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Magnetic particle based electrochemical sensing platform for PCR amplicon detection

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ABSTRACT

This work reports a novel electrochemical PCR detection platform using magnetic particles as a separation tool. A redox-active intercalator, anthraquinonemonosulfonic acid (AQMS), was firstly intercalated into biotin labeled PCR amplicons, and the resulting complex was then captured by streptavidin-coated magnetic particles (MPs) to form AQMS–DNA–MP conjugates. Subsequently, these conjugates were attracted to the bottom of the tube and separated from the solution by applying an external magnetic field, resulting in a significant reduction of the concentration of solution AQMS. The concentration changes of solution AQMS, which reflect the presence and quantity of PCR amplicons, were monitored by differential pulse voltammetry (DPV) on a chip electrode. PCR cycle number-dependent as well as the initial template DNA concentration-dependent performances were investigated. This electrochemistry based PCR detection platform is simple, convenient and inexpensive, and may have potential applications for practical sample monitoring.

1. Introduction

Polymerase chain reaction (PCR) is a technique capable of amplifying trace amounts of DNA into detectable products in a short time. Traditionally, the detection of PCR products relies on optical measurement, usually taking advantage of fluorescent labels [1,2]. However, these optics-based techniques usually require complicated and expensive equipments, which make it impossible to construct portable devices for point-of-care use. Electrochemistry based oligonucleotide and PCR detection, which utilizes external electro-active labeling such as enzyme tags, nanoparticles, and DNA intercalators [3–10], or the intrinsic electro-active moiety like guanine [11,12], offers an alternative approach, which has more potential to be developed into point-of-care DNA analysis tools.

Previously, our group has developed an electrochemical real-time PCR detection platform using ferrocene-labeled dUTP as an indicator [13,14]. Although this approach is elegant and proves to exhibit comparable detection sensitivity to that of the fluorescence counterpart, it inherently requires a probe immobilization step, which brings some inconvenience and, to some extent, limits the application of this technique. Therefore, there are strong demands to develop simple and effective detection platforms for point-of-care testing applications. We have previously reported an immobilization-free electrochemical DNA detection method using ferrocene-labeled PNA probes [15,16].

Here, we introduce a new immobilization-free electrochemistry based PCR detection approach utilizing the streptavidin-coated magnetic particles (MPs) and an electro-active intercalator AQMS. MPs are widely used in the fields of biochemistry, molecular biology, etc. They have been the critical components in the development of magnetic separation, biocatalysis, drug delivery and biosensing devices [17–21]. Micro- and nano-sized MPs have good biocompability and can be rapidly isolated from the solution by applying an external magnetic field. MPs modified with various recognition groups on the surface are able to capture specific biomolecules, and the resulting biomolecule-particle assemblies may create tremendous opportunities for fabricating high performance biosensing devices [22]. In biosensing applications, MPs were commonly employed as anchoring substrates for bio-immobilization, magnetic carriers of biomolecules, and efficient tools for controlling the electrochemical process on an electrode surface [23–25]. Many MP-based electrochemical DNA detection methods have been reported [26–30]. In these methods, the electrochemical signal comes from electro-active labels incorporated onto the surface of the MPs. The electrochemical signal can be measured directly by attracting the MPs to an electrode surface. The problem with this kind of measurement is that the MPs may interfere with the electron transfer, and therefore it will be difficult to obtain reliable and reproducible results. Alternatively, the MPs with incorporated

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electro-active labels can be magnetically separated and transferred to another solution, where the electro-active labels are released and the electrochemical signal produced by the electro-active labels dissolved in the solution phase is measured. As signals produced by supernatant species rather than surface-bound ones are measured, these "double-stage techniques" reduce the interference of MPs on the electrochemical measurement. However, they involve the transfer of the MPs from one solution phase to another and therefore at least one washing steps are required in the detection process. Our work reported here also takes advantage of measuring the signals produced by electro-active indicators in the solution phase (supernatant signals). However, only one solution phase is involved in our method and therefore no washing steps are needed in the detection process. This is realized because instead of measuring the amount of electro-active indicators incorporated onto the surface of the MPs, we measure the reduction of concentration of the electro-active indicator in the solution caused by its attachment to the MPs and subsequent magnetic separation. Fig. 1 shows the working principle of our method. An electro-active intercalator (i.e., AQMS) with preferential binding affinity to double-stranded DNAs is intercalated into the matrix of biotin-modified PCR amplicons after the PCR process. Then streptavidin-coated MPs is introduced to capture the biotin-modified and AQMS-incorporated PCR amplicons and the resulting AQMS–DNA–MP complex is separated from the solution by applying an external magnetic field, leading to a significant reduction in the concentration of AQMS in the solution, which is revealed by electrochemical measurement of the supernatant solution. Therefore, the presence of PCR amplicons is indicated by reduction of the AQMS signal. To our best knowledge, this is the first report of a MP-based electrochemical DNA detection method that measures supernatant signals while involving only one solution phase.

2. Experimental

2.1. Materials

Streptavidin-coated magnetic particles (100 nm, 10 mg/ml in PBS, 0.05% sodium azide) were purchased from Chemicell. The DNA template used in PCR (M13mp18 phage DNA) and the intercalator anthraquinone-2-sulfonic acid, sodium salt monohydrate (AQMS, 97% purity) were purchased from Sigma–Aldrich. Forward primer (5′-GTAAAACGACGGCCAG-3′) and biotin labeled reverse primer (5′-biotin-TTTTTTCAGGAAACAGCTATGAC-3′) were purchased from Integrated DNA Technologies (IDT), and the dNTPs were purchased from New England Bio-labs. Taq DNA polymerase and PCR buffer were purchased from Invitrogen. SSC buffer was prepared according to standard protocol. All other reagents used were of analytical grade, and deionized water was used throughout the experiment. The microchip used for electrochemical measurement was similar to the chip electrodes reported in our group's previous work [31] and its fabrication was done in Nanoelectronics Fabrication Facility (NFF) of our University. Electrochemical measurements were performed with an Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie). PCR was performed with a C1000^{TM} thermal cycler (Bio-Rad).

2.2. Procedure

2.2.1. Production of biotin labeled PCR amplicons

PCR mixtures with and without template were prepared. Briefly, the PCR mixture contained 1 × reaction buffer (20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8), 0.2 mM dNTPs, 0.2 μM forward primer, 0.2 μM biotin labeled reverse primer, 20 pg/μL, 2 pg/μL, or 0.2 pg/μL M13mp18 phage DNA template, and 0.02 units/μL Taq DNA poly-

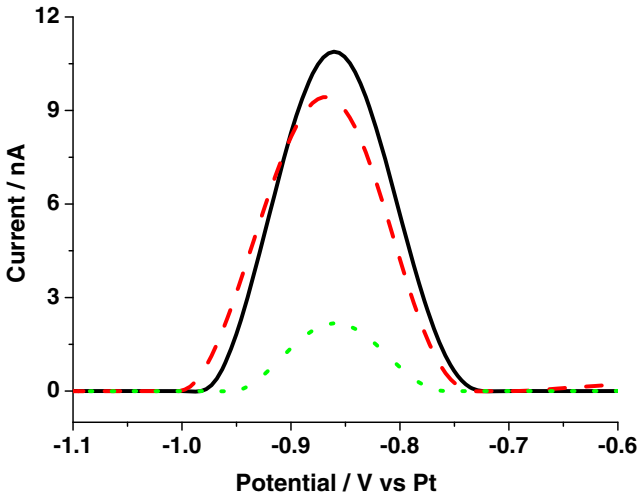

Fig. 2. Differential pulse voltammograms of AQMS in PCR mixture. Solid (—): before PCR and magnetic separation; dot (···): after PCR with DNA template and magnetic separation; dash (---): after PCR without DNA template and magnetic separation (negative control).

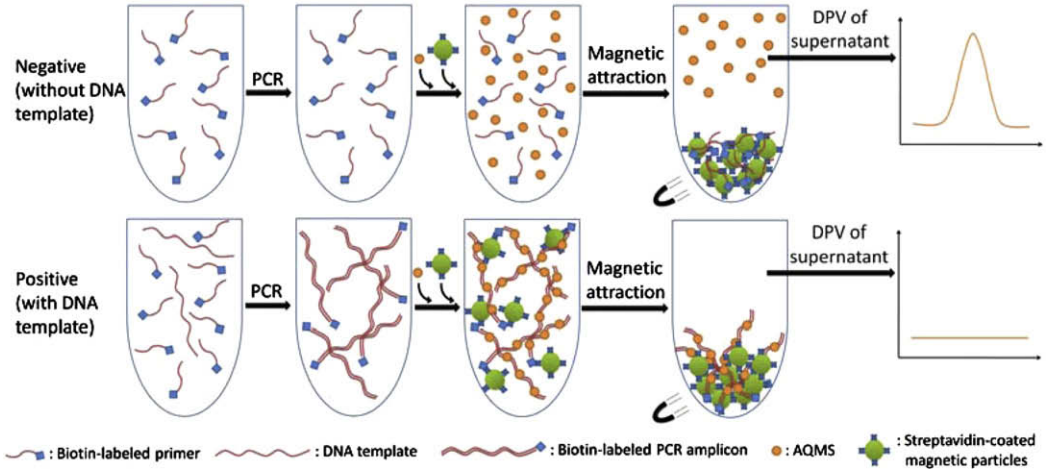

Fig. 1. Schematic representation of working principle of magnetic particle based electrochemical sensing platform for PCR amplicon detection.