Biosensors and Bioelectronics 28 (2011) 277–283

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Enzyme-amplified electrochemical biosensor for detection of PML–RARα fusion gene based on hairpin LNA probe

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ARTICLE INFO

Article history:
Received 8 May 2011
Received in revised form 14 July 2011
Accepted 14 July 2011
Available online 23 July 2011

Keywords:
Enzyme-amplified electrochemical biosensor
Hairpin locked nucleic acids
Acute promyelocytic leukemia
Fusion gene

ABSTRACT

In this study, an enzyme-amplified electrochemical biosensor was developed for detection of the promyelocytic leukemia/retinoic acid receptor alpha (PML/RARα) fusion gene in acute promyelocytic leukemia (APL). This new sensor employs a hairpin locked nucleic acids (LNAs) probe dually labeled with biotin and carboxyfluorescein molecule (FAM). The probe is immobilized at a streptavidin-modified electrode surface via the biotin–streptavidin bridge, and FAM serves as an affinity tag for the peroxidase conjugate binding. Initially, the immobilized hairpin probe was in the "closed" state in the absence of the target, which shielded FAM from being approached by the bulky anti-FAM-HRP conjugate due to the steric effect. Target binding opens the hairpin structure of the probe, the probe undergoes a significant conformational change, forcing FAM away from the electrode. As a result, the FAM label becomes accessible by the anti-FAM-HRP, and the target hybridization event can be sensitively transduced via the enzymatically amplified electrochemical current signal. This new biosensor demonstrates its excellent specificity for single-base mismatch and able to detect as little as 83 fM target DNA even in the presence of human serum. We also employed this sensor to directly detect PCR real sample with satisfactory results.

1. Introduction

APL is identified as the M_3 subtype of acute myeloid leukemia morphologically. It is characterized by selective expansion of immature myeloid precursors that are blocked at the promyelocytic stage (Wang et al., 2011). Cytogenetically, a translocation t(15;17)(q22;q21) is found in more than 95% APL patients, resulting in the formation of PML–RARα fusion gene (Qi et al., 2010). This gene rearrangement plays an important role in leukemogenesis through antagonizing retinoic acid signalling and the regulatory pathways mediated by APL. Thus, detection of PML–RARα fusion gene will afford an early diagnosis and monitor of the disease. The reported methods for the detection of PML/RARα fusion gene have included real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) (Devaraj et al., 1996), fluorescence in situ hybridization (FISH) (Han et al., 2009), flow cytometry (FCM) (Cullen et al., 2004), chromosome analysis (Yoo et al., 2006), etc. But there were some limitations in these methods, such as time-consuming, poor precision and expensiveness.

In recent years, there has been significant progress in developing biosensors for the rapid, cheap and accurate detections of specific gene sequence (Zhang et al., 2008). Various sensing strategies have been developed, aiming at the improvement of sensitivity and selectivity of electrochemical detection (Wang, 1998; Wei et al., 2008; Tosar et al., 2010). Among them, enzyme-based amplification strategies have been developed to improve the sensitivity of electrochemical DNA sensors (Wei et al., 2008). The selectivity of nucleic acid hybridization assays depends primarily on the selection of the probe and then of the hybridization conditions. Thus, the design of the probe is the most important pre-analytical step. Recently, Koshkin's group reported a novel oligonucleotide derivative, LNA (Koshkin et al., 1998). LNA nucleotides contain a methylene bridge between the 2′-oxygen and the 4′-carbon of the ribose moiety. The covalent bridge effectively 'locks' the ribose in the N-type (3-endo) conformation that is dominant in A-form DNA and RNA. Due to this chemical difference, LNAs differ from DNA molecules in several aspects: The covalent bridge results in a very high affinity for DNA and RNA complementary sequences, with each LNA substitution increasing the melting temperatures

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doi:10.1016/j.bios.2011.07.032

(T_m) by as much as 3.0–9.6 °C (James Yang et al., 2007); LNA/DNA hybrids are more destabilized by single-base mismatches than the corresponding DNA/DNA hybrids (Koshkin et al., 1998; Chen et al., 2008); low toxicity and enhanced triplex formation (Obika et al., 1998). Our laboratory has employed the LNA modified probe to detect target DNA and demonstrate that LNA probes hybridize with very high affinity to perfectly complementary targets, and at the same time the results also show an extraordinary specificity to discriminate the targets that differ by a single-base mismatch (Lin et al., 2009).

Electrochemical DNA sensors are able to sensitively detect DNA targets with high sequence specificity in pure DNA samples (Petrovykh et al., 2004). However, due to the complex background such as biological fluids or polymerase chain reaction (PCR) amplicons, conventional electrochemical detection methods do not meet the clinical diagnostic requirement of high signal-to-background ratio for DNA detection. Recently, some reports described a novel method of applying redox-labeled hairpin probes to enable oligonucleotide detection in various body fluids including serum and urine (Lubin et al., 2006). This method exploited variation in the steric effect due to the hybridization-induced conformational change for significant improvements in both sensitivity and specificity. With regard to early diagnosis and monitor of APL in the complex biological fluids of human serum, low copy numbers of PML/RARA fusion gene in serum demand highly sensitive sensors to detect signal. Inspired by above observation, we herein reported an enzyme-amplified electrochemical biosensor that employed hairpin LNA structured probes.

2. Experimental

2.1. Materials

All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China), and their base sequences are illustrated in Table S1. The hairpin probe for PML/RARα has a 5′-biotin and a 3′-FAM affinity label. It has six complementary bases at its 5′ and 3′ ends, which will form the stem at appropriate ionic strength. TMB (3,3′,5,5′ tetramethylbenzidine) was purchased from Neogen (Lexington, KY) in the format of a ready-to-use reagent (K-blue low activity substrate, H_2O_2 included). Anti-FAM-HRP was purchased from Southern Biotech. (Birmingham, USA). EZ-Link Amine-PEG_2-Biotin was obtained from Pierce biotechnology Inc., USA. Washing buffer was the mixture of 0.1 M NaCl and 10 mM PB (pH7.4). All solutions were prepared with Milli Q water (18.2 MΩ/cm resistivity) from a Millipore system.

2.2. Preparation of streptavidin-coated electrode

We had ever reported the method that a poly-calcon carboxylic acid (poly-CCA) modified GCE was fabricated by electropolymerization (Liu et al., 2008a,b). So poly-CCA membrane on GCE was formed according to the procedure previously described by the method. Before surface modification, the GCE was polished in sequential order with 1.0, 0.3 and 0.05 μm alumina (AlfaAesar, USA). The electrode was thoroughly washed with water, sonicated in ethanol, and finally dried thoroughly under N_2 flow. Briefly, GCE was polarized in 0.1 M H_2SO_4 by cyclic scanning between −0.40 and +1.60 V for 5 min at scan rate of 100 mV/s. Then the polarized electrode was carried out in 0.05 M NaOH containing 0.3 mM CCA solution by cyclic scanning between −0.40 and +1.80 V for 40 cyclic times at scan rate of 100 mV/s. After electropolymerization the GCE was modified with poly-CCA film, terminated by a carboxyl group. The terminal carboxylic acid groups of the poly-CCA/GCE were activated by immersion in the 50 mM PBS (pH 7.4) containing 200 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 50 mM N-hydroxysuccinimide (NHS) for 15 min. The sensors were rinsed with MilliQ water and dried with N_2 gas. The activated electrode surfaces were incubated with 5 mg/ml EZ-Link Amine-PEG_2-Biotin (in 0.1 M MES buffer, pH 5.0) for 10 min., followed by rinsing and drying. Ethanolamine–HCl (1.0 M, pH 8.5, Biacore) was loaded for inactivation of the unreacted EDC/NHS activated surface. Next, 0.5 mg/ml streptavidin (Vector Laboratories Inc., Burlingame, CA, USA) in PBS (pH 7.2) was incubated on the electrode for 10 min to produce streptavidin-coated electrode.

2.3. Fabrication of E-DNA sensors

The scheme for fabrication of E-DNA sensors is illustrated in Fig. 1. 6 μl of 1 μM dual-labeled LNA probe in 1.0 M PBS buffer (pH 7.0, including 100 mM NaCl, 10 mM $MgCl_2$) was immobilized onto the streptavidin-coated electrode for 6 h via the interactions

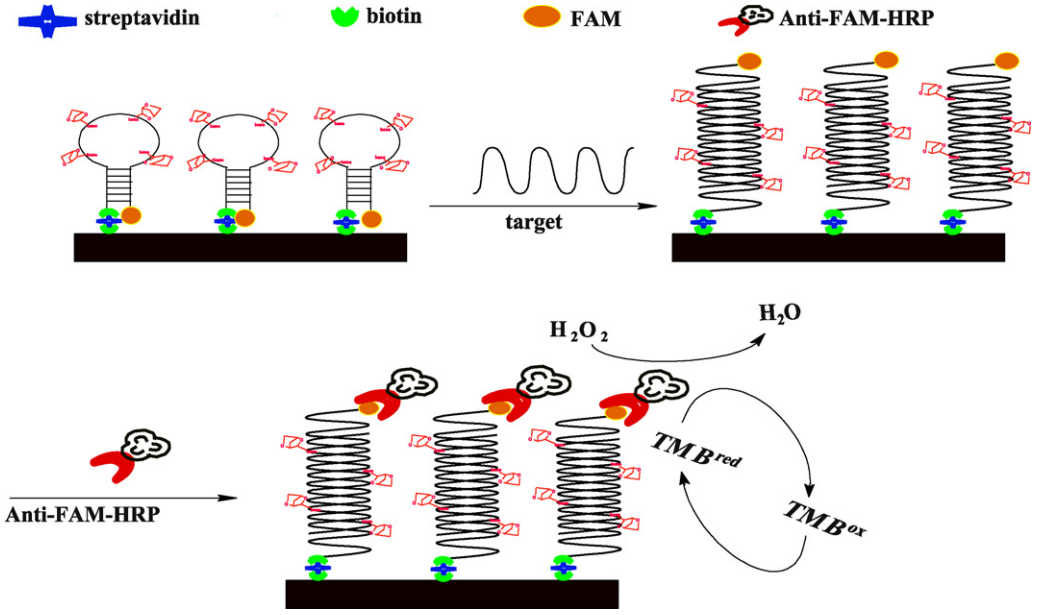

Fig. 1. Scheme for enzyme-amplified electrochemical LNA biosensor.

between streptavidin on the electrode surfaces and the biotin label on the probes. The sensor surface of modified LNA probes was incubated in different concentrations of complementary target for hybridization at 47 °C for 1 h. After being washed with ice-cold washing buffer, the sensors were incubated with 100 μl of PEG (0.05% polyethylene glycol 3350 in 0.1 M PBS) for 10 min to prevent any possible nonspecific binding. The sensors were again washed with washing buffer and dried with nitrogen, and then 5 μl of anti-FAM-HRP (0.5 U/ml in 0.1 M PBS buffer with 0.5% casein) was added and incubated for 15 min at room temperature. After removing any remaining HRP adsorped nonspecifically and subjected to electrochemical measurement. The same protocol as above-mentioned was applied for hybridization with mismatched sequences and also with non-complementary sequences. The electrochemical signal of the enzymatically produced TMB substrate was measured by cyclic voltammetry (CV) and amperometric *i–t* curve detection. CV was carried out at a scan rate of 100 mV/s. Amperometric detection was performed with a fixed potential of 100 mV and the steady state was usually reached and recorded within 100 s. All experiments were conducted in 1.0 M NaCl/10 mM PBS, pH 7.0, and human serum diluted with this buffer.

2.4. Detection of PML/RARα DNA

In order to test the real applicability of our new electrochemical DNA sensor, we challenged it with PCR amplicons for PML/RARα fusion gene. Templates cDNA of NB4 and real samples were kindly donated by Fujian institute of hematology, the affiliated union hospital of Fujian medical university. The PCR amplification was performed in a Bio-Rad PCR cycler. The amplification protocol is as follows, 3 min at 94 °C followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The reaction system was further incubated for 5 min at 72 °C to extend any incomplete products. After PCR amplification and purification, the PCR amplification products from positive real sample were sent to Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. (China) to determine the sequence. The PCR products were diluted by 10 times with buffered saline and thermally denatured by using a boiling water bath (5 min at +100 °C). The amplicon strands re-annealing was then retarded by cooling the sample in an ice-water bath for 5 min and subjected to electrochemical sensor detection following the same procedure used for synthetic oligonucleotides.

3. Results and discussion

3.1. Spectrophotometric characterization of DNA and LNA probes

Melting temperature (T_m) is the temperature at which double-stranded DNA is changed (50%) to single-stranded DNA under a given set of conditions. T_m value is an important parameter that denotes the thermal stability of double helix. The denaturation of dsDNA can be conveniently monitored by the sharp increase in absorbance at 260 nm (the absorbance maximum for DNA) using the spectrophotometer UV–Vis Varian Cary 100 (Varian). T_m for the hybridization of LNA with their complemental DNA were examined to confirm their potential for selective recognition of complementary sequences. A melting curve can be obtained by plotting the increase in absorbance versus the increase in temperature from 5 to 90 °C. As shown in Fig. 2 and in Table S2, the T_m value of the LNA probe (67.1 °C) was greatly higher than that of its corresponding DNA probe (50.4 °C). Comparing with the analogous DNA–DNA hybrids, T_m value for LNA binding to DNA was increased 16.7 °C, which was equivalent to 4.2 °C per LNA compared to DNA duplexes (You et al., 2006; Owczarzy, 2005). T_m value for LNA probe binding to single-base mismatch was decreased to 37.5 °C, DNA probe

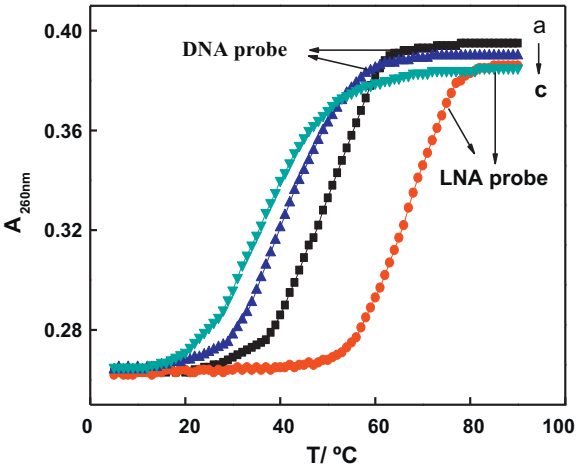

Fig. 2. Melting curves of DNA probe against complementary (a) and single-base-mismatch (b), LNA probe against complementary (c) and single-base-mismatch (d).

binding to single-base mismatch was 44.7 °C. Therefore, the affinity and specificity of the LNA probes can be confirmed by measurement of duplex T_m. The T_m value of the LNA probe binding to complementary target was 67.1 °C, while for single-base mismatch was decreased to 37.5 °C, which indicates that the difference of T_m between the complementary and mismatched sequences was a key factor for point mutation detection. Theoretically, the optimum hybridization temperature was about 20 °C lower than T_m (Chen et al., 2008). Therefore, the hybridization temperature was 47.1 and 17.5 °C for the complementary target and single-base mismatch, respectively. Accordingly, 47 °C was selected as hybridization temperature. At this temperature, hybridization could be occurred only for the complementary target strand but not for the single-base mismatch. Therefore, the hybridization specificity was dramatically increased.

3.2. Streptavidin-coated electrode and hairpin-induced specific amplification

A major concern of the sensor is the signal-to-background ratio. More sophisticated passivation of the surface may lead to reduced background that increases the signal-to-background ratio (Herrwerth et al., 2003; Gabriel et al., 2005). So, poly-CCA membrane on GCE for passivation of the surface was formed according to the procedure previously described by the method (Liu et al., 2008a,b). The characterization of electrochemically synthesized poly-CCA film was investigated by atomic force microscopy (AFM) and EIS. After electropolymerization for 40 cyclic times, the peak currents trended to be stable (figure not shown). The electron-transfer resistance (R_{et}) of the bare GCE was estimated to be 63 Ω. When a bare GCE was electropolymerized with CCA 40 cyclic times, R_{et} increased significantly (R_{et} = 3573 Ω). The poly-CCA film surface coverage (θ) on a bare GCE can be calculated from the EIS in terms of the equation (Liu et al., 2008a,b). $\theta = 1 - R_{et}^{Bare}/R_{et}^{CCA}$. The surface coverage ($\theta$) was calculated to be 98.3%. It is evident that a saturated monolayer of poly-CCA film on the bare GCE surface was formed. Such high-quality packing of electropolymerization film provided low background that was critical for the sensor detection (Gau et al., 2005). The GCE surface has been modified with carboxylic groups to form the CCA film (Wei et al., 2009). Amine-PEG_2-Biotin was attached to the conjugation site of the carboxylic groups of CCA film with the help of EDC/NHS. After that, streptavidin was coated on the biotinylated surface. Since streptavidin is a tetrametric protein containing four binding sites for biotin with extraordinarily high affinity, this configuration allows site-specific orientation of strep-

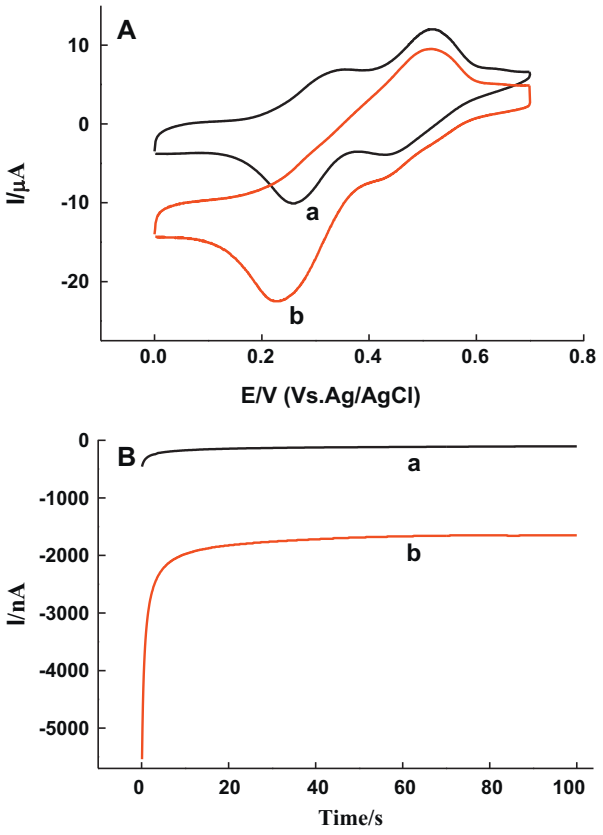

Fig. 3. Cyclic votammograms (A) and Amperometric measurements (B) of hairpin LNA probe modified GCE (a) and LNA–DNA modified GCE (b) in 1 ml TMB substrate.

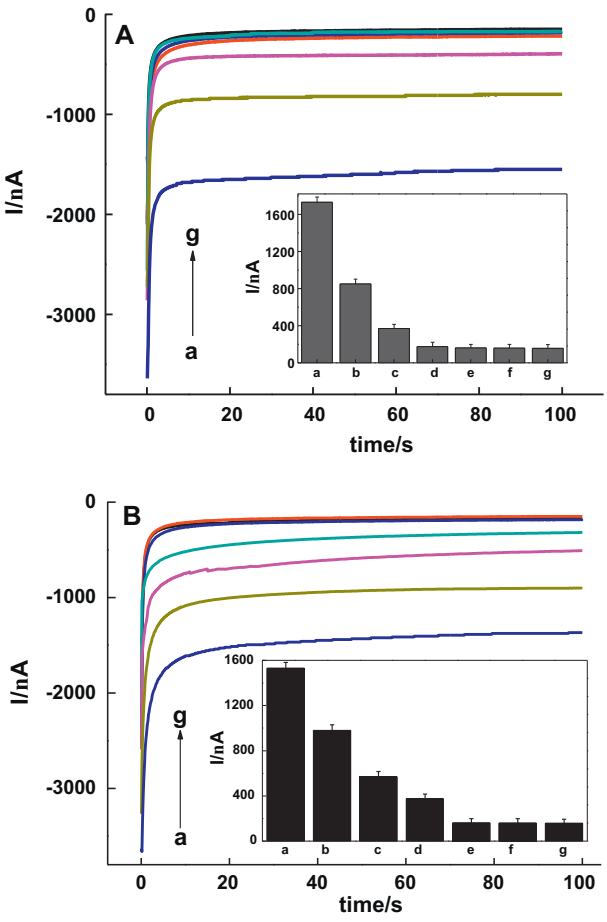

Fig. 4. Amperometric measurements of hairpin LNA probe (A) and DNA probe (B) modified GCE (g), and after hybridization with complementary target (a), single-base mismatch (b), three-base mismatch (c), five-base mismatch (d), seven-base mismatch (e), noncomplementary sequences (f).

tavidin and provides an ideal way to subsequent immobilization of biotinylated oligonucleotides. Hairpin LNA probes dually labeled with biotin and FAM could be facilely immobilized at streptavidin-coated electrode surfaces via the biotin–streptavidin bridge and transduced to electrochemical signals by using anti-FAM-HRP.

3.3. Electrochemical responses of the enzyme-based hairpin LNA-modified biosensor

The different responses of the hairpin LNA probe or heterogenesis LNA/target DNA double strands modified biosensors were studied under the same measurement condition. As shown in Fig. 3(A), we could find the reduction peak current of LNA/DNA double strands modified biosensor were more significant than that of hairpin LNA probe modified biosensor. The reason was that hairpin LNA probe was in the "closed" state when no complementary target was bound, anti-FAM-HRP conjugate could not be fixed onto the surface of electrode firmly. Because without complementary target binding, the proximity to the sensor surface creates steric hindrance, which inhibits signal amplification by preventing anti-FAM-HRP access to FAM. After washing nonspecific absorption and, there were few anti-FAM-HRP on the electrode surfaces. So the catalytic reduction reaction was not obvious, leading to weak reduction current signal. However, this built-in steric hindrance is removed after the bio-recognition component verifies the complementary target, making FAM accessible to Anti-FAM-HRP conjugate. Anti-FAM-HRP could be modified on the surface of GCE and efficiently catalyze reduction reactions of H_2O_2 with the redox reaction of TMB, leading to significant amplify of current signal (Wei et al., 2008).

The above results were further confirmed by amperometric measurements. As shown in Fig. 3B, in the "close" state we only found a relatively small and stable background amperometric current. Importantly, as we challenged the sensor with 10 nM complementary target DNA, which was expected to open the hairpin probe, a large amperometric signal was observed. This high signal gain is an inherent advantage of signal-on sensors as compared to signal-off ones. Accordingly, the novel biosensor can discriminate ss-LNA and heterogenesis LNA/DNA double strands very well.

3.4. Study on specificity of this novel electrochemical LNA biosensor

In order to evaluate the specificity of this novel E-DNA sensor, we challenged the hairpin LNA probes to hybridize with 10 nM various DNA sequences (complementary target, single-base mismatch, three-base mismatch, five-base mismatch, seven-bases mismatch, noncomplementary). For comparison, we also tested the same sensing scheme immobilizing DNA probe on the surface of streptavidin-coated electrode at the same condition. As using LNA probe, we found that a large amperometric signal (1750 nA, Fig. 4A curve a) was obtained after hybridization with complementary target, which was more than an order of magnitude compared with background amperometric current (141 nA, curve g). It indicated that LNA probes showed the excellent affinity for complementary target. This high signal gain is an inherent advantage of signal-on sensors as compared to signal-off ones. We interrogated the sequence specificity of the sensor by using single-base mismatched oligonucleotide. Interestingly, we found

that the signal was only 860 nA (curve b). That is, the signal for the fully complementary target was at least 2 times larger than that for the one-base mismatched oligonucleotide, suggesting that the enzyme-based E-DNA sensor has high sequence specificity toward even a single-base mismatch. We also employed a three-base mismatched oligonucleotide and found that the amperometric signal was only 370 nA (curve c). When the number of mismatch base was equal to 5, 7 or noncomplementary oligo, there were no significant differences from the background (curve g versus curves d, e, f), since no successful hybridization occurred due to the mismatch bases between the probe and these mismatch sequences. As using DNA probe, the signal for the fully complementary target was found to be about 1.5 times larger than that for the one-base mismatched oligonucleotide (Fig. 4B). These results clearly show the high selectivity of LNA probe for the complementary target and single-base mismatch sequences compared with DNA probe. The enhanced specificity can be attributed to the LNA probe, LNA probe is a DNA–LNA chimera, LNA monomer contains a methylene bridge that connects the 2′-oxygen with the 4′-carbon of the ribose ring. This bridge results in a locked 3′-endo conformation, reducing the conformational flexibility of the ribose and increasing the degree of local organization of the phosphate backbone. This entropic constraint leads to improving the binding to complementary DNA sequences (James Yang et al., 2007).

Our above observed results may also be studied by using fluorescence method. The fluorescent molecular beacons (MB) were designed by a combination of LNA hairpin structure and the ends of the stem of the hairpin were modified by FAM and quencher 4-(4-dimethylaminophenylazo) benzoyl (DABCYL). MB hybridized with complementary sequence, single-base mismatched sequence and non-complementary sequence, respectively. The fluorescence spectra are shown in Fig. S1. Before hybridization, the structure of MB kept hairpin, and DABCYL was close to FAM. So there were little fluorescent signals on the MB. When the MB interacted with the complementary sequence, the fluorescence increased significantly. This increase clearly showed that MB had successfully hybridized with its complementary sequence, and MB undergone a conformational change with the opening of the stem, leading to the distance between FAM and DABCYL was increased. Then the fluorescence intensities were increased. In the presence of single-base mismatched sequence, significantly decreased fluorescence signal was obtained as compared to the complementary sequence. This difference indicates that the complete hybridization was not accomplished due to the base mismatch. As expected, there was no significant difference in fluorescence intensity for its hybridization with non-complementary sequence. This means that the properties of MB do not change after its interaction with non-complementary sequence and the FAM and DABCYL are still held in close proximity to each other by the hairpin stem. These results demonstrate that the present approach has high specificity.

3.5. Study on selectivity and sensitivity of this electrochemical LNA biosensor in human sera

In order to evaluate the applicability of this electrochemical DNA sensor in clinically relevant samples, we challenged the sensor performance in human sera. Sera are highly complicated biological fluids containing large amounts of proteins and other molecules. Under these conditions, we could find the biosensor still kept high selectivity between perfectly complementary and mismatched sequences in the presence of 20% serum (diluted 20% with buffered saline). Significantly, we found that this E-DNA sensor was highly resistant to serum, with little alteration of the background noise and nearly the same hybridization signal for the 10 nM synthetic target even in the presence of 50% serum (Fig. 5A). Because PEG has the capacity to reduce protein adsorption and cell adhesion

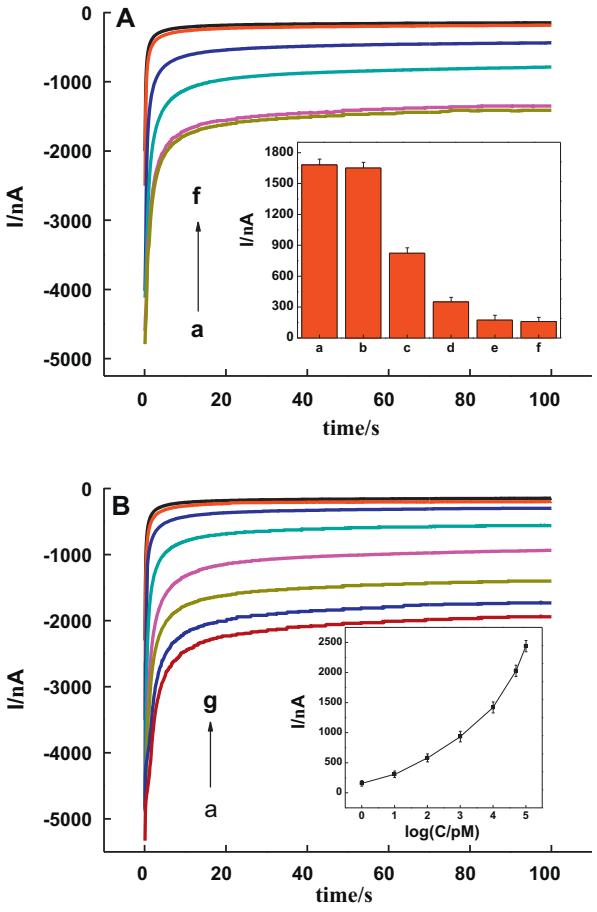

Fig. 5. (A) Amperometric measurements of hairpin LNA probe modified GCE (f) and after hybridization with complementary target (a), single-base mismatch (c), three-bases mismatch (d) in the presence of 20% serum; hairpin LNA probe modified GCE (e) and after hybridization with complementary target (b) in the presence of 50% serum. (B) Amperometric measurements for synthetic target DNA in 20% serum at a series of concentrations (from top to bottom): 0, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 50 nM and 100 nM. Inserts shows current plot versus logarithmic of target DNA concentration. Error bars show the standard deviations of measurements taken from at least three independent experiments.

on a variety of hydrophobic substrates (Lee et al., 1995; Vermette and Meagher, 2003). These results suggest the selectivity of the E-DNA sensor is, thus, sufficient to detect and identify target DNA sequences directly in clinically relevant materials and suggests the sensor is suitable for application in a wide range of real-world scenarios.

We challenged the sensor with the synthetic DNA target of a series of concentrations across the range of 1 pM to 100 nM in the presence of 20% serum. The amperometric signal was found to be a nonlinear logarithmic function related to the target concentration (Fig. 5B), spanning an impressive response region of at least 5 orders of magnitude. The detection limit was experimentally found to be 83 fM (>3 SD), which achieved even higher sensitivity than that of a previously reported sandwich-type sensor using linear capture probe (Miranda-Castro et al., 2007).

3.6. Detection of the real PCR sample

In order to test the real applicability of our new sensor, we further challenged it with a PCR amplicon from the PML/RARα fusion gene fragment of APL. The measured gene sequences results of PCR amplicon were shown in Fig. S2. We obtained the target sequences that could be directly sensed by LNA probe. Fig. 6A shows the result

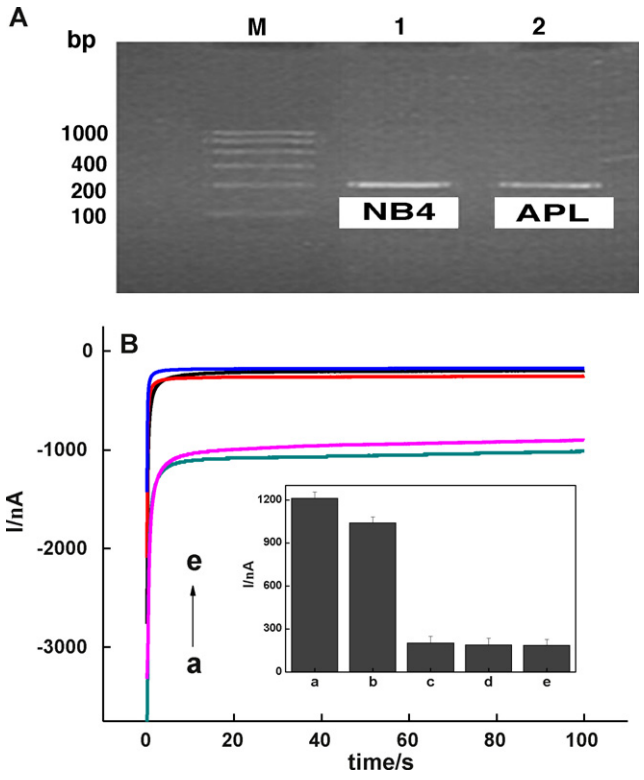

Fig. 6. (A) Electrophoresis of PCR products. The lanes from left to right: (1) DNA marker; (1) NB4 cell; (2) APL. (B) Amperometric curves of hairpin LNA probe modified GCE (e) and after hybridization with PCR blank buffer (PCR system without cDNA template) (d), NB4 cell PCR products (a), positive real sample PCR products (b), negative real sample PCR products (c). Inset B shows the bar graph of the peak current when the hairpin LNA probe hybridized with different PCR products. Error bars, ±relative standard deviation of three independent experiments.

of electrophoresis of PCR products. The PCR amplification products from NB4 cell and a positive real sample (see Fig. 6A, lane 1 and lane 2) show the light brands in the 1.5% agarose gel. As we all know, the NB4 cell is the positive cell strain of APL, which contains translocation t(15;17)(q22;q21), and the PCR amplification band from the positive real sample consistent with the band from the NB4 cell.

The denatured PML/RARα fusion gene fragment from APL was applied to the DNA sensor detection after PCR amplification. Fig. 6B shows the amperometric signals when the hairpin LNA probe for APL was immobilized and hybridized with real PCR samples in the hybridization step. Interestingly, our sensor performed equally well despite the fact that this target is much longer than the synthetic target as described above. The amperometric signals obtained from the hybridization of the hairpin LNA probe with PCR amplification of NB4 cell, the positive and negative real samples gave a mean average of 1210 nA with a RSD of 7.02%, 1060 nA with a RSD of 6.14% and 192 nA with a RSD of 7.02%, respectively. The obvious increases in the magnitude of the amperometric signals were obtained with NB4 cell or positive real samples. The increase of amperometric signals showed that the hybridization at the GCE surface occurred and steric inhibition was removed after the biorecognition component verifies the target specificity, making FAM accessible to the anti-FAM-HRP conjugate. So anti-FAM-HRP could be modified on the surface of GCE and efficiently catalyze reduction reactions of H_2O_2 with the redox reaction of TMB, leading to significant amplify of current signal. If the blood sample was negative, the negative real sample would not contain a target sequence complementary to the specific PML/RARα hairpin LNA probe. The hybridization between these negative real samples and the immobilized LNA probe would not occur, so the amperometric signal was nearly as high as the signal of probe. The results showed that the electrochemical biosensor was in good agreement with those obtained from the gel electrophoresis.

4. Conclusions

We reported an enzyme-amplified electrochemical biosensor that employed hairpin LNA probes for detection of the PML/RARα fusion gene. This new biosensor employs LNA probe dually labeled with biotin and FAM. The LNA probe immobilized at a streptavidin-modified GCE surface had a high ability to detect point mutation at 47 °C, which benefits from the use of hairpin LNA probe and the T_m is also the key factor in this case. Importantly, we found that the presence of biological fluids such as diluted serum did not sacrifice the performance of our sensor. This E-DNA sensor could effectively repel nonspecific adsorption of proteins, thus serving as an ideal sensor platform for real-world applications. We employed this sensor to directly detect PCR amplicons from NB4 and real samples with satisfactory results. So this electrochemical DNA sensor is a promising tool for the sensitive and portable detection of acute promyelocytic leukemia.

Acknowledgements

The authors gratefully acknowledge the financial support of the National High Technology and Development of China (863 Project: 2008AA02Z433), the National Natural Science Foundation of China (20805006 and 20975021), the Fujian Provincial University-Industry Cooperation Sciecne & Technology Major Program (2010Y4003), the Foundation of Fujian Key Laboratory of Hematology (2009J1004), the Natural Science Foundation of Fujian Province (2009J01023, 2010J06011, 2010J01032, 10J05019), the Scientific Research Major Program of Fujian Medical University (09ZD013) and the Foundation of Fujian Education Department (JA10155 and JA10126).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.032.

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