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A simple and ultrasensitive electrochemical DNA biosensor based on DNA concatamers†

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A simple, ultrasensitive and selective electrochemical DNA biosensor based on DNA concatamers is described, which can detect as low as 100 aM target DNA even in complex samples.

Detection of an ultralow amount of DNA is extremely important in the fields ranging from virus detection to the diagnosis of genetic diseases.[1] To date, numerous amplified optical detection techniques, based on target amplification or signal amplification, have been developed for the ultrasensitive sequence-specific detection of DNA, including polymerase chain reaction (PCR),[2] rolling circle amplification (RCA)[3] and some DNA machines.[4] It is well known that, although the PCR and RCA methods can be used for DNA amplification and detection of exceedingly low levels of DNA, some inherent issues still cannot be avoided, for example, complex operations, costly optical labels, and dedicated instrumentation. Electrochemical approaches, being simple, portable, low-cost, and highly sensitive, are well suited for DNA detection.[5] Several recent papers have reviewed the development of electrochemical DNA biosensors.[6] Among these sensors, some novel detection paradigms based on enzyme amplification[7] even can rival PCR for sensitivity.[8] However, many of these enzyme-based electrochemical DNA biosensors greatly increase the experimental cost and complexity.

Most recently, Zuo and co-workers reported an ingenious electrochemical sensor for DNA detection.[9] They used signal probes that hybridize to two regions of a target DNA (Fig. 1B). Thus hybridization of signal probes and target DNA can create supersandwich construction, which can lead to a significantly amplified signal compared with the traditional sandwich assay (Fig. 1A) and achieve the detection limit of 100 fM (100 pM for the traditional sandwich assay). However, since numerous target DNA should participate in the construction of the supersandwich, the detection limit of this protocol would be restricted.

Herein we develop a new DNA biosensor by introducing an auxiliary probe that can hybridize to two different regions of a signal probe and self-assemble with signal probes to form a long DNA concatamer. Then even a single target DNA can trigger the connection of a long concatamer to a capture probe on the electrode surface, instead of participating in the concatamers. This biosensor can detect as low as 100 aM target DNA, with a sensitivity similar to that of PCR. Fig. 1 is a comparison of three detection protocols. In a traditional DNA sandwich assay (Fig. 1A), a single target molecule hybridizes with a single signal probe. In our assay (Fig. 1C), the auxiliary probe (AP) is used to hybridize with two different regions of the redox-tagged signal probe (SP). In the presence of target DNA, one terminus of a target DNA can hybridize with the capture probe (CP) immobilized on the working electrode, and the other terminus can hybridize with SP. When AP is added, continuous hybridization reactions between alternating AP and SP will lead to long concatamers containing a great deal of SP and AP. Therefore, the target DNA can trigger the linkage of the long concatamers with numerous redox tags to the gold electrode to produce a remarkable amplified electrochemical signal. Accordingly, we can achieve a simple and ultrasensitive biosensor for DNA detection.

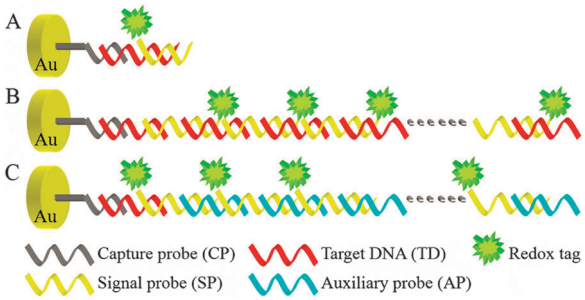

Fig. 1 Schematic representation of three electrochemical biosensors for DNA detection: (A) traditional DNA sandwich biosensor; (B) supersandwich biosensor by Zuo *et al.*; (C) DNA concatamer based biosensor.

Oligonucleotide sequences used for the detection are as follows. The 38-base target DNA (5′-GCTAGAGATTTTCCACACTG *ACTAA AAGGG TCTGA GGG*-3′) is from the HIV-1 U5 long terminal repeat sequence. The oligonucleotide sequence of the capture probe (CP) is 5′-CAGTGTGGAAAATCTCTAGC-$(CH_2)_6$-SH-3′. The sequence of the ferrocene labeled signal probe (SP) is 5′-**TACTC CCCCA GGTGC** *CCC TCAGA CCCTT TTAGT*-ferrocene-3′. The sequence of the auxiliary probe (AP)

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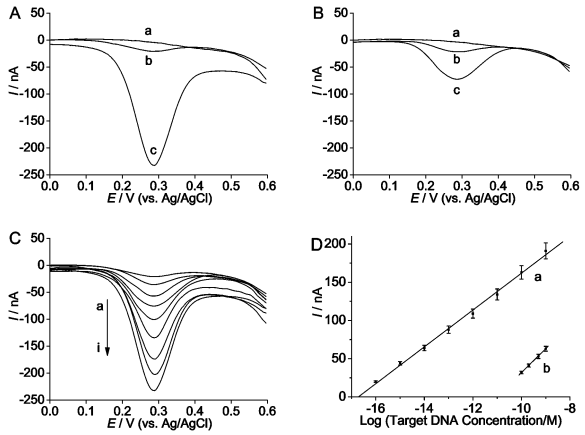

Fig. 2 (A) Differential pulse voltammetry (DPV) responses of the DNA concatamer based biosensor. The concentration of target DNA (TD) is 1 nM. The concentrations of SP and AP in the detection are 1 μM. The electrode surface is modified with various oligonucleotides: (a) CP, (b) CP + SP + AP, (c) CP + TD + SP + AP. (B) DPV responses of the traditional DNA sandwich biosensor. The electrode surface is modified with: (a) CP, (b) CP + SP, (c) CP + TD + SP. (C) DPV response curves of the concatamer based biosensor at different target DNA concentrations: (a) 0 M, (b) 100 aM, (c) 1 fM, (d) 10 fM, (e) 100 fM, (f) 1 pM, (g) 10 pM, (h) 100 pM, (i) 1 nM. 1 M $NaClO_4$ is used as the electrolyte. (D) Linear relationship between peak current and the logarithm of the target DNA concentration. Line (a) belongs to the concatamer based biosensor, while (b) belongs to the traditional sandwich biosensor. The illustrated error bars represent the standard deviation of five repetitive measurements at each concentration.

is 5′-**GCACC TGGGG GAGTA** *ACTAA AAGGG TCTGA GGG*-3′. The sequence of the signal probe of the traditional DNA sandwich assay is 5′-*CCC TCAGA CCCTT TTAGT*-ferrocene-3′. The sequence of the single-base mismatch target (1MT) is 5′-GCTAG AGATT **G**TCCA CACTG ACTAA AAGGG TCTGA GGG-3′ and the sequence of the two-base mismatch target (2MT) is 5′-GCTAG AGATT **GG**CCA CACTG ACTAA AAGGG TCTGA GGG-3′ (the underlined bold letter means the mismatch site). The 35-base non-complementary sequence (NC) is 5′-CCTTT TAGTC AGTGT GGAAA ATCTC TAGCA GTGGC-3′.

To fabricate the electrochemical DNA biosensor based on DNA concatamers, first of all, the 3′ termini of CP were attached to a gold electrode by gold–thiol chemistry and no reduction peak could be observed (Fig. 2A, a). In the absence of target DNA, although the added SP and AP can be self-assembled to form long DNA concatamers in the solution, the concatamers cannot link to the electrode surface. Thus only a small reduction peak was observed (Fig. 2A, b), due to the nonspecific adsorption of the SP. When target DNA was introduced, its 5′ terminus hybridized with the CP and the 3′ terminus hybridized with a concatamer. Then, the long DNA concatamer, self-assembled by numerous AP and ferrocene tagged SP, was firmly immobilized on the gold electrode *via* target DNA and CP. Thus a large number of ferrocene tags along each DNA concatamer produced a magnificently amplified electrochemical signal (Fig. 2A, c). The traditional sandwich assay, in contrast, yielded a much smaller signal under the same experimental conditions because of its 1 : 1 ratio of signal probes to targets (Fig. 2B, c).

The biosensor based on DNA concatamers can significantly improve the detection limit relative to the traditional sandwich assay. As shown in Fig. 2D, the traditional sandwich assay only obtains a detection limit of 100 pM. In contrast, the DNA concatamer based biosensor can detect as low as 100 aM without optimizing conditions (practical measurement indicated in Fig. 2C), and the peak current monotonically increases along the increasing target DNA concentration. The peak current of the DNA concatamer based sensor exhibits a linear correlation to the logarithm of the target DNA concentration across the eight-decade range from 100 aM to 1 nM (linear correlation coefficient is 0.9967). We believe that the dramatically amplified signal is related to the conformation of the long DNA concatamers. Our experimental results have proved that numerous AP and ferrocene tagged SP can be self-assembled to form long DNA concatamers and a single target DNA can trigger the connection of a long DNA concatamer to the electrode surface. Thus the long DNA concatamers bring numerous ferrocene tags closer to the electrode surface to produce the significantly amplified signal. Long-range charge transfer in DNA may also contribute to high sensitive detection of target DNA.[10] After hybridization, the orderly base stack of the obtained dsDNA can improve the efficiency of DNA-mediated charge transport and allow efficient electron transfer between the labeled ferrocene and electrode, thus leading to an increased electrochemical response.

We also employed atomic force microscopy (AFM) to characterize the long DNA concatamers. We fabricated the sensor on a gold electrode. In the absence of target DNA, the self-assembled long DNA concatamers were easily detached from the electrode surface when the electrode was thoroughly rinsed with water. So the AFM image (Fig. 3B) was similar to that of the electrode modified with CP and MCH (Fig. 3A). However, in the presence of target DNA, the long concatamers were linked to the CP *via* target DNA and difficultly detached. As a result, long curved DNA chains with the length more than 5.0 μm can be observed (Fig. 3C and D). According to the geometrical parameters of double stranded DNA, the separation between adjacent base pairs along the DNA axis is 0.34 nm.[11] Thus the AFM images (Fig. 3C and D) indicate that more than 15 000 base pairs were involved in a long DNA concatamer. The AFM height images also show that the average height of the long concatamer is approximate to 2 nm (Fig. 3, a–d), which is the expected magnitude for the diameter of the dsDNA helix. These results verify the connection of the long DNA concatamers to the surface of the gold electrode.

Compared with other electrochemical methods[5] for DNA detection, this concatamer based sensor is really superior in sensitivity with a wide linear range. Moreover, this sensor achieves its remarkable detection limit without sacrificing its specificity. In this work, the complementary target DNA (TD), the single-base mismatch target (1MT), the two-base mismatch target (2MT), and the non-complementary sequence DNA (NC) were used to study the sequence-specificity of the sensor. Fig. 4A shows the comparison of the peak currents of the blank and the four different target DNA strands with the same concentration of 1 nM. The sensor provides 6 : 1 differentiation between TD and 1MT, suggesting that this sensor was highly selective.

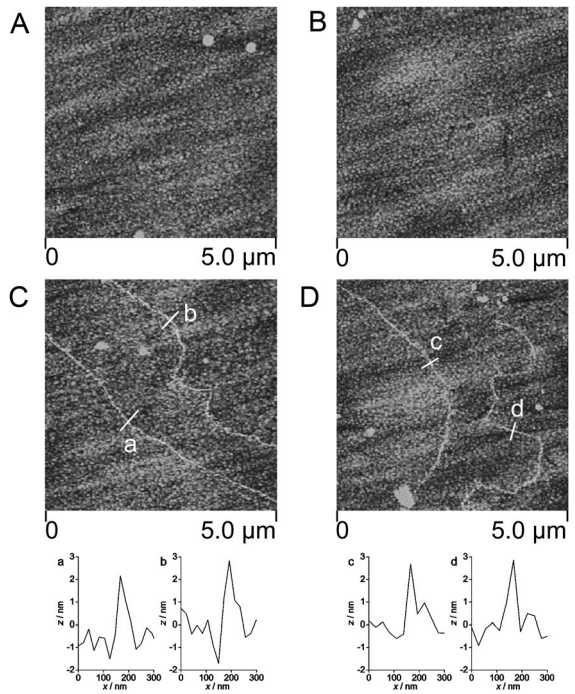

Fig. 3 AFM height images of electrode modified with MCH and various oligonucleotides: (A) CP; (B) CP + SP + AP. (C) and (D) both are assembled by CP + TD + SP + AP. (a), (b), (c) and (d) are associated height profiles.

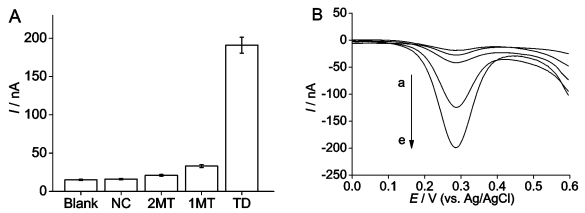

Fig. 4 (A) Bar chart of the DPV responses of the blank and the four various DNA sequences. The illustrated error bars represent the standard deviation across five repetitive experiments. (B) DPV response curves of the concatamer based biosensor in the diluted cell lysates at different target DNA concentrations: (a) 0 M, (b) 100 aM, (c) 1 fM, (d) 1 pM, (e) 1 nM.

To investigate the feasibility of our proposed sensor in real physiological media, we challenged this concatamer based sensor in a complex sample matrix of 1 : 1 diluted HeLa cell lysates. Under the same experimental conditions, the signal (Fig. 4B) became a little lower compared with the peak current observed in 1 M $NaClO_4$ (Fig. 2C), due to the interference of the sample matrix in the hybridization of the target DNA to the capture probe. The DPV curves showed that despite multicomponent nature of the cell lysates, the DNA concatamer based biosensor performed excellently.

In conclusion, our innovative biosensor, which makes use of target DNA as a trigger to connect long concatamers with numerous ferrocene tags to the gold electrode, can be used for amplified detection of DNA and detect as low as 100 aM without optimizing conditions. This detection limit is similar to that of PCR. This novel electrochemical DNA biosensor is also suitable for the detection of target DNA in the complex samples. Furthermore, this DNA biosensor, no need of any kind of enzyme or sophisticated equipment, is really simple and inexpensive. In view of these advantages, this new electrochemical DNA biosensor has a great potential in the area of DNA diagnostics and clinical analysis.

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