A NEW SOLUTION-PHASE ELECTROCHEMICAL DNA DETECTION PLATFORM WITH TARGET RECYCLING-BASED SIGNAL AMPLIFICATION

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ABSTRACT

We report a rapid, ultrasensitive, immobilization-free and signal-on electrochemical DNA biosensor with excellent discrimination ability for single-nucleotide polymorphisms. It achieves recognition signal generation by the specific cleavage function of exonuclease III and the diffusion different between oligonucleotides and mononucleotides towards a negatively charged ITO electrode surface. Meanwhile, signal can be further amplified by target recycling resulting from the probe digestion process. Combining the advantages of immobilization-free electrochemical detection and isothermal signal amplification strategy, the presented approach may represent a promising path toward direct DNA detection at the point of care.

KEYWORDS

Exonuclease iii, immobilization-free, electrochemical, DNA detection.

INTRODUCTION

Electrochemistry-based biosensors which are able to detect ultralow concentrations of specific DNA sequences have received particular attention over the past decade, because of the fact that they can provide rapid, simple and low-cost on-field detection. Up to now, most of existing electrochemical DNA biosensors require a probe immobilization step which is usually laborious, time-consuming and with low DNA hybridization efficiency, therefore limiting their practical application. To overcome this bottleneck for electrochemical DNA sensing, our group has recently developed a new immobilization-free platform for electrochemical DNA detection [1-2], in which all the DNA hybridization occurs in a solution phase instead of on a substrate-solution interface. Based on this unique platform, we reported an electrochemical melting curve analysis technology at microTAS 2009 [3] and a signal-on immobilization-free electrochemical detection strategy for DNA and polymerase at microTAS 2010 [4]. One of the issues of this solution-phase detection strategy is that its sensitivity is not as high as the traditional immobilization-based ones, as a result of the diffusion-controlled nature of the detection scheme. In order to improve the detection sensitivity, integration with signal-amplification mechanisms such as target recycling is considered to be a promising solution.

In this study, we demonstrate a versatile exonuclease III-assisted electrochemical biosensor for direct detection of DNA in homogenous solution with excellent sensitivity and selectivity. This DNA biosensor employs a methylene blue-labeled electrochemical molecular beacon (MeMB) with a protruding 3' terminus as the signaling probe and negatively charged indium tin oxide (ITO) electrode as the working electrode. It takes the advantage of strand-specific exonuclease activity of exonuclease III to achieve selective enzymatic digestion of MeMB signaling probe upon the hybridization with target sequence. Exonuclease III is an enzyme which has a specific exo-deoxyribonuclease activity for duplex DNAs in the direction from 3' to 5' terminus. However, its activity on single-stranded DNA and duplex DNAs with 3'-protruding end is limited. In another way, the enzyme's preferred substrates are blunt or recessed 3' terminus. As Fig. 1 showed, in the presence of target DNA, the MeMB probe with protruding 3' terminus recognizes and hybridizes with the target DNA to form a probe/target duplex in the form of a 3'-blunt end at the MeMB probe and a 3'-protruding end at target DNA. In this way, exonuclease III specifically recognizes this structure and selectively digests the MeMB probe from the 3' terminus, releasing methylene blue-labeled mononucleotide (MeNT). Unlike the negatively-charged MeMB probe, the released MeNT, because of the less extent of electrostatic repulsion with a negatively charged substrate surface, would diffuse freely to the negatively charged ITO working electrode, contributing to an increase of electrochemical signal. Meanwhile, the target DNA dissociates from the duplex and recycles to hybridize with a new MeMB probe, leading to gradual digestion of a large amount of MeMB probes and significantly amplify the electrochemical signal to achieve high sensitivity.

EXPERIMENT

The electrochemical measurements were conducted on an indium tin oxide (ITO) coated glass chip (Fig. 2), which is similar to the one previously reported by our group for the immobilization-free sequence-specific electrochemical detection of DNA. [4]. The chip has four patterned circular ITO spots serving as working electrodes, a Pt counter electrode and a Pt pseudo-reference electrode. For the immobilization-free detection of sequence-specific DNA, 50 μL of 1 × NEBuffer 1 containing 2 μM MeMB probe (5'–AGGAAGACGTACGTATCTTCCTTTTGTT-methylene blue-C-3'), 100 units of exonuclease III and varying concentrations of the target DNA (5'-GAACAAAAGGAAGATACGTACGAGAAGGAAAAATC-3'), 100 nM of the single-base-mismatched DNA (5'-GAACAAAAGGAAGTTACGTACGAGAAGGAAAAATC-3') or 100 nM of the three-base-mismatched DNA (5'-GAACAAAAGGAACTAACGTACGAGAAGGAAAAATC-3') was incubated

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at 37 °C for a certain period of time. Then, for each differential pulse voltammetric (DPV) scanning, 1.5 µL of the mixture solution was pipetted onto the chip to cover the Pt counter electrode, the Pt pseudo-reference electrode and one of the ITO working electrodes.

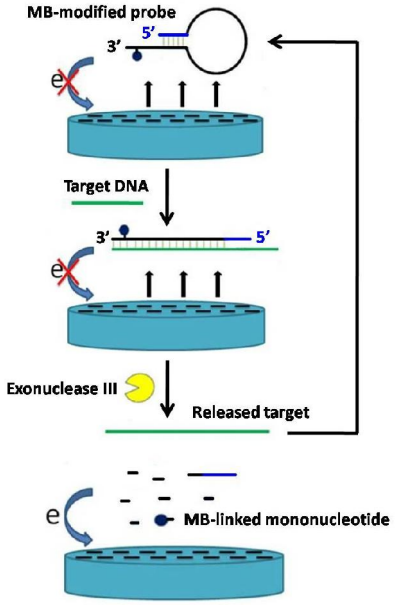

Figure 1: The working principle of the exonuclease III-assisted electrochemical DNA biosensor.

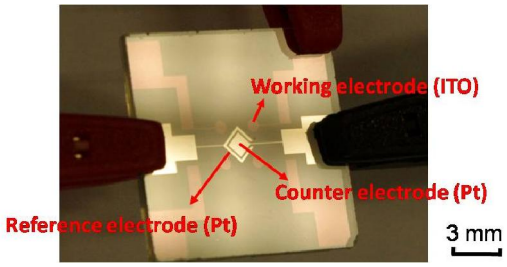

Figure 2: Schematic showing the chip for electrochemical measurements.

Due to the cyclic digestion process of the MeMB probes, the designed electrochemical DNA biosensor showed a very good performance on sensitivity. The sensitivity was investigated by challenging it with different concentrations of the target DNA. The samples were all incubated at 37 oC for one hour, and then, the response of the peak current of methylene blue was measured with DPV scans. As Fig. 3 showed, we readily achieved a 20 pM experimental detection limit of target DNA without any background subtraction. Above this concentration, the average peak-currents showed a good linear correlation with the concentration of the target DNA in the range of 20-300 pM. To further improve detection sensitivity, nano-structured redox cycling electrode arrays will be incorporated into the platform, which is currently under investigation in our group (Fig. 4).

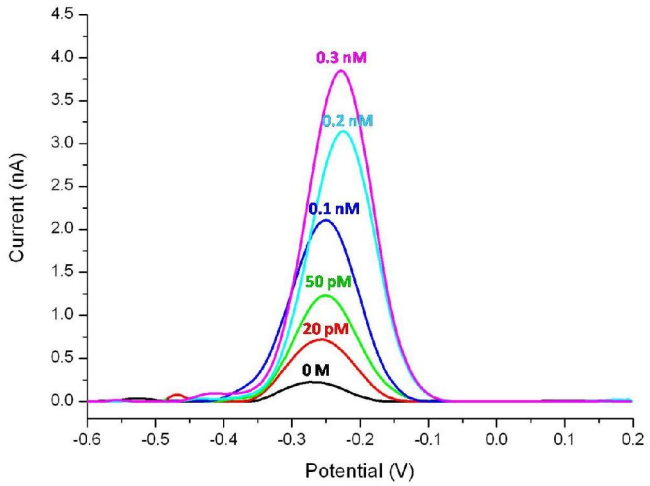

Figure 3: DPV scans of reaction mixtures containing 2 µM signaling probe, 2 unit/µL exonuclease III and varying concentrations of target DNA after incubation at 37 ^{o}C for 60 min.

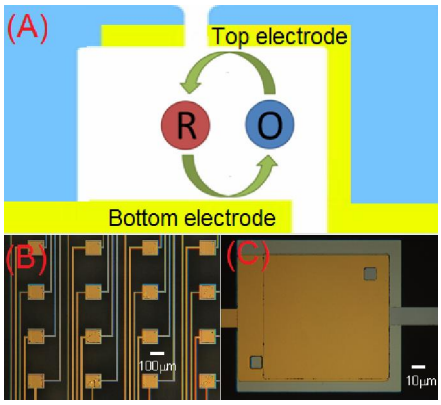

Figure 4: (A) Schematic showing the signal amplification mechanism of redox cycling dual electrodes; (B) Photo of the redox cycling electrode array; (C) Photo of a redox cycling dual electrode.

Unlike traditional electrochemical DNA biosensors immobilizing probes on the electrode which hampers the selectivity, our electrochemical DNA biosensor requires no probe immobilization step and all the hybridization is occurred in homogenous solution phase. Thus, it exhibits an excellent selectivity. In order to demonstrate this point, we challenged our sensor using 100 nM perfectly matched targets, single-base-mismatched targets and three-base-mismatched targets, respectively. As Fig. 5 showed, the resulting signal was 71% of the signal obtained with the perfectly matched target for single-base-mismatched targets and 49% for three-base-mismatched targets. This result shows the potential for this electrochemical DNA biosensor to discriminate between single nucleotide polymorphisms (SNP).

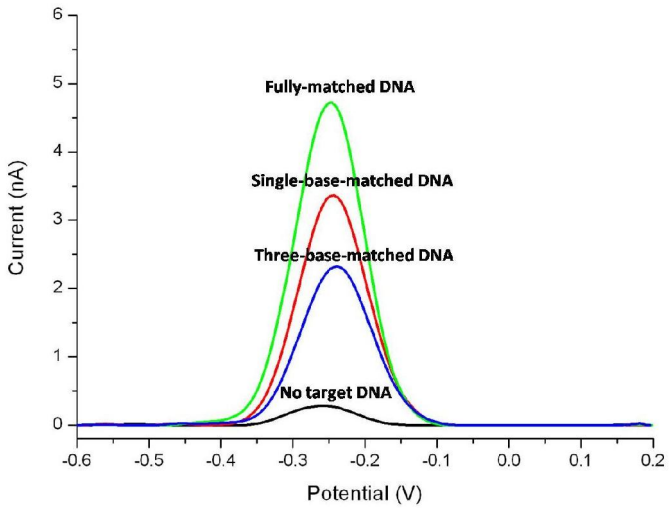

Figure 5: DPV scans of reaction mixtures containing 2 μM signaling probe, 2 unit/μL exonuclease III and 100 nM fully-matched target DNA, 100 nM single-base-mismatched DNA, 100 nM three-base-mismatched DNA or 0 μM target DNA in 1 × NEBuffer 1, after incubation at 37 ^{o}C for 60 min.

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