DNAzyme units is positively related to the

CL intensity, and thus the Exo III-assisted target recycling leads to significant amplification of the signal.

To evaluate the amplification function of the proposed sensing system in Scheme 1, the target-induced CL signals in the presence and the absence of Exo III were recorded by using a CL analyzer, which collected the total luminescence intensity. As shown in Figure 1, an obvious signal enhancement was

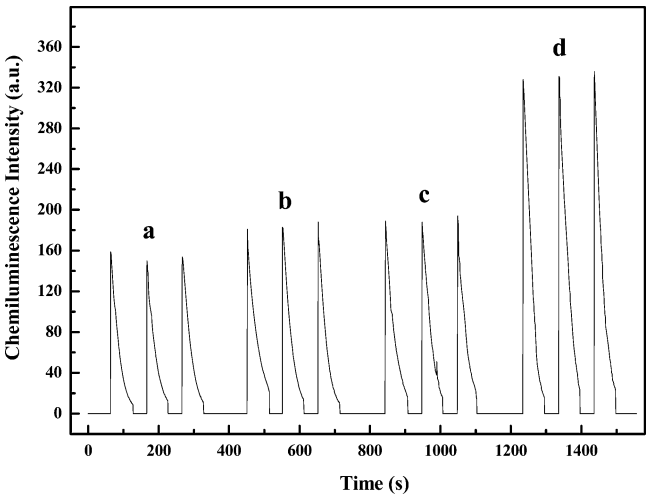


Figure 1. Measurement results of the CL biosensing system under different conditions: (a) hemin, (b) probe + hemin, (c) probe + target + hemin; and (d) probe + target + Exo III + hemin. Experimental conditions: 0.1 μM probe, 1.0 pM target, 25 U Exo III, 2 μM hemin, 0.4 mM luminol, and 1.0 mM H_2O_2.

observed due to Exo III-catalyzing target DNA recycling upon the addition of 1.0 pM target DNA (curve d). In contrast, the CL intensity of 1.0 pM target DNA in the absence of Exo III (curve c) was similar to the background (curve b). In the above experiment, we found the system was obscured by the high background which could be considered as the CL intensity observed in the absence of target DNA while in the presence of hemin. According to the principle of this sensing system, the G-quadruplex–hemin DNAzyme cannot form in the absence of target DNA. However, hemin itself can catalyze the H_2O_2–luminol CL reaction.[33] Generally, for the full formation of G-quadruplex–hemin DNAzyme, an excess of hemin (2 μM hemin was used in this system) is added into the system, which undoubtedly results in a high background signal. The high background would seriously affect the sensitivity. Thus, to overcome the problem of high background is of great significance to further improve the detection sensitivity of this sensing platform.

SWCNTs provide a high intrinsic surface area without intraparticle diffusional limitations. Some polycyclic aromatic hydrocarbons and heterocyclic molecules (such as porphyrins and phthalocyanins) can be effectively adsorbed on the surface of SWCNT through π–π stacking interactions between the aromatic group and SWCNTs sidewalls.[35] In our recent works,[25,35] we found that free hemin could be efficiently adsorbed on SWCNTs, whereas the G-quadruplex–hemin DNAzyme could not be adsorbed on SWCNTs. Inspired by the above work,[25] SWCNTs were employed in this study to circumvent this problem of high background. The principle of the improved proposal is illustrated in Scheme 1B. After the Exo III-assisted target recycling amplification process, DNAzyme sequences (region I) of the hairpin probe are released and accumulated, then they can self-assemble with hemin to form the catalytic DNAzyme units. The superfluous hemin, which does not complex with G-quadruplex, can be absorbed efficiently on SWCNTs due to the strong affinity of SWCNTs with hemin. Thus, the superfluous hemin is removed by simple ultracentrifugation, resulting in significant reduction of the background. The DNAzyme sequences (region I) form G-quadruplex–hemin DNAzyme, which can catalyze the oxidation of luminol by H_2O_2 to generate CL signals. Subsequently, we tested whether the SWCNTs act on the sensing system. As shown in Figure 2, after the Exo III-assisted

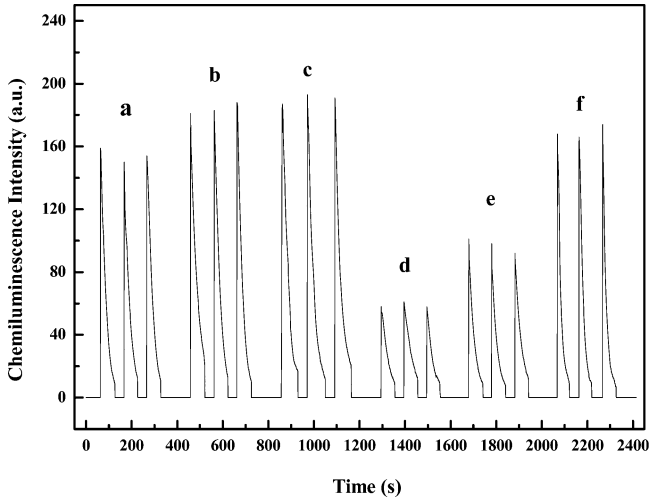


Figure 2. Measurement results of the CL biosensing system under different conditions: (a) hemin; (b) probe + hemin; (c) probe + target + Exo III + hemin; (d) hemin + SWCNTs; (e) probe + hemin + SWCNTs; and (f) probe + target + Exo III+ hemin + SWCNTs. Experimental conditions: probe 0.1 μM, target 0.2 pM, Exo III 25 U, hemin 2 μM, luminol 0.4 mM, H_2O_2 1.0 mM, and SWCNTs 35 μg/mL.

target recycling amplification process, the CL intensity of 0.2 pM target DNA (curve c) in the absence of SWCNTs is almost the same as the background (curve b). In contrast, the presence of SWCNTs could significantly decrease the background (the CL intensity changed from ca. 185 to 90), and the CL intensity of 0.2 pM target DNA showed almost no change in the presence of SWCNTs. It is confirmed that SWCNTs could markedly reduce the background of this CL biosensing system, which is propitious to detect the analyte at low concentration.

All in all, combination of exonuclease III-assisted signal amplification and carbon nanotubes-assisted reducing background could remarkably improve the sensitivity of G-quadruplex DNAzyme-based CL biosensing for DNA detection.

CD Characterization. Evidence for the Exo III-assisted target recycling amplification was provided by CD spectra analysis. The CD spectra of the system were shown in Figure S1 (Supporting Information); the CD spectrum of the hairpin probe displayed a negative peak at ca. 240 nm and a positive peak at ca. 280 nm (curve a), which are characteristic from double-stranded DNA.[36] After interacting with target DNA, the intensity at about 260 nm increased, and the intensity around 280 nm decreased (curve b). A negative peak at ca. 240 nm and a small positive peak at ca. 260 nm are characteristic for a parallel G-quadruplex system,[37] indicating the formation of parallel G-quadruplex from hairpin probe in the presence of

dx.doi.org/10.1021/ac402728d | Anal. Chem. 2013, 85, 11494–11500

target DNA and hemin. Upon adding Exo III to the system, the peak intensity at 260 nm continued to increase while the peak at 240 nm decreased further (curve c). Also, the peak at 280 nm became very weak and disappeared at last. This was because Exo III was able to stepwise remove mononucleotides from 3′-hydroxyl termini of double-stranded DNA, and the sequence of the MB probe was released, thus forming a more parallel G-quadruplex structure. In addition, the purpose of adding SWCNTs into this system is to absorb and to remove superfluous hemin by simple ultracentrifugation, and at this rate SWCNTs should not absorb and influence the G-quadruplex–hemin DNAzyme. To validate the conclusion, 35 μg/mL of SWCNTs was added into the DNAzyme solution, and after ultracentrifugation the CD spectrum of the supernatant solution was measured. There were a sharp positive peak at ca. 260 nm and a negative peak at 240 nm in the CD spectra (Figure S2, Supporting Information), which is typical of a parallel quadruplex DNA conformation. Obviously, the structure of G-quadruplex–hemin DNAzyme did not change after addition of SWCNTs. Meantime, the peak intensity at 240 and 260 nm showed almost no change before and after addition of SWCNTs, suggesting that G-quadruplex–hemin DNAzyme was not absorbed on SWCNTs surface.

Optimization of Experimental Conditions. To achieve the best sensing performance, the stem length of the MB probe was optimized. The stem length of the hairpin probe has a remarkable effect on the sensitivity.[38] When the stem is too short, the hairpin probe is not stable enough and can be easily opened by hemin, resulting in a high background signal. However, a too long stem will lead to very strong hybridization so that the target DNA finds it hard to open the hairpin probe, which will reduce the sensitivity. To investigate the influence of the stem length, three hairpin probes with stem lengths of 7 (P_1), 8 (P_2), and 9 (P_3) base pairs were used in this system. The ratio of I/I_0 (where I and I_0 are the CL intensity of the sensing system in the presence and absence of target DNA, respectively) was used to evaluate the sensitivity of the assay. As shown in Figure S3 (Supporting Information), it could be seen that I/I_0 of P_2 was larger than that of P_1 and P_3. Hairpin probe P_2 performed better than the other two hairpin probes, so the stem length of 8 base pairs was used in the later experiments.

In this proposed assay, the sensitivity can be dramatically increased by using the Exo III-assisted target recycling amplification. As such, the amount of Exo III is the key factor in this system. The experimental results showed that the sensitivity for detecting target DNA increased with increasing Exo III concentration in the range of 10–30 U. Taking into account the consumption of enzyme, 25 U Exo III in the final solution was used. The effect of the incubation time of DNA and Exo III was investigated. When the assay was conducted at 25 °C, the CL signal increased with increasing incubation time, and after 1 h the CL signal reached the maximum. The continuously increasing signal indicates that the designed Exo III-assisted target recycling was indeed taking place. Thus, the incubation time was fixed at 1 h. We also investigated the effect of the amount of SWCNTs on the sensitivity. The experimental results in Figures S4 and S5 (Supporting Information) showed that the highest signal-to-noise ratio was obtained when 200 μL SWCNTs (35.0 μg/mL) was used with a 30 min interaction time of SWCNTs and hemin.

Furthermore, the performance of the developed CL biosensing for DNA was still strongly influenced by the CL conditions (such as hemin concentration, luminol concentration, H_2O_2 concentration, and CL media pH). Different assay conditions were investigated by fixing the 0.1 μM hairpin probe and 10 pM target DNA. The experimental results showed that the optima concentrations of hemin, luminol, and H_2O_2 were 2 μM, 0.4 mM, and 1.0 mM, respectively. The optimal CL buffer solution pH on the sensing system was 9.

Analytical Performance of the CL Biosensing for DNA. Under the optimized conditions, experiments were carried out by adding target DNA with different concentrations into the CL system to examine whether the CL change could be used for DNA quantification. As shown in Figure 3, the CL intensity

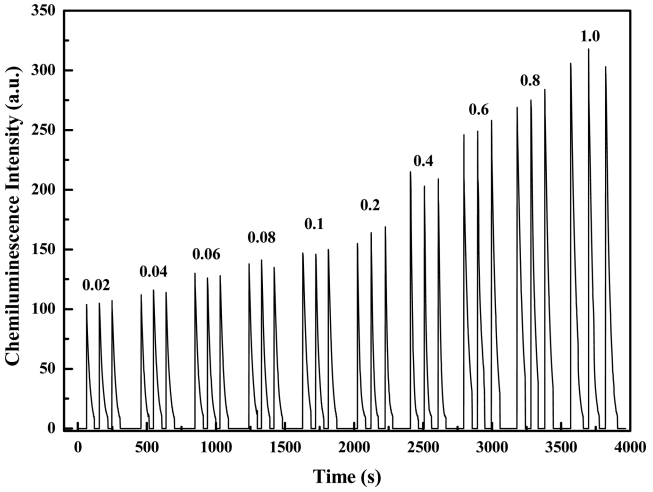


Figure 3. Measurement results of target DNA with the concentration in the presence of Exo III. Experimental conditions: Probe 0.1 μM, Exo III 25 U, hemin 2 μM, luminol 0.4 mM, H_2O_2 1.0 mM, and SWCNTs 35.0 μg/mL.

increases with the increasing target DNA concentration, to reveal a linear relationship in the target DNA concentration range from 0.02 to 1.0 pM (Figure S6, Supporting Information). The limit of detection (taken to be 3 times the standard deviation in the blank solution) was 12 fM, which was about 100-fold lower than that of the previously reported DNAzyme-based CL sensor for DNA detection without Exo III-assisted amplification.[11,16,21] Control experiments (Figure 1) showed that this DNAzyme-based CL system in the absence of Exo III and SWCNTs could not distinguish 1.0 pM target DNA and background. Furthermore, for comparison, we also measured the CL signals of this Exo III-assisted target recycling system in the absence of SWCNTs, and the detection limit was 0.1 pM. Thus, such high sensitivity of this proposed method can be attributed to two factors: the amplification efficiency of Exo III and the reducing background ability of SWCNTs. Moreover, this DNAzyme-based CL biosensing system is far more sensitive than the reported DNAzyme-based colorimetric biosensing system with Exo III-assisted amplification.[32]

The specificity of this proposed strategy for DNA detection was investigated by testing the CL response to other DNA molecules, including a single-base mismatched DNA (T_1), two-base mismatched DNA (T_2), and the noncomplementary DNA (T_n). Figure 4 shows the CL intensity changes with target DNA and other mismatched DNA strands. The completely complementary target DNA (T), T_1, T_2, and T_n were distinctly discriminated under the same detection conditions. These results indicate the high specificity of this proposed CL strategy for DNA detection.

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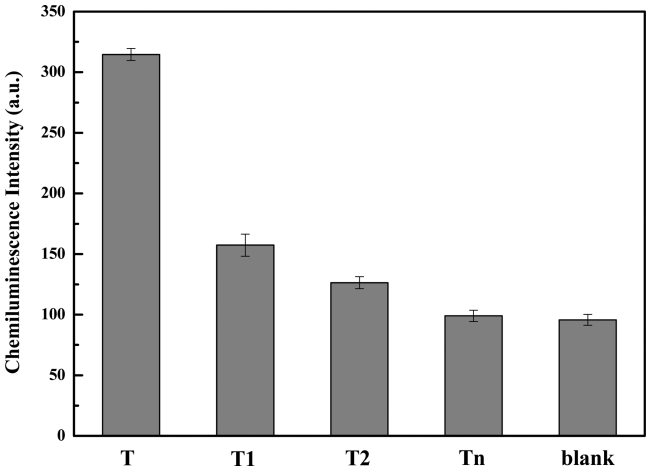


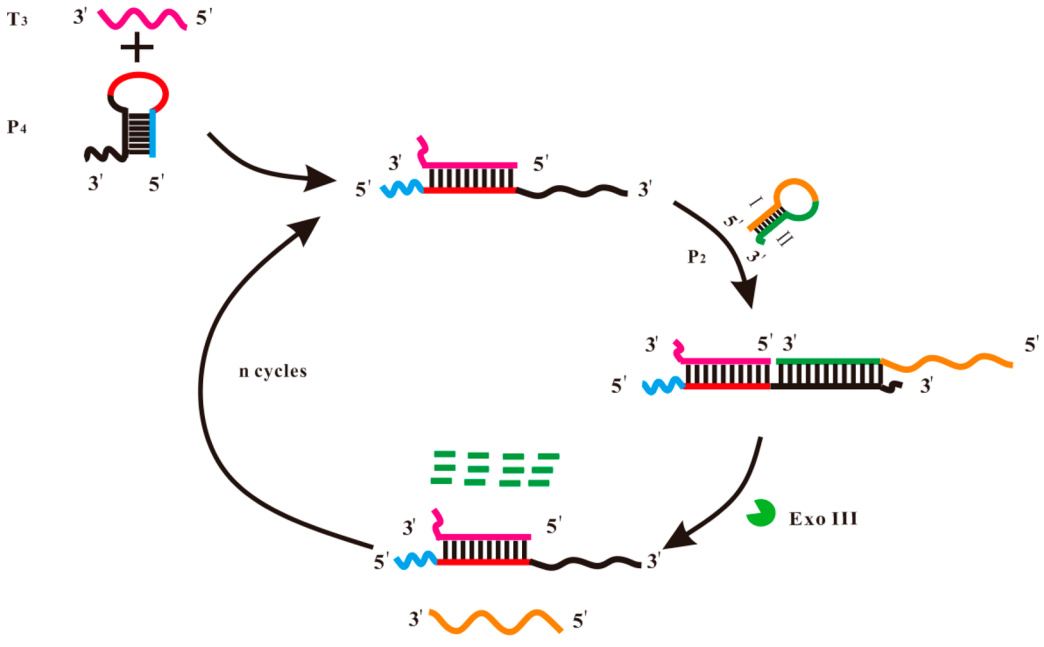
Figure 4. Specificity of the assay for DNA detection by hybridizing probe DNA with different target DNA: completely complementary target DNA (cDNA), single-base mismatched DNA (T_1), two-base mismatched DNA (T_2), noncomplementary DNA (T_n), and the absence of DNA (blank), respectively. Experimental conditions: 0.1 μM Probe, 25 U Exo III, 2 μM hemin, 0.4 mM luminol, 1.0 mM H_2O_2, and 1.0 pM target DNA. Error bars represent the standard deviations of three independent measurements.

Based on the sensing principle illustrated in Scheme 1A, this G-quadruplex DNAzyme-based CL biosensing strategy can be used to detect the target DNA, which must contain a part of the G-rich sequence. However, this apparent limitation is resolved by introducing another MB probe. As shown in Scheme 2, an unlabeled MB probe (P_4), which recognizes the target DNA (T_3), was introduced into the system. The probe contains the target DNA recognition domain (red) and the complementary sequences (black) to region II of the probe illustrated in Scheme 1A (P_2). The complementary sequence is partially caged in the stem region of the hairpin. Upon hybridization with target DNA (T_3), the P_4 is opened to form a double strand. The opening of P_4 can release the complementary sequence to region II and enable it open the hairpin probe P_2 with the DNAzyme sequence. Exo III can bond to the duplex region and stepwise hydrolyze the mononucleotides of P_2 from the blunt 3′-terminus in the T_3–P_4–P_2 duplex structure, resulting in the release of the open hairpin duplex T_3–P_4 that can be substituted for target to further hybridize to an additional P_2 and leading to amplification of the signal. Because the recognition domain (red) of P_4 can be predesigned, the system can realize versatile sensing of different target DNAs. We used the general DNA sensing platform for detecting T_3 (model target with random sequences), and the results was shown in Figure S7 (Supporting Information). Therefore, the sensing platform shown in Scheme 1 can be modified by using another MB probe to analyze the target with a random sequence.

CONCLUSION

A highly sensitive and simple G-quadruplex DNAzyme-based CL biosensing for DNA detection has been demonstrated in this study. In this proposed strategy, Exo III-assisted target recycling is used to amplify the detection signal, and meantime SWCNTs are applied to reduce the background. Thus, this proposed method exhibits high sensitivity toward target DNA with a detection limit of 12 fM, which is far more sensitive than most of the DNA assays reported so far, and holds a great potential for early diagnosis in gene-related diseases. The MB probe does not need labeling or modifying, and the present MB method is a turn-on model. So, the method is cheap, simple, and easy to operate. Furthermore, the assay is based on CL detection, requiring no sophisticated experimental techniques and instruments. The features make the proposed assay particularly useful for point-of-care applications in the developing world. More importantly, it opens a promising approach to develop an autocatalytic target DNA recycling strategy based on Exo III for the fabrication of an ultrasensitive biosensor.

Scheme 2. Schematic Illustration of the General DNA Sensing Platform, Using a Predesigned Hairpin Structure and Exo III-Assisted Target Recycling Amplification Process



dx.doi.org/10.1021/ac402728d | Anal. Chem. 2013, 85, 11494–11500

■ ASSOCIATED CONTENT

Supporting Information

Table of sequences of the used oligonucleotides (Table S1) and figures showing CD spectra of different samples (Figure S1), CD spectra of the supernatant solution in the presence and absence of SWCNTs (Figure S2), effect of the different sequences on the sensing system (Figure S3), effect of SWCNTs (35.0 μg/mL) volume on the sensing system (Figure S4), effect of SWCNTs incubated time on the sensing system (Figure S5), calibration curve corresponding to the CL intensity increase upon analyzing different concentrations of target DNA (Figure S6), and measurement results of target DNA with the different concentration (Figure S7). 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.

■ ACKNOWLEDGMENTS

This work was supported financially by the National Natural Science Foundation of China (Nos. 21075080 and 21275096) and Program for Innovative Research Team in Shaanxi Province.

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