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Dual-probe electrochemical DNA biosensor based on the "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification for detection of double-strand DNA of PML/RARα related fusion gene

Kun Wang [a,c], Yun Lei [a,e], Guang-Xian Zhong [a,d,e], Yan-Jie Zheng [a,e], Zhou-Liang Sun [c], Hua-Ping Peng [a,e], Wei Chen [a,e], Ai-Lin Liu [a,e,*], Yuan-Zhong Chen [b,e,**], Xin-Hua Lin [a,e,*]

[a] Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou 350004, China
[b] Fujian Institute of Hematology, the Affiliated Union Hospital of Fujian Medical University, Fuzhou 350000, China
[c] Department of Pharmacy, the First Affiliated Hospital of Xiamen University, Xiamen 361003, China
[d] Department of Orthopaedics, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350004 China
[e] Nano Biomedical Technology Research Center, Fujian Medical University, Fuzhou 350004, China

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ABSTRACT

Taking advantage of "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification, a dual-probe electrochemical DNA (DE-DNA) biosensor was designed to detect double-stranded DNA (dsDNA) of acute promyelocytic leukemia (APL) related gene. Two groups of detection probes were designed, and each group was composed of a biotinylated capture probe and an assisted probe. They were separately complementary with two strands of target dsDNA in order to prevent the reannealing of the two separate strands from target dsDNA. First, thiol functionalized capture probes (C1 and C2) were severally assembled onto two different gold electrodes, followed by hybridizing with target dsDNA (S1a–S1b) and assistant probes to form two Y-junction-structure ternary complexes. Subsequently, restriction sites on the ternary complexes were digested by *Rsa I*, which can release S1a, S1b and biotins from the electrode surfaces. Meanwhile, the released S1a and S1b can further hybridize with the unhybridized corresponding detection probes and then initiate another new hybridization–cleavage–separation cycle. Finally, the current signals were produced by the enzyme-catalyzed reaction of streptavidin-horse reddish peroxidase (streptavidin-HRP). The distinct difference in current signals between different sequences allowed detection of target dsDNA down to a low detection limit of 47 fM and presented excellent specificity with discriminating only a single-base mismatched dsDNA sequence. Moreover, this biosensor was also used for assay of polymerase chain reaction (PCR) samples with satisfactory results. According to the results, the power of the DE-DNA biosensor as a promising tool for the detection of APL and other diseases.

1. Introduction

As a sub type of acute myeloid leukemia (AML), acute promyelocytic leukemia (APL), which is designated M3 in the French-American-British (FAB) classification, is characterized by a translocation between the promyelocytic leukemia gene (PML) on chromosome 15 and the retinoic acid receptor-α (RARα) gene on chromosome 17 (Rowley et al., 1977). The reciprocal translocation t (15;17) (q22;21) leads to the expression of the promyelocytic leukemia/retinoic acid receptor alpha (PML/RARα) fusion protein (Borrow et al., 1990; Andres et al., 2003). This single gene rearrangement is a unique marker gene in APL and plays an important role in leukemogenesis through antagonizing retinoic acid signaling and the regulatory pathways mediated by APL (Daniela et al., 1995; Larson et al., 1984). Thus, the detection of PML/RARα fusion gene is significant in early diagnosis, monitoring of minimal residual disease and prognosis about APL. Currently, the monitoring and testing methods of clinical diagnosis and prognosis

* Corresponding authors at: Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou 350004, China. Tel./fax: +86 591 22862016.
** Corresponding author at: Fujian Key Lab of Hematology, Fujian Institute of Hematology, Fujian Medical University Union Hospital, Fuzhou 350001, China Tel.: +86 591 83351966; fax: +86 591 83324116.
E-mail addresses: ailinliu@mail.fjmu.edu.cn (A.-L. Liu), chenyz@mail.fjmu.edu.cn (Y.-Z. Chen), xhl1963@sina.com (X.-H. Lin).

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about fusion gene of APL have mainly included flow cytometry (FCM) (Wolfgang et al., 2010), fluorescence in situ hybridization (FISH) (Choughule et al., 2009), chromosome analysis (Tirado et al., 2003) and real-time quantitative reverse transcription PCR techniques (RT-PCR) (Koshy et al., 2012). But there are some limitations in these methods, such as low sensitivity, poor precision, time-consuming and expensiveness. Therefore, it is an urgent need to develop a new rapid, effective and convenient method with high selectivity and sensitivity to detect PML/RARα fusion gene.

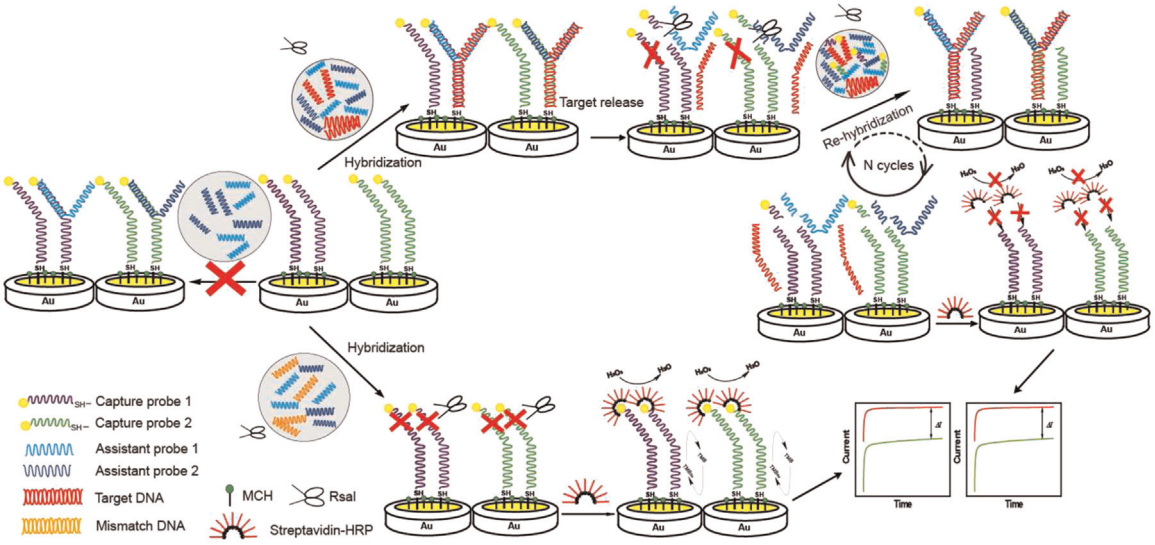
In recent years, deoxyribonucleic acid (DNA) testing techniques play a more and more important role in the clinical diagnosis and biomedical research with the development of molecular biotechnology. Among them, electrochemical DNA (E-DNA) biosensors have prominent advantages of high sensitivity, simplicity, compatibility, miniaturization and low cost (Cagnin et al., 2009; Ronkainen et al., 2010). Thus, it has already become a hot research area and demonstrated a good development prospect for the diagnosis of genetic diseases and other biological analysis (Zhang et al., 2010; Sheng et al., 2010; Wang et al., 2010; Pournaghi-Azar et al., 2010).

A typical electrochemical DNA biosensor can be prepared by immobilizing only one kind of DNA probe on an electrode and using electroactive substances to monitor the hybridization events between the DNA probes and target DNA (Eric, 2004; Peter, 2005). There were several E-DNA biosensors reported for the detection of the synthetic single-stranded (ss-) PML/RARα fusion gene in our research group (Zhong et al., 2011; Liman et al., 2012; Ailin et al., 2011; Yun et al., 2013; Wang et al., 2011). However, the target DNA segments derived from clinical samples exist in form of native double-stranded (ds-) fragments (e.g., double-stranded DNA or DNA/RNA complexes) which are stable in vitro. So the double-stranded targets have to be denatured in some ways (e.g., thermal denaturation, chemical reagent treatment) before hybridization with DNA probes which have a complementary sequence to the DNA sample. However, the reannealing of the two separate strands from target dsDNA would have an impact on the recognition between the DNA probes and the corresponding complementary target strand during subsequent hybridization of capturing target double-stranded DNA (dsDNA) by single kind of DNA probe. In an attempt to avoid this situation, some reported electrochemical detection methods would employed asymmetric PCR technique to generate an excess of single-stranded target DNA amplicon that could be directly detected by probe conjugated to the electrode surface (Gang et al., 2008; Rebeca et al., 2007; Kagan et al., 2006). Nonetheless, compared to symmetric PCR, asymmetric PCR has poor amplification efficiency. The studies showed that the efficiency of a symmetric PCR could reach around 90%, while the efficiency of asymmetric PCR was just 70% under the optimized condition (Gyllensten and Allen, 1993). So it is potential to propose a direct detection approach of native dsDNA segments based on E-DNA biosensor platform in order to offer better specificity, sensitivity and simplicity for clinical samples in the future.

In order to overcome the limitations of single-probe electrochemical DNA biosensor, a novel sensing strategy based on double-probe detection was designed in our research. This sensing strategy contained two different sequences of DNA probes which were separately complementary to the corresponding strand in target dsDNA. The two kinds of DNA probes modified onto the different electrodes could achieve to recognize the two corresponding complementary sequences of target dsDNA in the same hybridization system after thermal denaturation. Compared with single-probe E-DNA biosensor, this dual-probe electrochemical DNA (DE-DNA) biosensor can effectively reduce the influence of reannealing of the two separate strands of target dsDNA and improve the efficiency of probe–target hybridization.

As the key point for the design of this novel sensing strategy, the selectivity between DNA probes with complementary sequences has an important impact on the performance of double-probe E-DNA biosensors. Nakayama and co-workers have reported a novel nucleic acid probe detection technique called as junction-probe (Shizuka et al., 2008). This technique contains a pair of probes (including a capture probe and an assistant probe), which do not hybridize with each other at a specific temperature, can be made to anneal to each other in the presence of a target sequence by the formation of a Y-junction-structure ternary complex. As a new nucleic acid probe detection technique, the E-DNA biosensors based on the junction-probe strategy were reported to show better specificity ability for the detection of single-base mismatch (Jing et al., 2009; Yanli et al., 2009). Additionally, signal amplification is also a way to improve the performance of E-DNA biosensors. Restriction enzymes are endonucleases from eubacteria and archea that recognize a specific DNA sequence. Recently, some E-DNA biosensors based on endonuclease assisted electrochemistry signal amplification were reported for the direct detection of small amounts of DNA with limits of detection (Hsieh et al., 2010; Liu et al., 2011, 2009; Natalie and Jacqueline 2013). The strategy of this biosensor was as follow: the capture probe modified on the electrode can hybridize with the target DNA sequence to form the target DNA/capture DNA duplexes structure which contained restriction site. Then, the structure would be cleaved into two pieces so as to make the target DNA released by enzyme endonuclease via identifying restriction site. Following, the released target DNA can identify another capture probe and start another hybridization–cleavage–separation cycle. Finally, the detection signal would be amplified via several repeated cycles (hybridization–cleavage–separation) since the target DNA can be recycled in many repeated cycles. Therefore, the choice of restriction sites with specific sequences was very important in this strategy. However, the sites were located on the capture probe/target DNA duplexes in most of these E-DNA biosensors, so it was required that the target DNA should contain special sequences. For this reason, these highly sensitive E-DNA biosensors only could detect the sequence-specific target DNA. It would limit the application of these approaches.

In order to overcome the above deficiencies, a novel sensing strategy based on double probes detection was designed by combining “Y” junction structure and restriction endonuclease assisted cyclic enzymatic amplification for detection of PML/RARα fusion gene dsDNA in this study (Scheme 1). Two Y-junction-structure ternary complexes were formed by hybridization, both of which contained a biotin labeled capture probe, an assistant probe and a strand from target dsDNA (C1–A1-Sa and C2–A2-Sb). In the sensing mode, two groups of biotin labeled capture probes (C1 and C2) were separately complementary to partial segments of the two corresponding strands in target dsDNA. C1 and C2 could be severally modified onto electrode surfaces in the two channels of dual-probe E-DNA biosensor in order to realize to detect the two corresponding complementary strands in target dsDNA at the same time. After the thermal denaturation, the two modified electrodes were simultaneously incubated into the hybridization system contained target dsDNA (Sa and Sb) and assistant probes (A1 and A2). Both of the assistant probes (A1 and A2) were not labeled and contained two regains: one regain that was complementary to part of the corresponding strand in target dsDNA (Sa or Sb) and the other that was complementary to partial segment of the corresponding biotin labeled capture probe (C1 or C2). Although capture probes might hybridize with the corresponding assistant probes to form nonspecific duplexes (C1–A1 and C2–A2), the melting temperature of these duplexes were lower than that of the ternary “Y” junction structures formed by the probes and target DNA (C1–A1-Sa and C2–A2-Sb). Therefore, the two groups of detection probes (C1/A1 and C2/A2) could recognize and catch



Scheme 1. Scheme of the procedure dual-channel electrochemical DNA sensor array based on the "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification for detection of synthetic PML/RARα fusion gene dsDNA.

the specific strands from target dsDNA well to form two stable Y-junction-structure ternary complexes onto the different Au electrode surface only if there were target dsDNA in the reaction system. As a result, the restriction sites for restriction endonuclease *Rsa I* were located on one arm of the "Y" junction structure formed by the assistant probe/capture probe duplexes and would be subsequently cleaved by *Rsa I*. The capture probes (C1 and C2) were both digested into two pieces, and the biotin labeled piece dissociated from the Au electrode. At the same time, the corresponding target ssDNA (Sa or Sb) was also released from the capture probe (C1 or C2) modified on Au electrode. Subsequently, the dissociated target ssDNA (Sa or Sb) could hybridize with another corresponding capture probe (C1 or C2) and assistant probe (A1 or A2), and a new round of hybridization–cleavage–separation cycle was initiated. Ultimately, each target ssDNA could go through many repeated cycles, resulting in the cleavage of many capture probes. The number of biotin modified on the Au electrode surface would decrease, and streptavidin-horse reddish peroxidase (Streptavidin–HRP) connected with biotin labeled at the end of capture probe via streptavidin–biotin affinity binding would also reduce. So the enzymatically amplified electrochemical current signal for detection of target DNA decreased. If the reaction solution was in the absence of target dsDNA, capture probe and assistant probe can't form the "Y" junction structure. Meanwhile, *Rsa I* would be unable to cleave capture probe into two pieces and the biotinylated piece could not dissociate from the surface of electrode. This was because the capture probe would not hybridize with the corresponding assistant probes and predominately exist as single-stranded at the certain hybridization reaction temperature. So plenty of streptavidin-HRP could be modified onto the electrode surface and catalyzed 3, 3', 5, 5' tetramethylbenzidine (TMB) substrate to amplify the current signal effectively. Target dsDNA was detected according to the change of the current signals.

2. Experimental

The details of the experimental section are shown in Supporting Information.

3. Results and discussion

3.1. The feasibility of the dual-probe electrochemical DNA biosensor

According to Scheme 1, the designed elements of the dual-probe E-DNA biosensor based on the "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification were shown for detection of dsDNA of PML/RARα related fusion gene. In order to ensure the specificity of the designed probes, the thermodynamic parameters of all oligonucleotides used in the study were calculated using integrated DNA technologies (http://mfold.rit.albany.edu/ (Zuker 2003). Two groups of detection probes (C1/A1 and C2/A2) were designed based on the melting temperatures in our system according to the target dsDNA sequence (Shown in Supporting Information, Table S2). Therefore, in order to characterized the before and after restriction endonuclease digestion process of the electrochemical biosensor, the different stages of the dual-probe E-DNA biosensor preparation were tested by cyclic voltammetry and electrochemical impedance spectroscopy (EIS), respectively (Shown in Supporting Information, Fig. S1).

Electrochemical responses of the DE-DNA biosensor were also investigated in the research (Fig. 1). Fig. 1(A) shows the cyclic voltammogram of different modified dual-probe electrodes was tested in TMB substrate by dual-channel electrochemical analyzer. There were two pairs of redox peaks at the surface of HRP/capture probes (HRP/C1 and HRP/C2) modified electrodes exposed in TMB substrate, and the prominent catalytic reduction peaks were both found in CV of HRP/C1 and HRP/C2 electrodes (Fig. 1(A), curve (a)). From Fig. 1(B), the intensity of current signals were shown more directly from channel 1 (curve (a), 16900 nA) and channel 2 (curve (a), 17,100 nA). This reflected that HRP had been fastened onto the different capture probes modified electrodes via streptavidin–biotin affinity binding. Along with the catalysis of HRP, TMB was oxidized into a colored compound by H_2O_2, and thousands of reduction reactions of H_2O_2 could be efficiently catalyzed, leading to significantly amplified electrochemical current signals (Volpe et al., 1998).

In the absence of target dsDNA, the catalytic reduction peak current slightly decreased after restriction endonuclease treatment (Fig. 1(A), curve (b)). The values of current signals were shown in Fig. 1(B) (channel 1: curve (b), 14,600 nA; channel 2: curve (b), 14,600 nA). That may be implied that a small amount of

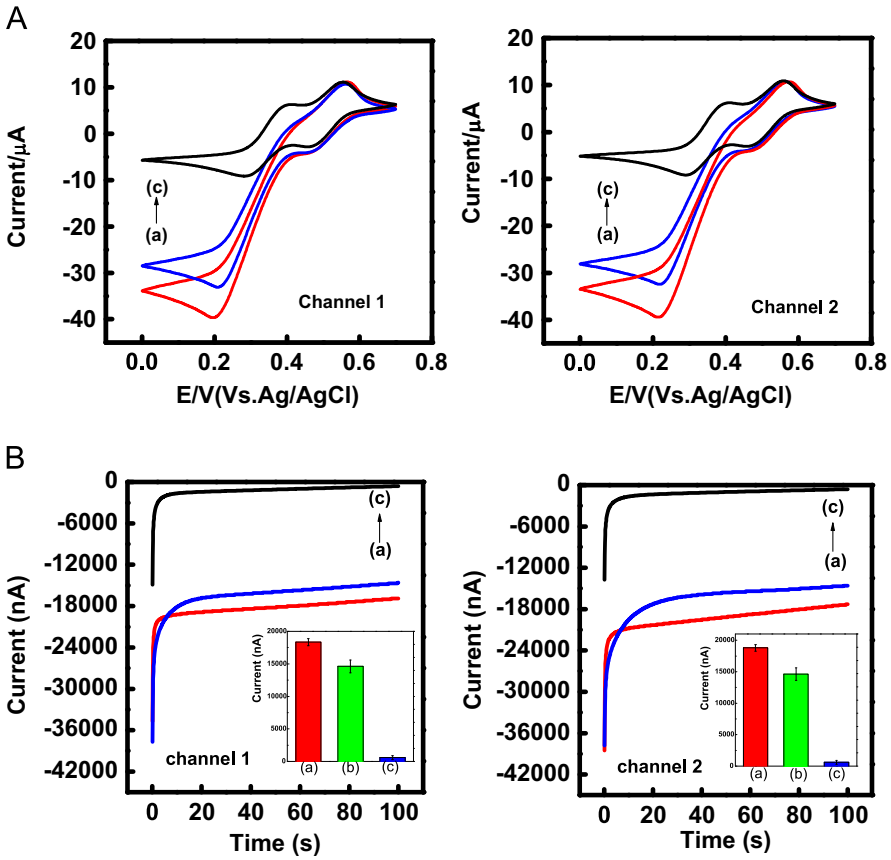


Fig. 1. Cyclic voltammogram (A) and *i–t* curve (B) of dual-channel electrodes: (a) capture probe modified on the Au electrode; (b) after restriction endonuclease reaction for 90 min in the absence of target dsDNA; (c) after restriction endonuclease reaction for 90 min in the presence of 10 nM target dsDNA.

assistant probes (A1 and A2) might be hybridized with the capture probes (C1 and C2) in the absence of target DNA. Obviously, the probability was very low, since only a few base pairs between capture probe and assistant probe were present. The melting temperature (T_m) for C1–A1 and C2–A2 were both lower than the hybridization reaction temperature (seen in supplementary information S3). So capture probes and assistant probes could not form the stable duplexes (C1–A1 and C2–A2) under the reaction temperature. When 10 nM target dsDNA was present, both of the catalytic reduction peak currents in channel 1 and channel 2 decreased obviously after restriction endonuclease treatment (Fig. 1 (A), curve (c)). The values of current strength were 590 nA and 610 nA, respectively (Fig. 1(B), curve (c)). It is implied that two kinds of Y-junction-structure ternary complexes were formed and the restriction endonuclease could recognize and cleave the corresponding restriction sites located on the arms of the two Y-junction-structure ternary complexes which were formed by C1–A1 and C2–A2 respectively. So the biotin labeled pieces dissociated from the Au electrode and there were fewer HRP could be fixed onto the surface of the electrode and induced the catalytic reduction of H_2O_2 with the redox reaction of TMB at the electrode surface. It was noted that the current (c) did not decrease to approximately zero (Fig. 1 (B)). The possible reason is that a small amount of assistant probes remain associated with the capture probes to form C1-A2 and C2-A1 duplex structures due to a few base pairs between C1-A2 and C2-A1. However, there were no restriction sites on these duplex structures, so the biotin was still modified on the electrode. Streptavidin–HRP can conjugate with biotinylated capture probes via affinity bond to be modified on the surface of electrode. And the catalytic reduction of H_2O_2 with the redox reaction of TMB at the electrode surface happened (Volpe et al., 1998; Fanjul et al., 2004; Das and Hecht, 2007). But the

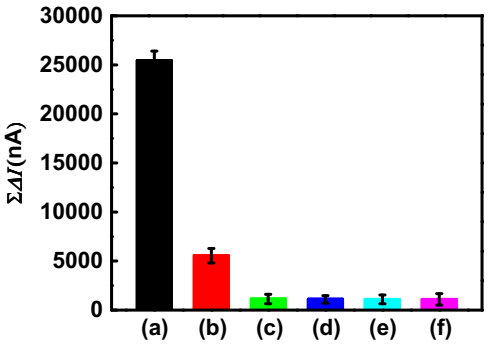


Fig. 2. Bar chart of sum of current difference value ($\sum^{\Delta} I$) of dual-channel electrochemical DNA biosensor array hybrid with 10 nM complementary dsDNA sequence (a), single-base mismatch dsDNA sequence (b), three-bases mismatch dsDNA sequence (c), five-bases mismatch dsDNA sequence (d), PML partial dsDNA sequence (e) and RARα partial dsDNA sequence (f), respectively.

phenomenon did not affect the DE-DNA biosensor discriminated whether there were target dsDNA in the reaction system. So the method was shown the good specificity on the detection of dsDNA. According to the results, it's potential that this DE-DNA biosensor could be used to detect dsDNA of PML/RARα related fusion gene after optimizing the reaction condition.

3.2. Study on the selectivity of the dual-probe E-DNA biosensor

Several different kinds of dsDNA sequences (see Table S1 for details in Supporting Information) including the complementary target dsDNA (S1), single-base mismatch dsDNA (S2), three-base mismatch dsDNA (S3), five-base mismatch dsDNA (S4), PML

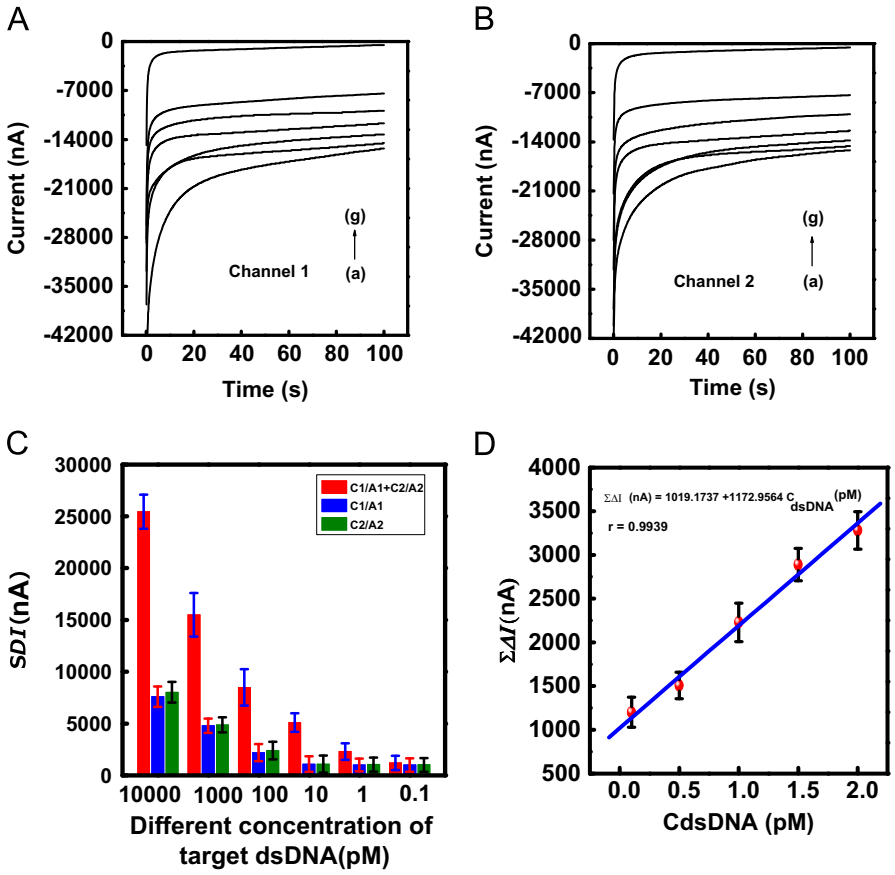


Fig. 3. *i–t* Curves of C1–A1–S1a (channel 1: (A)) and C2–A2–S1b (channel 2: (B)) in "Y" junction probes based dual-channel electrochemical DNA array biosensor hybridized with synthetic target dsDNA at a series of concentrations from dual channels. (From (a) to (g): 0 pM, 0.1 pM, 1 pM, 10 pM, 100 pM, 1000 pM and 10,000 pM); (C) Comparison of bar chart of sum of current difference value ($\sum^{\triangle}I$) for dual-channel electrochemical DNA array biosensor modified with two groups of "Y" junction probes (C1–A1–S1a+C2–A2–S1b: red) or with single group of "Y" junction probes (C1–A1–S1a: blue or C2–A2–S1b: olive) after hybridization with different concentration target dsDNA species under the same condition; (D) the plot of $\sum^{\triangle}I$ vs target dsDNA concentration (From 0.1 pM to 2 pM). Error bars show the standard deviations of measurements taken from at least three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

partial dsDNA (S5) and RARα partial dsDNA (S6) were chosen to study the selectivity. Detection of these dsDNA was investigated by amperometric technique, which provided a rapid and direct measure of the current associated with the HRP catalyzed electrochemical process (Wang et al., 2011). Fig. 2 showed $\sum^{\triangle}I$ ($\sum^{\triangle}I = {}^{\triangle}I_1$ (channel 1) $+ {}^{\triangle}I_2$ (channel 2), ${}^{\triangle}I = I_{\text{blank}} - I_{\text{dsDNA}}$, where I_{blank} and I_{dsDNA} referred to the current signal from single channel obtained before and after the capture probes modified electrodes were simultaneously incubated in 10 nM of target DNA and assistant probes, respectively) of these six dsDNA sequences, The same amounts of six dsDNA sequences (10 nM) were detected in the reaction system. Among these kinds of dsDNA sequences, $\sum^{\triangle}I$ of the complementary dsDNA was much higher than those of the other mismatch sequences. Compared with the value of $\sum^{\triangle}I$ of complementary dsDNA, the value of $\sum^{\triangle}I$ of the single-base dsDNA was just around 1/5. This result indicated that "Y" junction probe exhibited an enhanced discriminatory power for the complementary and single-base mismatch target dsDNA sequence, outperforming the DNA probe reported by other reports (Zhong et al., 2011; Mahmoud and Mojtaba, 2015; Anqi et al., 2014; Wei et al., 2013; Wang et al., 2011). When the number of the mismatch bases was equal or greater than 3 bp ($\geq$ 3 bp, S3, S4, S5 and S6), the detected current signals (I_{dsDNA}) were similar to the current signal of the blank background (I_{blank}). So these sums of current signals were very small, which meant the hybridization reaction did not occur and the dual probe E-DNA biosensor was able to discriminate between target dsDNA and mismatch dsDNA with high specificity.

3.3. Study on the sensitivity of the dual-probe E-DNA biosensor

Electrochemical responses of the complementary target dsDNA with different concentration were also quantitatively analyzed in the reaction system. In order to show the superiority of dual-probe detection mode for detection of dsDNA, the different single-probe modified E-DNA biosensors (C1/A1 or C2/A2) were investigated to detect the same concentration range of the related dsDNA. Fig. 3 (A) and (B) shows *i–t* curves of C1–A1–S1a (channel 1: (A)) and C2–A2–S1b (channel 2: (B)) in dual-probe E-DNA biosensor hybridized with target dsDNA at a series of concentrations from dual channels. The dual-probe E-DNA biosensor was challenged with the target dsDNA of a series of concentration across the range of 0.1 pM to 10 nM, spanning a response region of 5 orders of magnitude (Fig. 3(C)). It is worthwhile to point out that this dual-probe detection mode used for dsDNA detection achieved even higher gain than the E-DNA biosensors based single-probe (C1/A1:blue or C2/A2: olive in Fig. 3(C)). Under the optimal condition of the hybridization, while detecting the complementary DNA standard concentration range of target dsDNA from 0.1 pM to 2 pM, there was a good linear relationship between the current signal and the complementary DNA concentration, the lower limit was 47 fM (Fig. 3(D)). This sensitivity was comparable to or better than that of the other method for dsDNA detection reported by our research group (Yun et al., 2013). The results were indicated that the dual-probe E-DNA biosensor could prevent the reannealing of the two separate strands from the same target dsDNA efficiently and realized sensitive, specific and quantitative determination of

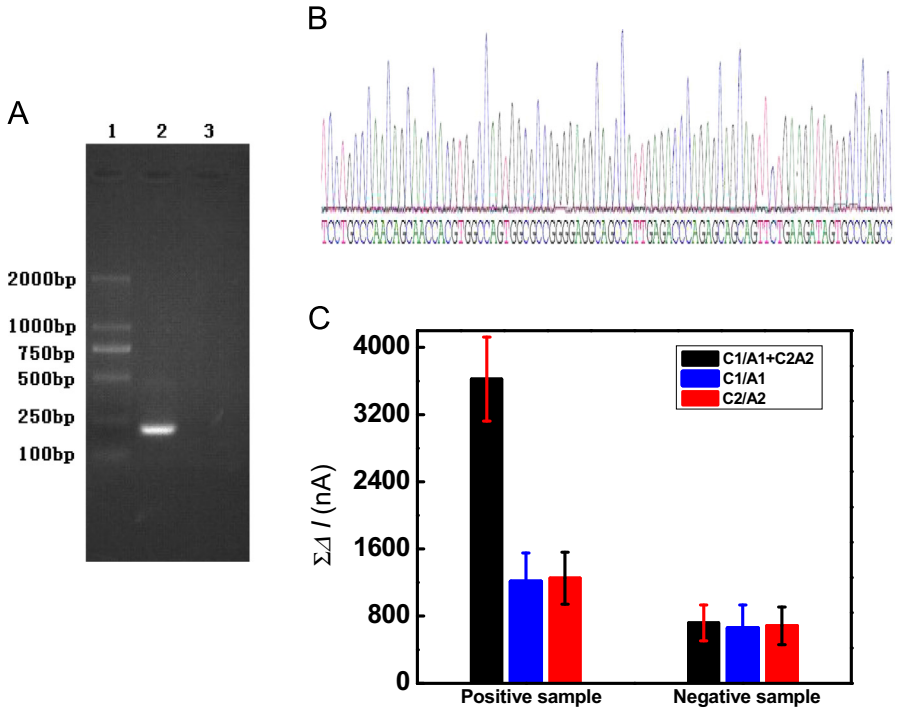


Fig. 4. (A) Electrophoretogram of PCR amplified products. The lanes from left to right: Lane 1: DL2000 DNA marker (the brands from up to down: 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp and 100 bp); lane 2: positive real sample; lane 3: negative real sample. (B) The partial result of gene sequencing. (C) shows the bar graph of $\sum\Delta I$ from dual channels when different groups of detection probes hybridized with different samples (C1/A1+C2/A2:black, C1/A1:Blue and C2/A2: Red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

synthetic PML/RARα fusion gene dsDNA fragment in APL.

3.4. Detection of PCR products

In a preliminary evaluation study of the dual-probe E-DNA biosensor in APL detection, we studied the performance of electrochemical analysis with PCR amplification. Fig. 4(A) shows the result of electrophoresis of PCR products. The PCR amplification products from NB4 cell (see lane 2) showed the light bands in the 1.5% agarose, while the PCR products from negative sample (lane 3) did not show any bands. As the related positive cell strain, NB4 cell, which contains the gene segment we want to detect, is also studied (Fig. 4(B)). From the result of gene sequencing, we can know that the PCR product from positive real sample contains the target gene fragment we need. After hybridization of the dual-probe E-DNA biosensor with the PCR products (500 times dilution by Tris–HCl reaction buffer) from NB4 cell, the $\sum^{\triangle}I$ (3624 nA, Fig. 4 (C)) obtained was more than an order of magnitude compared with that of negative sample (721 nA). Meanwhile, the $\sum^{\triangle}I$ of positive sample was comparable to or greater than that of the single-probe E-DNA biosensors used for the detection of the same products. It was indicative of the presence of the expected target. It was worth noting that in order to ensure the detected target existing in the single-stranded form, the symmetric PCR products were pretreated with the same approach used in the detection of dsDNA. The results obtained from the electrochemical DNA biosensor were in good agreement with those of the gel electrophoresis.

4. Conclusion

In summary, a facile and sensitive signal-off dual-probe E-DNA biosensor based on the "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification was constructed for detection of dsDNA of PML/RARα related fusion gene without the use of helicase enzymes, alkali or denaturants (DMSO or formamide). On the basis of the related sequence of PML/RARα fusion gene, two groups of detection probes were designed in order to enhance the hybridization efficiency between the detection probes and target dsDNA. Each group was composed of a capture probe and an assisted probe, and they were separately complementary with partial sequences of the two strands from the target dsDNA. The design could achieve to prevent the re-annealing of the two separate strands from target dsDNA well. According to the design, two different "Y" junction structures were formed by hybridization of the cooperating detection probes and the specific strand from target dsDNA. The dual-probe E-DNA biosensor showed good sensitivity and selectivity via the "Y" junction structure and restriction endonuclease assisted cyclic enzymatic amplification. The results showed that the dual-probe E-DNA biosensor could distinguish the complementary sequence from different mismatch dsDNA sequences very well and detect target dsDNA with a low limit of detection of 4.7×10^{-14} mol/L, which were better than the single-probe E-DNA biosensors used for the detection of the same samples. Moreover, this biosensor has also been used for assay of PCR real sample with satisfactory result. The work further supported the power of this dual-probe E-DNA biosensor will be achieved to detect quantitative target sequences from real samples directly and solve the actual problem of the early precise diagnosis and prognosis monitoring of APL and other diseases. In future work, we would implement this detection strategy in an integrated PCR-electrochemical system for quantitative analysis application.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.04.071.

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