Biosensors and Bioelectronics 42 (2013) 486–491

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

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

Caifeng Ding *, Nannan Wang, Jian Zhang, Zhenfeng Wang

State Key Laboratory Base of Eco-chemical Engineering; Key Laboratory of Biochemical Analysis, Ministry of Education; College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China

ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form
21 September 2012
Accepted 3 October 2012
Available online 26 October 2012

Keywords:
Rolling circle amplification (RCA)
DNA
Cancer cells
Nanoparticle (NP)
Differential pulse voltammetry (DPV)

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

* Corresponding author. Tel./fax: +86 532 84022750.
E-mail address: dingcaifeng2003@163.com (C. Ding).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.10.015

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. $C_6H_5Na_3O_7 \cdot 3H_2O$ 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 $lUmL^{-1}$ 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

with a magnetic field. Then, 0.2 M solution of EDC (150 μL) was added to the MBs for 40 min to activate the MBs. Second, 10 μL capture DNA 1(1.0 μM) was added into the above MBs. The MBs and capture DNA 1 were incubated for 12 h at 37 °C with gentle shaking. The formed MB–capture DNA 1 were separated. Then target DNA (100 μL, 0.1 μM) were added. The mixture was incubated at 37 °C for 1 h and then separated. Finally, primer DNA (10 μL 1.0 μM) was added into the MB–DNA biocomplex and incubated at 37 °C for 1 h. After all, the MB–DNA biocomplex was separated from the solution with a magnetic field. All above MBs were washed three times with Tris–HCI buffer (200 μL, 0.17 M NacL, pH 8.0, 0.05% Tween 20, 5 mM).

2.7. The procedure of RCA

First, 10 μL circle DNA (1.0 μM) was added into the above MB–DNA biocomplex and incubated at 37 °C for 1 h with gentle shaking. The formed biocomplex was separated from the incubation solution and washed three times with Tris–HCI buffer (200 μL). Second, *Φ*29 DNA polymerase (10 U) and dNTPs (100 μM) were added and incubated at 37 °C for 2 h with gentle shaking. Finally, the products of RCA were cut off by DNA endonucleases at 60 °C for 2 h. Then they were separated with a magnetic field and the solution was reserved (Konry et al., 2009; Zhao et al., 2006, 2008; Li et al., 2009; Schweitzer et al., 2002; Wang et al., 2004).

2.8. Fabrication of the CL biosensor

A gold electrode was polished carefully with alumina slurries (1, 0.3, 0.05 μm) and washed ultrasonically with deionized and doubly distilled water. Then it was electrochemically cleaned in 0.5 M H_2SO_4 solution by cyclic potential scanning between 0.3 and 1.5 V until a standard CV was obtained. Subsequently, the gold electrode was rinsed with deionized and doubly distilled water and absolute ethanol in turn. Subsequently the pretreated electrode was immersed in 100 μL capture DNA 2 (1.0 μM) and incubated for 1 h. In order to avoid consequent nonspecific adsorption in the following hybridization steps, the modified electrode was immersed in the MCH solution for 1 h to block the uncovered gold surface. The modified electrode was immersed into the products of DNA endonucleases with the different concentration for 2 h. Then the modified electrode was immersed into Au nanoprobes for 24 h. In order to avoid consequent nonspecific adsorption, all of the above electrodes were washed with 0.1 M phosphate buffer containing 1‰ SDS (pH 7.0) after each step of fabrication process.

2.9. Electrochemical detection

Electrochemical detection was detected according to our former work with some modifications (Zhang et al., 2008a,b). All Electrochemical detections proceeded in a 10 mL beaker with three electrode systems (working electrode, reference electrode, auxiliary electrode), and they were inserted into $KClO_4$ (2 mL, 0.1 M). The positive differential pulse voltammetry (DPV) scan was performed after a 15 s rest period (without stirring) from 0.2 to 0.8 V (vs. Ag/AgCl), with a pulse amplitude of 50 mV and a pulse width of 50 ms. The anodic stripping peak current ($i_{p.\ a}$) located at ca. 0.42 V was taken as the analytical response.

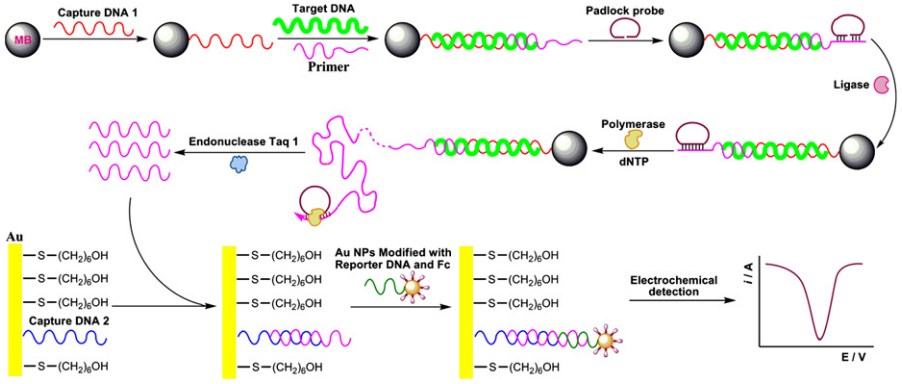
3. Results and discussion

The electrochemical principle used for the amplified sensing of target DNA was shown in Scheme 1. Capture DNA 1 was connected to MBs for 12 h. And target DNA was tethered to capture DNA 1 for 1 h. Then, one end of primer DNA was connected to target DNA, the other end was connected to circle DNA through complementary base-pairing reactions for 1 h. In the presence of dNTPs and *Φ*29 DNA polymerases, the RCA was initiated to produce micrometer-long single-strand DNA for 2 h, which contained hundreds of tandem-repeat sequences. Finally Taq I DNA enzymes were added to the MB–DNA biocomplex and incubated for 2 h at 65 °C with gentle shaking. The long RCA products were then digested to a large amount of short single strand DNA with the same sequence as transfer DNA (t DNA).

After magnetic separation, t DNA was collected to fabricate the electrochemical biosensor with capture DNA **2** self-assembled on the surface of the Au electrode. Another end of t DNA hybridized with the signal DNA loaded on the surface of Au NPs. The quantity of the t DNA was monitored by differential pulse voltammetry (DPV) scan.

3.1. Characterization of the process of RCA

To confirm the efficiency of the RCA replication as expected, DNA samples were analyzed by agarose gel (1.0%) electrophoresis experiment as shown in Fig. S4 in Supporting Information. The nucleotides sequence of RCA products (Fig. S4A, line 2, 3) were longer than marker DNA after 2 h RCA reactions. Compared to the primer DNA, the long DNA products were generated by successive RCA steps. In the presence of Taq I, the long DNA molecules were indeed converted into much shorter DNA fragments (Fig. S4B, line 2, 3).



Scheme 1. Schematic diagram of RCA and electrochemical detection of DNA.

3.2. Characterization of signal DNA probe

The UV-visible spectrum was performed to characterize the conjugation between the AuNPs and DNA and Fc as shown in Fig. S5. Curve d exhibited both the characteristic absorbance of DNA (curve a) and Fc (curve b) at about 520 nm. It also included the characteristic absorbance of AuNPs (curve c) at about 440 nm. The results indicated that the signal DNA and Fc had been successfully labeled on the Au NPs.

3.3. Characterization of DNA sensors

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were performed on a model 660C electrochemical analyzer (CH Instruments). CV imaging and EIS were adopted to demonstrate the structure of the DNA sensors, as shown in Fig. S6 in the Supporting Information. The CV curves showed the changes of the electrode behavior after each step. As shown in Fig. S6A, a step-by-step modification on the Au electrode was accompanied by a decrease in the amperometric response and an increase in the peak-to-peak separation between the cathodic and anodic waves, showing the electron transfer kinetics of $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ obstructed. After the Au electrode was assembled with capture DNA and treated with MCH, the electron transfer between the assembled monolayer and electrode surface was inhibited. When the capture DNA hybridized with DNA–Au NPs probe, the peak currents of the redox couple of $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ decreased again. As described above, capture DNA, Au NPs could make it difficult for the electron transfer.

EIS can also give detailed information on the impedance changes in the modification pro-cess. The semicircle portion of the impedance spectra, corresponding to the electron-transfer-limited process, became larger and larger after each modification step, as shown in Fig. S6B. Both CV and EIS results were consistent with the fact that the electrode was fabricated as expected.

3.4. The selectivity of the present method

To demonstrate the principle of the present assay, the selectivity was determined as to whether the biosensor could differentiate between target and non-target DNA. The results established that the electrochemical signal of the target DNA (Fig. 1, curve c) was significantly higher than that of non-target DNA (curve b) which was approximately the same as the blank solution (curve a). These results indicated that the DNA biosensor was of higher sensitivity and bounded selectively to the target DNA.

3.5. Application of the strategy for the detection of DNA

Under the optimal conditions, the DPV peak current was proportional to the DNA concentration ranging from 1.0×10^{-17} to 8.0×10^{-16} M with a correlation coefficient, R, of 0.9969 ($n=10$) (Fig. 2). The regression equation, I (μA)$=0.9939+1.2666C$ (M), was used, in which I was the current intensity, and C was the DNA concentration. A series of eleven duplicate measurements of 2.0×10^{-17} M, were used for estimating the precision, and the relative standard deviation (RSD) was 4.8%; this showed good reproducibility. The detection limit for DNA concentration was

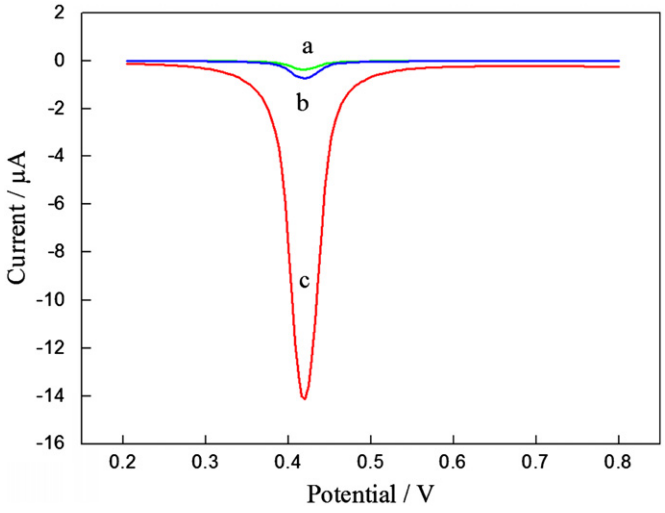


Fig. 2. A. DPV curve of DNA sensor in $KClO_4$ for hybridizing different concentrations of target DNA; **B.** DPV curve of the DNA sensor, where the definition of signal is the same as that in Fig. A.

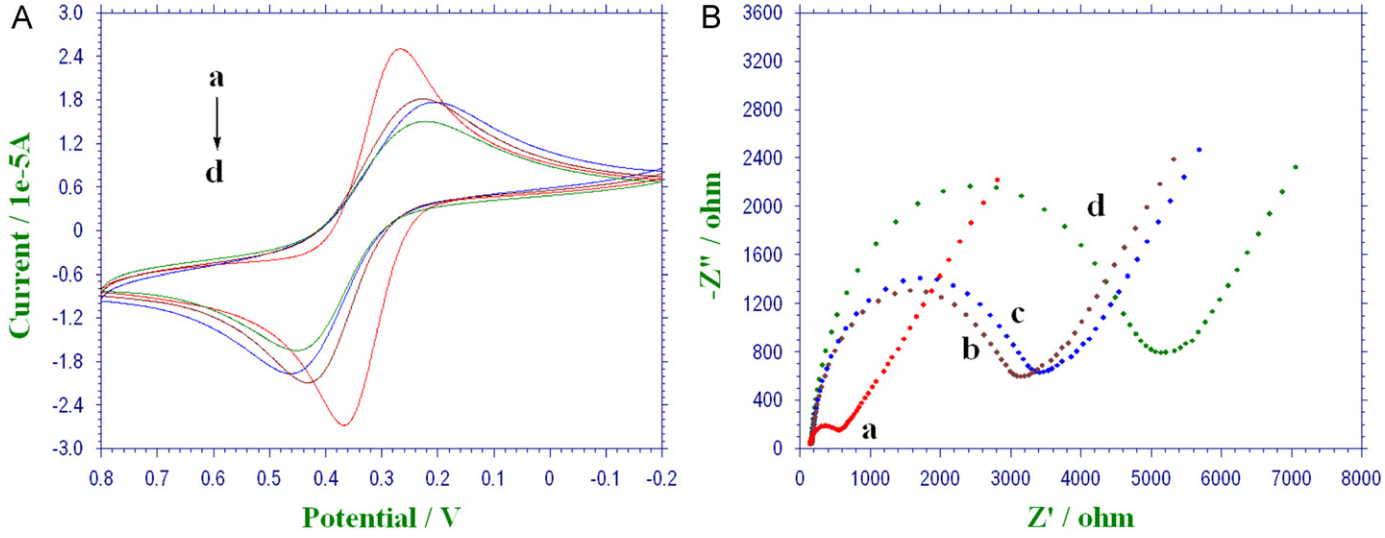
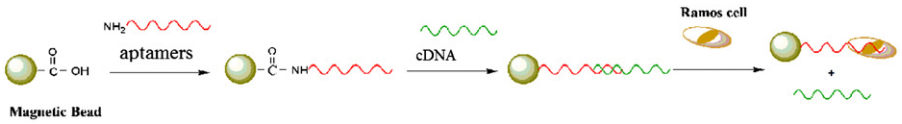


Fig. 1. Sensitivity of the DNA biosensor: a, blank solution; b, non-target DNA sequences; c, target DNA sequences; (all the concentrations of target DNA in b and c were 1.0×10^{-16} M).



Scheme 2. Schematic diagram of reaction of Ramos cells and aptamer.

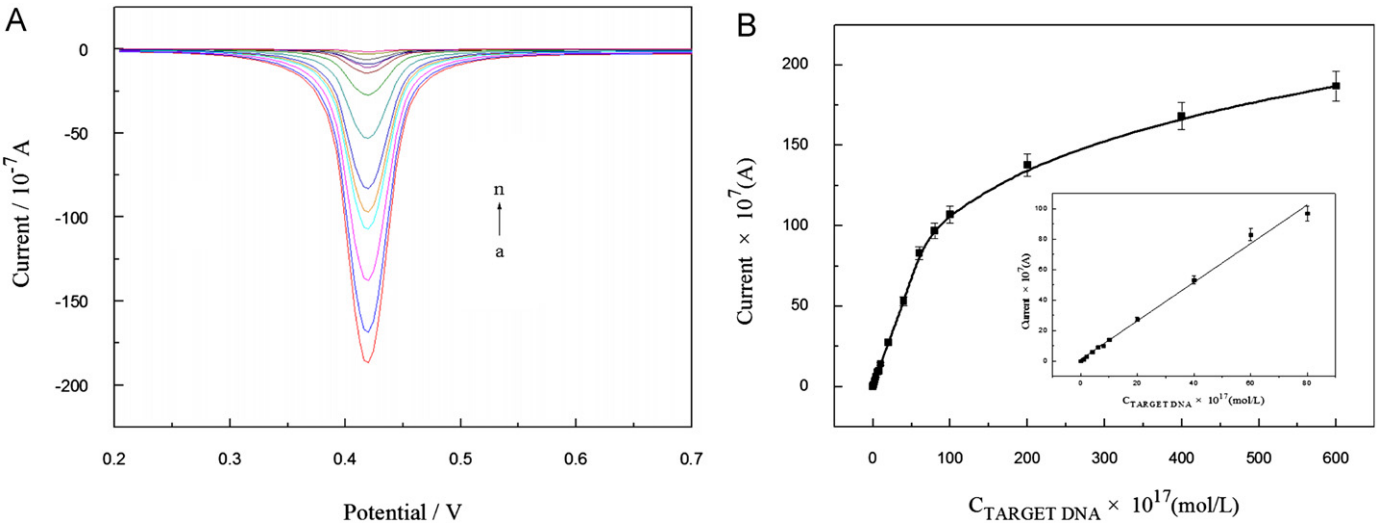


Fig. 3. A. Dose-responses between the DPV peak currents and the different concentrations of the Ramos cells: 100, 200, 400, 600, 800 and 1000 cells mL^{-1}; **B.** Liner relationship between the DPV peak currents and the different concentrations of the Ramos cells: 100, 200, 400, 600, 800 and 1000 cells mL^{-1}.

calculated to be 2.8×10^{-18} M at 3σ. The low detection limit was ascribed to the signal amplification of RCA.

3.6. *Application of the strategy for the detection of cells*

As shown in Scheme 2, the binding of amino-modified aptamers of Ramos cells with carboxyl-group-coated MBs was carried out by using a slightly modified procedure (Colin et al., 2008). Then the MB–DNA biocomplex was formed after the aptamers hybridizing with complementary DNA (cDNA). In the presence of Ramos cells, cDNA could be released from the biocomplex due to the stronger affinity between the aptamers and its targets. The concentration of the cDNA, which indirectly reflected the amount of the Ramos cells, was detected as described in Scheme 1 as target DNA.

Under the optimal conditions, the DPV peak current was proportional to the cell concentration ranging from 10^2 to 10^3 cells mL^{-1} with a correlation coefficient, R, of 0.9993 (n=5) as shown in Fig. 3. The regression equation, I (mA)=I=1.7725+ 0.1111C (cells mL^{-1}), was used, in which I was the current intensity, and C was the cell concentration.

4. Conclusions

A versatile method was developed for ultrasensitive detection of DNA (down to 2.8×10^{-18} M) by combining the RCA technique with the biofunctionalization of Au NPs. By integrating DNA hybridization principle, nanobiotechnology, and electrochemical detection, this primary research opened new horizons for integrating different disciplines and would promote the basic and clinical research. The proposed RCA-NPs strategy, which had been applied to the detection of cancer cells with the detect limitation of 100 Ramos cells mL^{-1}, would become a powerful tool for genome research and clinical diagnostics.

Acknowledgment

This work was supported by the National Nature Science Foundation of China (No. 20975059), National Basic Research Program of China (No. 2012CB722606), the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT), the Excellent Young Scientists Foundation of Shandong Province (JQ201005), the National Nature Science Foundation of Shandong Province (No. ZR2009BZ005), the University Doctoral Program Foundation for Youth Teacher, the Ministry of Education of China (20093719120003), and the Fund Project for Shandong Key Technologies R&D Program (2010GGX10417).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.10.015.

References

Bakht, J., Shafi, M., Khan, AM., 2001. Pakistan Journal of Science 4, 516–518.
Blackburn, E.H., 1991. Nature 350, 569–573.
Brakmann, S., 2004. Angewandte Chemie 116, 5851–5855.
Cao, Y.W.C., Jin, R., Mirkin, C.A., 2002. Science 297, 1536–1540.
Christian, B., Christelle, T., Wang, L., Bruce, A., Katherine, H., Arvind, K., Gary, B., David Boykin, W., 2001. Biochemistry 40 (33), 9770–9779.
Colin, D.M., Joshua, E.S., Tang, Z.W., Wu, Y.R., Suwussa, B., Tan, W.H., 2008. Analytical Chemistry 80, 1067–1072.
Cheglakov, Z., Weizmann, Y., Basnar, B., Willner, I., 2007. Org. Biomol. Chem. 5, 223–225.
Daubendiek, S.L., Ryan, K., Kool, E.T., 1995. JACS 117, 7818–7819.
Du, D., Ju, H.-X., Zhang, X.-J., Chen, J., Cai, J., Chen, H.-Y., 2005. Biochemistry 44, 11539–11545.
Ding, C.F., Zhong, H., Zhang, S.S., 2008. Biosensors and Bioelectronics 23, 1314–1318.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
Fang, Y., Brendan, P., Orner, 2006. Chemical Biology 1 (9), 557–558.

Fire, A., Xu, SQ., 1995. Proceeding of the National Academy of Sciences 92 (10), 4641–4645.
Grabar, K.C., Smith, P.C., Musick, M.D., Davis, J.A., Walter, D.G., Jackson, M.A., Guthrie, A.P., Natan, M.J., 1996. Journal of the American Chemical Society 118, 1148.
Ho, H.A., Dore, K., Boissinot, M., Bergeron, M.G., Tanguay, R.M., Boudreau, D., Leclerc, M., 2005. American Chemical Society 127, 12673–12676.
Hu, J., David, R., 2007. Biochemistry 46 (25), 7581–7589.
Konry, T., Hayman, R.B., Walt, D.R., 2009. Analytical Chemistry 81 (14), 5777–5782.
Larsson, C., Koch, J., Nygren, A., Janssen, G., Raap, AK., Landegren, U., Nilsson, M., 2004. Nature Methods 1 (3), 227–232.
Li, N., Jablonowski, C., Jin, H.L., Zhong, W.W., 2009. Analytical Chemistry 81, 4906–4913.
Lizardi, P.M., Huang, X., Zhu, Z., Bray-Ward, P., Thomas, DC., Ward, DC., 1998. Nature Genetics 19 (3), 225–232.
Lohmann, JS., Stougaard, M., Koch, J., 2007. BMC Biotechnol 7 (1), 49.
Miao, W., Bard, A.J., 2004. Analytical Chemistry 76, 5379–5386.
Murakami, T., Sumaoka, J., Komiyama, M., 2009. Nucleic Acids Research 37, 3.
Nilsson, M., Malmgren, H., Samiotaki, M., Kwiatkowski, M., Chowdhary, BP., Landegren, U., 1994. Science 265 (5181), 2085–2088.
Pavlov, V., Shlyahovsky, B., Willner, I., 2005. Journal of the American Chemical Society 127, 6522–6523.
Pickering, J., 2002. Nucleic Acids Research 30 (12), 60.
Rawle, Robert J., Selassie, Cynthia R.D., Johal, Malkiat S., 2007. Langmuir 23 (19), 9563–9566.
Risch, N., Merikangas, K., 1996. Science 273 (5281), 1516–1517.
Schweitzer, B., Roberts, S., Grimwade, B., Shao, W., Wang, M., Fu, Q., Shu, Q., Laroche, I., Zhou, Z., Tchernev, V.T., Christiansen, J., Velleca, M., Kingsmore, S.F., 2002. Nature Biotechnology 20, 359–365.
Schweitzer, B., Wiltshire, S., Lambert, J., O'Malley, S., Kukanskis, K., Zhu, Z., Kingsmore, S.F., Lizardi, P.M., Ward, D.C., 2000. Proceedings of the National Academy of Sciences of the United States of America 97, 10113–10119.
Smolina, I., Lee, C., Frank-Kamenetskii, M.D., 2007. Applied and Environmental Microbiology 73, 2324–2328.
Smolina, I., Kuhn, H., Lee, C., Maxim, D., 2008. Bioorganic & Medicinal Chemistry 16, 84–93.
Tian, C., Richard, M. Breyer, Hak Jun Kim, Murthy, D., David, B., Anne Karpay, Charles, R., 2006. JACS 128 (15), 5300–5300.
Tuerk, C., Gold, L., 1990. Science 249, 505–510.
Woolley, Adam T., Hadley, Dean, Landre, Phoebe, deMello, Andrew J., Mathies, Richard A., Northrup, M.Allen, 1996. Analytical Chemistry 68 (23), 4081–4086.
Wang, G.L., Yuan, J.L., Gong, B.L., Matsumoto, K., Hu, Z.D., 2001. Analytica Chimica Acta 448, 165–172.
Wang, G., Maher, E., Brennan, C., et al., 2004. Genome Research 14 (11), 2357–2366.
Zhang, M., Mamatha, D., Baher, M., Francis, X., Zhang, J., Lynn, S., David, M., 2004. Langmuir 20 (19), 8015–8021.
Zhang, S.S., Xia, J.P., Li, X.M., 2008a. Analytical Chemistry 80, 8382–8388.
Zhang, S.S., Zhong, H., Ding, C.F., 2008b. Analytical Chemistry 80, 7206–7212.
Zhao, W.A., Ali, M.M., Brook, M., A., Li, Y.F., 2008. Angewandte Chemie International Edition 47, 6330–6337.
Zhao, W.A., Gao, Y., Kandadai, S.A., Brook, M.A., Li, Y.F., 2006. Angewandte Chemie International Edition 45, 2409–2413.
Zhou, L., Ou, L.J., Chu, X., Shen, G.L., Yu, R.Q., 2007. Analytical Chemistry 79 (19), 7492–7500.