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An electrochemical DNA biosensor based on the "Y" junction structure and restriction endonuclease-aided target recycling strategy†

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Based on the "Y" junction structure and restriction endonuclease-aided target recycling strategy, an electrochemical biosensor for DNA detection was developed. This universal biosensor was suitable for detecting different sequences of target DNA by changing the sequence of capture and assistant strands.

Sensitive and selective detection of DNA sequences plays a significant role in clinical diagnosis and biomedical studies.[1–3] Electrochemical DNA biosensors have attracted significant attention due to their simplicity, sensitivity and selectivity.[4,5] Recently, some electrochemical DNA biosensors based on isothermal signal amplification have been developed for the direct detection of small amounts of DNA with impressive limits of detection (LOD).[6–11] Chen *et al.* introduced a novel electrochemical biosensor for the detection of DNA based on the nicking endonuclease assisted detection strategy.[6,7] Liu *et al.* developed a sensitive and signal-on electrochemical DNA biosensor based on nicking endonuclease assisted electrochemistry signal amplification.[8] Hsieh *et al.* reported a label-free, isothermal electrochemical DNA biosensor based on the phenomenon of target recycling.[9] Wu *et al.* demonstrated a label-free electrochemical sensor for specific DNA detection based on an exonuclease III-aided target recycling strategy.[10]

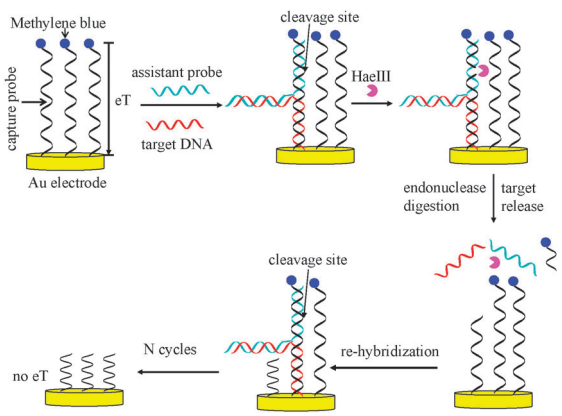
In the above approaches, the target should first hybridize with the capture probe immobilized on the electrode, and then a restriction site, which could be specifically recognized by enzyme, was created. Secondly, the enzyme only cleaved the capture probe into two pieces and the intact target was released. Thirdly, the released target could hybridize with another capture probe to initiate the next cycle. The signal could be enhanced obviously by the repeated cycles of hybridization, cleavage and dissociation since the target could be reused for many cycles.

In most of these works, cleavage sites with specific sequences were necessary. However, these sites were located on the target DNA/capture DNA duplexes, and the targets should contain special sequences. For this reason, these highly sensitive approaches only detected the sequence-specific target DNA. It would limit the application of these approaches.

Here, we introduced a universal electrochemical biosensor which was suitable for detecting different sequences of target DNA. The cleavage site was isolated from target with the aid of a "Y" junction structure.[12,13] The signal was amplified based on the isothermal enzyme-assisted target recycling strategy.

As shown in Scheme 1, a "Y" junction structure was formed by hybridization of a methylene blue (MB) labeled capture probe, an assistant probe, and a target DNA. First, MB labeled capture probes self-assembled on a gold electrode with Au–S linkages. Then, when target DNA was introduced into the system, it was complementary to the assistant probe at one-half of the segment and complementary to MB labeled capture probes at the other half-segment, resulting in the formation of a stable duplex complex. Meanwhile, the assistant probe also hybridized with the capture probe. As a result, the cleavage site was located on the assistant probe/capture probe duplexes, and isolated from target DNA.

Next, restriction endonuclease HaeIII was added for digesting the assistant probe/capture probe duplexes. The capture DNA was cleaved into two pieces and the MB labeled piece dissociated from the gold electrode. At the same time, the target DNA



Scheme 1 Schematic of an electrochemical DNA biosensor based on the "Y" junction structure and restriction endonuclease-aided target recycling strategy.

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Table 1 Sequences of oligonucleotides used

Name	Sequences
Capture probe	5′-MB-TTT GGG GCC CCT TCT CGA TTT TTT TTT T-C_6H_{12}-SH-3′
Complementary DNA (cDNA)	5′-TC GAG AAG TGC GTG CTG TAA-3′
Single base mismatch DNA (smDNA)	5′-TC GAG AAG TGC C**T**G CTG TAA-3′
Double base mismatch DNA (dmDNA)	5′-TC GA**C** AAG TGC GTG CA**A**G TAA-3′
Three base mismatch DNA (tmDNA)	5′-TC G**T**G AAG TG**G** GTG CT**C** TAA-3′
Random DNA (nonDNA)	5′-AG TGG TCC CCC AAC TCC TCT-3′
Assistant probe 1	5′-TTA CAG CAC GCA GGG CCC C-3′
Assistant probe 2	5′-TA CAG CAC GCA GGG CCC C-3′
Assistant probe 3	5′-A CAG CAC GCA GGG CCC C-3′
Assistant probe 4	5′-CAG CAC GCA GGG CCC C-3′

The locations of mismatched sites in smDNA, dmDNA and tmDNA are underlined.

dissociated from the capture probe. Subsequently, the released target DNA could hybridize with another capture probe and an assistant probe, then initiate the next cycle of cleavage. Eventually, each target DNA could go through many cycles, resulting in the cleavage of many capture probes. Since the capture probes were labeled with MB, the above processes could be monitored using alternating current voltammetry, *i.e.* the peak current of MB decreased as a result of the cleavage of many capture probes. Target DNA was detected according to the change of the peak current of MB. The sequences of oligonucleotides used here are listed in Table 1.

The feasibility of this electrochemical DNA biosensor was first investigated (Fig. 1). When the MB labeled capture probes were immobilized on the Au electrode, an obvious MB peak was observed (curve a). In the absence of cDNA, the peak current slightly decreased after restriction endonuclease treatment (curve b). This phenomenon implied that a small amount of assistant probes might be hybridized with the capture probes in the absence of target DNA. Obviously, the probability was very low, since only 7 base pairs between the capture probe and assistant probe were present. When 1 nM cDNA was present, the peak current decreased obviously after restriction endonuclease treatment (curve c). The results showed that this biosensor could be used to detect DNA. It was noted that the current c did not decrease to approximately zero. The possible reason is that a small amount of target DNA strands remain associated with the capture strand due to the 7 base pairs between the capture probe and the target.

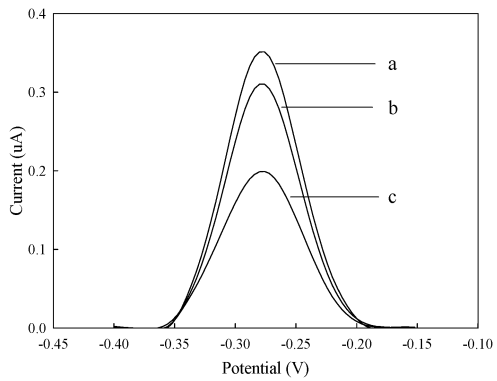

Fig. 1 Alternating current voltammogram of (a) capture DNA modified on the Au electrode; (b) after restriction endonuclease treatment for 60 min in the absence of cDNA; (c) after restriction endonuclease treatment for 60 min in the presence of 1 nM cDNA.

The surface coverage of electrode was one of the crucial parameters in the design of a sensitive and selective DNA biosensor.[14,15] Electrodes with capture DNA of different surface coverage were interrogated in this work. Fig. S1 (ESI†) shows the signal/background (S/B) ratio corresponding to low surface coverage (3.37×10^{12} molecules cm^{-2}), medium surface coverage (4.79×10^{12} molecules cm^{-2}) and high surface coverage (8.66×10^{12} molecules cm^{-2}) for the detection of 10 nM cDNA. The decrease of peak current that resulted by enzyme cleavage in the presence of target DNA was recorded as signal (S). The decrease of peak current that resulted by enzyme cleavage in the absence of target DNA was recorded as background (B). As shown in Fig. S1 (ESI†), the highest S/B ratio could be obtained at medium surface coverage. The possible reason was that low surface coverage would capture few target DNA strands and high surface coverage would decrease the efficiency of DNA hybridization. Therefore, medium surface coverage was selected in the following experiments to ensure improved hybridization efficiency.

In our design, the form of "Y" junction structure depended on simultaneous recognition of target DNA by the assistant probe and capture probe, thus it was necessary to optimize the sequence of assistant probe since the sequences of capture probe and target were all defined. Fig. 2 shows the S/B ratio corresponding to different assistant probes. The highest S/B ratio could be obtained when the assistant probe 3 was used. The possible reason was as follows: for longer assistant probes, it was easy to hybridize with capture probes modified on the electrodes in the absence of cDNA, so a high background resulted; for shorter assistant probes, it was hard to hybridize with capture probes modified on the electrodes even if cDNA was present, so a low signal was achieved. Therefore, the assistant probe 3 was selected in the following experiments.

Some factors, such as HaeIII concentration, reaction temperature and reaction time, also influenced the sensitivity of this biosensor. Here, the optimal HaeIII concentration, temperature and time were 0.1 U μL^{-1}, 40 °C and 60 min, respectively (shown in Fig. S2–S4 of ESI†). Under the optimized conditions, different concentrations of target DNA were detected. Fig. 3A shows that the MB peak current gradually decreased as the cDNA concentration increased from 1 pM to 10 nM. Fig. 3B shows the relation between the S/B ratio and the concentration of target DNA. According to the 3σ rule,[16] the detection limit was 14 pM. This sensitivity is comparable to or better than that of many reported methods for DNA detection.[9,13,17–21]

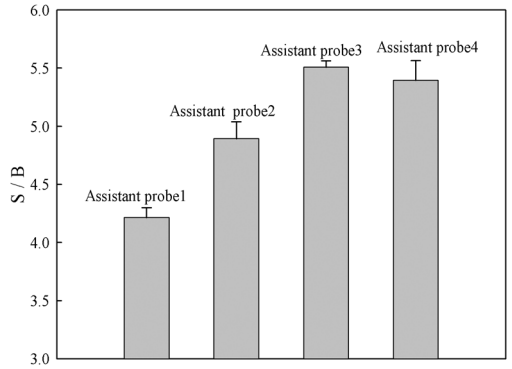

Fig. 2 Signal/background ratio corresponding to different assistant probes. The error bars represent the standard deviation of six measurements. Concentration of cDNA: 10 nM; concentration of HaeIII: 0.1 U μL^{-1}; concentration of assistant probe: 1 μM; surface coverage: 4.79×10^{12} molecules cm^{-2}; temperature: 37 °C; reaction time: 60 min.

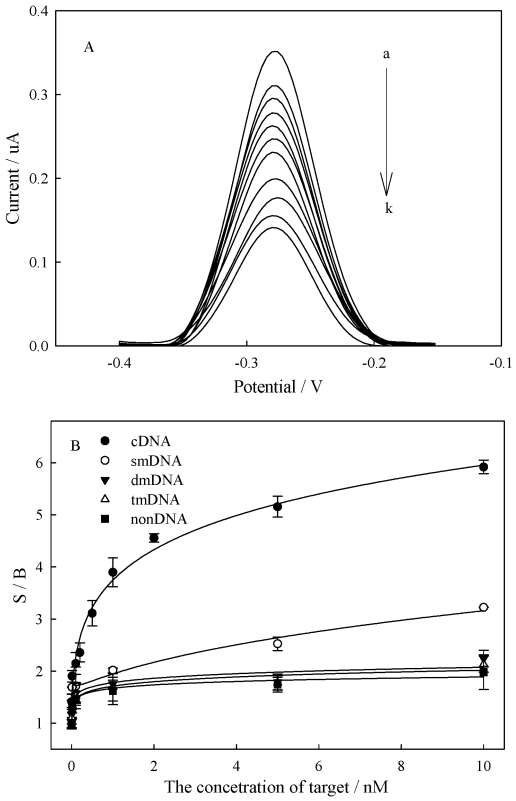

Fig. 3 (A) Alternating current voltammograms for complementary DNA at different concentrations. (a) Alternating current voltammogram of the capture probe modified on the electrode. From b to k: 0 pM, 1 pM, 10 pM, 0.1 nM, 0.2 nM, 0.5 nM, 1 nM, 2 nM, 5 nM, 10 nM DNA. (B) Plots of S/B *vs.* target DNA concentration. Error bars show the standard deviations of measurement taken from six experiments. Surface coverage: 4.79×10^{12} molecules cm^{-2}; concentration of HaeIII: 0.1 U μL^{-1}; assistant probe: assistant probe 3; concentration of assistant probe: 1 μM; temperature: 40 °C; reaction time: 60 min.

Different kinds of target DNAs including cDNA, single base mismatch DNA (smDNA), double base mismatch DNA (dmDNA), three base mismatch DNA (tmDNA) and random DNA (nonDNA) were chosen to investigate the selectivity of this biosensor. As shown in Fig. 3B, for dmDNA, tmDNA and nonDNA, the S/B ratio was observed to be very low. It was due to low hybridization efficiency for these targets. For smDNA, the S/B ratio increased slightly as the concentration of smDNA was increased. When the concentration of target DNA was 5 nM, the S/B ratio was *ca.* 5.2 for cDNA. It was more than twice higher than that of smDNA (*ca.* 2.5). Therefore, this biosensor showed good selectivity.

In summary, a simple signal-off electrochemical DNA biosensor based on the "Y" junction structure and HaeIII-aided target recycling strategy was introduced. The biosensor showed good sensitivity and selectivity. Different from those amplified DNA biosensors using enzyme labels or nanolabels, a simple and commercially available MB-labeled DNA was used in this biosensor. Compared to other electrochemical DNA biosensors based on an enzyme assisted target recycling strategy, our design could be suitable for the detection of different sequences of target DNA by changing the sequence of capture and assistant strands. It was because an assistant probe was used and the cleavage site of restriction endonuclease was isolated from target DNA.

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Notes and references

1 A. Sassolas, B. D. Leca-Bouvier and L. J. Blum, *Chem. Rev.*, 2008, **108**, 109–139.
2 Q. Guo, X. Yang, K. Wang, W. Tan, W. Li, H. Tang and H. Li, *Nucleic Acids Res.*, 2009, **37**, e20.
3 Y. He, K. Zeng, X. Zhang, A. S. Gurung, M. Baloda, H. Xu and G. Liu, *Electrochem. Commun.*, 2010, **12**, 985–988.
4 S. Cagnin, M. Caraballo, C. Guiducci, P. Martini, M. Ross, M. SantaAna, D. Danley, T. West and G. Lanfranchi, *Sensors*, 2009, **9**, 3122–3148.
5 N. J. Ronkainen, H. B. Halsall and W. R. Heineman, *Chem. Soc. Rev.*, 2010, **39**, 1747–1763.
6 J. Chen, J. Zhang, J. Li, F. Fu, H. H. Yang and G. Chen, *Chem. Commun.*, 2010, **46**, 5939–5941.
7 J. Chen, J. Zhang, Y. Guo, J. Li, F. F. Fu, H. H. Yang and G. Chen, *Chem. Commun.*, 2011, **47**, 8004–8006.
8 Z. Liu, W. Zhang, S. Zhu, L. Zhang, L. Hu, S. Parveen and G. Xu, *Biosens. Bioelectron.*, 2011, **29**, 215–218.
9 K. Hsieh, Y. Xiao and H. T. Soh, *Langmuir*, 2010, **26**, 10392–10396.
10 D. Wu, B. C. Yin and B. C. Ye, *Biosens. Bioelectron.*, 2011, **28**, 232–238.
11 Y. Chen, B. Jiang, Y. Xiang, Y. Chai and R. Yuan, *Chem. Commun.*, 2011, **47**, 12798–12800.
12 S. Nakayama, L. Yan and H. O. Sintim, *J. Am. Chem. Soc.*, 2008, **130**, 12560–12561.
13 R. Kong, X. Zhang, L. Zhang, Y. Huang, D. Lu, W. Tan, G. Shen and R. Yu, *Anal. Chem.*, 2011, **83**, 14–17.
14 A. A. Lubin, B. Vander Stoep Hunt, R. J. White and K. W. Plaxco, *Anal. Chem.*, 2009, **81**, 2150–2158.
15 X. Yang, Q. Wang, K. Wang, W. Tan, J. Yao and H. Li, *Langmuir*, 2006, **22**, 5654–5659.
16 C. Liu, Y. Lin, C. Huang and H. Chang, *Biosens. Bioelectron.*, 2009, **24**, 2541–2546.
17 L. Cui, G. L. Ke, W. Y. Zhang and C. J. Yang, *Biosens. Bioelectron.*, 2011, **26**, 2796–2800.
18 J. H. Kim, R. A. Estabrook, G. Braun, B. R. Lee and N. O. Reich, *Chem. Commun.*, 2007, 4342–4344.
19 X. L. Zuo, F. Xia, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 1816–1818.
20 H. J. Lee, Y. Li, A. W. Wark and R. M. Corn, *Anal. Chem.*, 2005, **77**, 5096–5100.
21 S. Zhang, Z. Wu, G. Shen and R. Yu, *Biosens. Bioelectron.*, 2009, **24**, 3201–3207.

