Rolling circle amplification combined with nanoparticle aggregates for highly sensitive identification of DNA and cancer cells

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ABSTRACT

An electrochemical biosensor based on rolling circle amplification (RCA) and nanoparticle aggregates for highly sensitive identification of DNA and cancer cells were established in this work. First, a "sandwich-type" DNA complexes containing target DNA was constructed on the surface of the magnetic beads. Second, one part of the primer in the "sandwich-type" DNA complexes induced the RCA in the system. Then the long RCA products were digested to construct another "sandwich-type" DNA complex for the electrochemical detection. Differential pulse voltammetry (DPV) peaks with high signal intensity were obtained, and the signal intensities were found to be dependent on the amount of Fc, which is related to the concentration of target DNA. Under the optimum conditions, the electrochemical signal intensity was increased with the increase of the concentration of target DNA. A detection limit of 2.8×10^{-18} M of target DNA was achieved. Combined with aptamers technology, the proposed signal amplification strategy was also used for the identification of cancer cells with the detection limit of 100 Ramos cells mL^{-1}.

1. Introduction

As the carriers of genetic information in all cells and in many viruses, nucleic acids might provide useful information for disease diagnosis and pharmacogenomics (Risch et al., 1996). Accordingly, the sensitive, rapid and reliable methods for nucleic acid detection were highly desired in molecular biology and clinical diagnostics, especially in early diagnosis of a variety of infectious and hereditary diseases. Great efforts have been made to develop the more sensitive methods for the detection of DNA with the aim of making portable and affordable devices (Cao et al., 2002). Many optical (Cao et al., 2002; Pavlov et al., 2005; Ho et al., 2005), chemiluminescence (Miao and Bard, 2004; Zhang et al., 2008a,b; Ding et al., 2008), surface plasmon resonance (Fang et al., 2006), quartz crystalmicrobalance (Rawle et al., 2007), inductively coupled plasma mass spectrometric (ICPMS) (Zhang et al., 2004; Hu et al., 2007), atomic absorption spectrometric (Wang et al., 2001), electrochemiluminescent (Blackburn et al., 1991), and electrochemical (Zhang et al., 2008a,b) techniques have been used for detecting and quantifying sequence-specific DNA. However, the detection limit of DNA of these methods was still not as low as expected. In the DNA detection process, the amplification steps were important to realize the high sensitivity. These amplification systems included polymerase chain reaction (PCR) protocols (Woolley et al., 1996), and the using of nanoparticles (Brakmann, 2004; Du et al., 2005).

In recent years, an alternative nucleic acid amplification technique, rolling circle amplification (RCA), was adapted to the detection of DNA instead. This amplification method is achievable at constant temperature (e.g., 60 °C) simply by mixing circular single-stranded DNA probe, DNA polymerase and nicking enzyme. Unlike conventional nucleic-acid amplification reactions such as PCR, this reaction does not require exogenous primer, which often causes primer dimerization or non-specific amplification. (Murakami et al., 2009) RCA was a simple isothermal enzymatic process that can be used to generate very long single-stranded DNA (ssDNA) molecules with tandem repeats (Schweitzer et al., 2000, 2002). In RCA, a small nucleic acid circle hybridizes with a primer, which in turn extended around the circle, ultimately displacing the original primer and continuing to produce long nucleic acid products (Fire et al., 1995; Daubendiek et al., 1995). The robustness, high potential, and simplicity of RCA soon made it a powerful DNA diagnostic technology among other isothermal amplification techniques for probe/signal amplification. The utility of RCA as a method for signal amplification was initially demonstrated with great success in nucleic-acid diagnostics (Nilsson et al., 1994; Lizardi et al., 1998; Bakht et al., 2001; Christian et al., 2001; Larsson et al., 2004; Pickering et al., 2002; Smolina et al., 2007; Smolina et al., 2008; Lohmann et al., 2007). The research groups of Willner and Mao recently used the RCA

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technique to generate repetitive units of a reporter DNAzyme for the highly sensitive detection of DNA (Cheglakov et al., 2007; Tian et al., 2006). To date, techniques for the analysis of RCA product have included radiolabeling, UV absorbance, conventional gel electrophoresis, fluorescence, and electrochemical detection (Zhou et al., 2007).

Herein, we reported a novel electrochemical assay for the quantitative detection of sequence-specific DNA and cancer cells. Aiming at the detection of low abundant of target, this work integrated an advanced amplification, RCA, with the Au NPs loaded with Fc, to design a signal amplification strategy for ultrasensitive detection of DNA. Combined with the amplification of aptamers and the magnetic separation technology, the proposed signal amplification strategy was also used for the identification of cancer cells with the detection limit of 100 Ramos cells mL^{-1}. What is more, RCA is a simple amplification method with high sensitivity and specificity owing to the stringent strand matching requirement for ligation and its high amplification efficiency in contrast with PCR which is considered to be too complicated for a diagnostic setting as they require sophisticated equipment and skilled operators to perform the assays (Konry et al., 2009).

2. Experimental

2.1. Reagents

All of synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd. (China). Sequences of the oligonucleotides are listed in Table 1.

Φ29 DNA Polymerase (10 u/μL) was purchased from Fermentas (America). dNTPs were purchased from TAKARA biotechnology (dalian) CO., LTD. Endonuclease Taq I was purchased from SBS Genetech. MCH was obtained from Fluka (America). 6-SH-ferrocene (Fc) and HAuCl$_4 \cdot 3H_2O$ were purchased from j&k. C$_6$H$_5$Na$_3$O$_7 \cdot 3H_2O$ was purchased from Acros organics. Magnetic Beads (0.5–1 μm) were obtained from Beisile Chromatography Technology Development Center (tianjin).All the reagents were analytical grade and used without further purification.

0.01 M PBS buffer (pH 7.4) was prepared for dissolving DNA. 0.1 mol/L imidazole-HCl buffer (pH 7.0), and 0.1 M SDS Phosphates buffer (pH 7.0) were used for washing the surface of electrodes; 5 mM Tris–HCI buffer(0.17 moM NaCl, pH 8.0, 0.05% Tween 20) was prepared for cleaning separated Magnetic Beads. DTT solution of 1 mM was diluted by Tris–HCl (0.5 M, pH 8.0), MCH solution of 1 mM was diluted by ethnol.

2.2. Apparatus

CHI 660C electrochemical workstation (CH Instruments, Inc America) was used for the detection of DPV, CV and EIS. It consisted of a three-electrode-system structure, among them,

the gold electricity (4.0 mm) was working electrode, Ag/AgCl electrode was reference electrode and platinum electrode was working as auxiliary electrode.

2.3. Cell

Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) were obtained from the Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IUmL^{-1} penicillin Streptomycin. The cell density was determined by using a hemocytometer, and this was performed prior to any experiments. Approximately one million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and redispersed in cell media three times and were then redispersed in cell media buffer (1 mL). During all experiments, the cells were kept in an ice bath at 4 °C (Colin et al., 2008).

2.4. Preparation of Au NPs

Au NPs were prepared according to the method reported previously with a slight modification (Grabar et al., 1996; Tuerk and Gold, 1990; Ellington and Szostak, 1990). HAuCl$_4$ and trisodium citrate solutions were filtered through a 0.22 μm microporous membrane filter prior to use, and then 1.0 mL of 1% trisodium citrate was added to 100 mL of boiling 0.01% HAuCl$_4$ solution and stirred for 10 min at the boiling point. The final Au NPs prepared by this method had an average diameter of approximately 20 nm by TEM as shown in Fig. S1. The prepared colloid Au NPs were stored in brown glass bottles at 4 °C.

2.5. Modification of Au NPs with Fc and probe DNA

The process of modification of Au NPs with Fc and probe DNA was performed according to the reference with the working of Wang Jun. First of all, signal DNA need to be activated by DTT for 0.5 h. Subsequently, about 500 μL of the prepared gold colloid solution was added to 200 μL 1.0×10^{-5} M signal DNA (1 μM) and 100 μL Fc(5 mM) dissolved by n-hexane, and the mixture was shaken for 24 h. Finally, the Au NPs modified with Fc and probe DNA was collected by centrifugation at10,000 g for 30 min, and were then washed twice with n-hexane (500 μL), dispersed in PBS (200 μL). The solution of prepared Au NPs was stored at 4 °C(Tuerk and Gold, 1990; Ellington and Szostak, 1990).

2.6. Fabrication of the MB–DNA biocomplex

The binding of linker DNA with carboxyl-group-coated MBs was carried out by using the following procedure. First, carboxyl-group-coated MBs (20 μL) were transferred into a 1.5 mL Eppendorf tube and were washed three times with imidazole-HCl buffer (200 μL; 0.1 M, pH 6.0), while physically retaining the particles

Table 1
The Sequences of the DNA.

DNA	Sequence
DNA probe	5′-TTG TCA ATG TAA AGA AGG TGA ATA-SH-3′
target DNA	5′-ACT GCT AGA CAT TTT CCA CAC TGA CTA AAA GGG TCT GAG GGA-3′
Capture DNA 1	5′-TGG AAA ATC TCT AGC AGT CGT-NH2-3′
Circle DNA	5′-p-TAG CAC GGA CAT ATA TGA TGG TAC AGT CGA TAA GTG GAA GAA ATG TAA CTG TTT CCT TC-3′
Primer DNA	5′-CA TTG ACA AAG GAA GAT CGT GCC TGT TTT TTT TCC CTC AGA CCC TTT GTA-3′
Capture DNA 2	5′-SH-GCT GAC ATG GTA GTA TAT ACA GGC-3
S1	5′-AAA AAA TAC AGA ACA CCG CGT-NH2-3′
S2	5′-CGG TGT TCT GTA TTT TTT TTT TTT TTT AAA GGG TCT GAG GGA-3′
Aptamer	5′-TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG TCT CCT CCC GGT G-NH2-3