Available online at www.sciencedirect.com
ScienceDirect
Biosensors and Bioelectronics 23 (2008) 1588–1594
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Construction, electrochemically biosensing and discrimination of recombinant plasmid (pEThIL-2) on the basis of interleukine-2 DNA insert

Mohammad Saeid Hejazi [a,b], Mohammad Hossein Pournaghi-Azar [c,*], Esmaeel Alipour [c], Farrokh Karimi [d,e]

[a] *Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
[b] *Drug Applied Research Center and Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[c] *Electroanalytical Chemistry Laboratory, Faculty of Chemistry, University of Tabriz, Tabriz, Iran*
[d] *Department of Biotechnology, Faculty of Agriculture, University of Maragheh, Maragheh, Iran*
[e] *Research center for pharmaceutical nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran*

Received 19 November 2007; received in revised form 8 January 2008; accepted 14 January 2008
Available online 31 January 2008

Abstract

Construction, electrochemically biosensing and discrimination of recombinant pEThIL-2 plasmid, with 5839 bp size, on the basis of interleukine-2 (IL-2) DNA insert are described. Plasmid pEThIL-2 was constructed by PCR amplification of IL-2 encoding DNA and subcloning into pET21a(+) vector using *Bam*HI and *Sac*I sites. The recombinant pEThIL-2 plasmid was detected with a label-free DNA hybridization biosensor using a non-inosine substituted probe. The proposed sensor was made up by immobilization of a 20-mer antisense single strand oligonucleotide (chIL-2) related to the human interleukine-2 gene on the pencil graphite electrode (PGE) as a probe and then the sensing of recombinant pEThIL-2 plasmid was conducted by anodic differential pulse voltammetry (ADPV) based on guanine oxidation signal. Selectivity of the detection was assessed with pET21a(+) non-complementary plasmid, with 5443 bp size, lacking IL-2 encoding DNA. Different factors such as electrode activation conditions and washing strategy were tested in order to eliminate the nonspecific adsorption of pET21a(+). We have found that the PGE activation for 300 s produces a condition in which desorption of nonspecifically adsorbed plasmids from the electrode surface can be achieved by 300 s washing of the electrode in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl. Diagnostic performance of the biosensor is described and the detection limit is found to be 10.31 pg/μL.

Keywords: DNA biosensor; Interleukine-2; Recombinant plasmid; Pencil graphite electrode; Non-inosine substituted probe

1. Introduction

Interleukine-2 (IL-2) is a cytokine that stimulates immune response by activating a wide range of immune system cells, including helper T cells, B cells, macrophages and natural killer cells (Jeffrey et al., 1992; Robb, 1984), resulting in protection against bacterial (Seow et al., 1997; Anderson et al., 1987) and viral disease (Weinberg et al., 1987). Due to its key role in immune system some clinical applications, including cancer therapy and promotion of immune response to vaccines (Perrint et al., 1988; Weinberg and Merigan, 1988; Meuer et al., 1989) have been approved for this drug. Commercial human interleukin-2 is produced as a recombinant protein by recombinant *Escherichia coli* cells harboring IL-2 DNA plasmid (Seow et al., 1997; Robbens et al., 1995). Moreover, IL-2 DNA has been used to construct recombinant viruses (Kittel et al., 2005). Human IL-2 DNA containing recombinant viruses have been utilized in cancer gene therapy studies (William and Qun, 2005).

During the past decade, many groups have focused their studies on the detection of DNA hybridization using fluorescence techniques, mass spectroscopy (Sauer, 2006), quartz-crystal microbalance (Willner et al., 2002; Patolsky et al., 2000), surface plasmon resonance spectroscopy (Liu et al., 2005; Wang et al., 2004), and electrochemical methods (Diculescu et al.,

* Corresponding author. Fax: +98 411 3340191.
E-mail address: pournaghiazar@tabrizu.ac.ir (M.H. Pournaghi-Azar).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.01.021

2005; Palecek et al., 2002; Wang, 2000; Boon et al., 2000; Pournaghi-Azar et al., 2006; Sabzi et al., 2008). However, the electrochemical methods have attracted more attention than other methods because they are highly sensitive, inexpensive, easy-to-use, portable and compatible with microfabrication technologies.

Electrochemical DNA hybridization detection methods are mainly classified into two direct and indirect protocols. Signal transduction induced directly from oxidation of guanine or adenine moieties in DNA strands (label-free detection) makes the principle of DNA hybridization detection in direct strategy. While indirect DNA hybridization detection method is based on incorporation of an electroactive label. On the basis of literature investigation, label-free monitoring based on guanine moiety oxidation signal of probe or target on conventional carbon electrodes in electrochemical DNA biosensor, seems to be a simple, less time-consuming and more applicable strategy in comparison with the others (Yang and Thorp, 2001; Wang et al., 2003).

Recently, we developed a label-free DNA hybridization biosensor using a non-inosine substituted probe such as antisense strand of human-interleukine-2 gene (chIL-2) and renewable PGE. The optimum conditions for probe immobilization on the electrode surface and hybridization event between chIL-2 probe and its complementary oligonucleotide (hIL-2) were reported previously (Pournaghi-Azar et al., 2007). As the utilized probe contained only one guanine and seven cytosine bases, it allowed (i) providing at least a detectable electrochemical signal for direct optimizing of the probe immobilization conditions and (ii) obtaining an enhanced electrochemical signal during hybridization with target DNA containing numerous guanine bases. This strategy eliminated the time-consuming external indicator accumulation as well as obviated the electrode surface regeneration step.

There are many reports for developing of electrochemical DNA hybridization biosensor but most of them related to detection of short oligonucleotide target DNA (Pournaghi-Azar et al., 2007; Xu et al., 2006; Wong and Goodling, 2006; Cai et al., 1996). In comparison, the numbers of reported DNA biosensors for detection of DNA as recombinant plasmid are so little. Considering that IL-2 encoding DNA is incorporated in recombinant plasmids and recombinant viral vectors for IL-2 production (Robbens et al., 1995; Devos et al., 1983; Cerretti et al., 1986; Williams et al., 1988) and its delivery in the body (Kittel et al., 2005), respectively, and drawing attention to this point that in both cases IL-2 encoding DNA is accompanied by other DNA fragments and genes (necessary for the function and delivery of IL-2 encoding DNA), we decided to biosense IL-2 DNA within a context of unrelated DNA fragments and genes. In order to achieve this aim we constructed a vector containing IL-2 encoding DNA (pEThIL-2) and develop a DNA-based biosensor for detection of the constructed plasmid and discrimination of it from original vector (pET21a(+)) with 93% homology at nucleic acid level. It should be noted that pET21a(+) vector contains 5443 bp and pEThIL-2 vector is composed of 5839 bp from which 5438 bp are identical between two vectors.

Here for the first time, we describe the direct electrochemical biosensing of the recombinant pETIL-2 plasmid monitoring of the guanine oxidation signal using differential pulse voltammetry (DPV) technique.

2. Materials and methods

2.1. Materials

Luria-Bertani (LB) (Bacto-tryptone 10 g/L, yeast extract 5 g/L, and NaCl 10 g/L with pH 7.0, all were purchased from Merck, Germany) was used as the culture medium, and ampicilline (100 mg/mL) was added into the medium when required to maintain selection pressure. T4 DNA ligase, DNA size marker, *Sac*I, *Bam*HI, *Nde*I and *Xho*I restriction enzymes were purchased from Fermentas and *Pfu*Turbo DNA polymerase was supplied from Clontech company and DNA gel extraction kit from Roche company. DH5α strain of *E. coli* was used for amplification of the plasmids. The retroviral-based r-PWhIL-2B7.MA plasmid encoding precursor IL-2 (a kind gift from Dr. Joop Gaken, Department of Hematological Medicine, Rayne Institute, King's College London, London), was used as the template DNA for amplification of mature human IL-2 encoding DNA, the prokaryotic expression vector pET21a(+) was used for cloning of amplified DNA.

The pencil graphite was obtained as pencil lead – composed of natural graphite, a polymeric binder and clay – from Rotring Co. Ltd., Germany (R 505210 N) of type H. All leads had a diameter of 2.0 mm and were used as received. A 20-mer oligonucleotide corresponding to antisense strand of human IL-2 gene (chIL-2) was used as the probe. This oligonucleotide was supplied (as lyophilized powder) from MWG-Biotech, with the following sequence: 5′-CTA AAT TTA GCA CTT CCT CC-3′.

The stock solution of the oligonucleotide (100 μg/mL) was prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of the oligonucleotide were prepared using 0.50 M acetate buffer (pH 4.80) solution containing 20 mM NaCl. pET21a(+) plasmid (5443 bp) was used as the backbone for construction of pEThIL-2 plasmid containing human interleukine-2 encoding DNA. pET21a(+) plasmid was prepared using alkaline lysis mini-preparation method from recombinant DH5α strain of *E. coli* cells with a concentration of 1.77 μg/μL, without any manipulation. More diluted solutions of the plasmids were prepared using 10 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl. Other chemicals were of analytical reagent grade. The distilled, deionized and sterilized water was used in all solution preparation. Each measurement consisted of the immobilization/hybridization/detection cycle carried out on a fresh PGE surface. All the experiments were performed at room temperature in an electrochemical cell.

2.2. IL-2 encoding DNA amplification

Plasmid r-PWhIL-2B7.MA was used as the template DNA for IL-2 encoding DNA amplification using polymerase chain reaction (PCR) technique with *Pfu*Turbo DNA polymerase

(clontech) in the presence of designed forward FhIL-2 (5′-CAG GAT CCG CAC CTA CTT CAA GTT-3′) and reverse RhIL-2 (5′-CTG AGC TCT TAA GTC AGT GTT GAG ATG AT-3′) primers. The forward primer designed so that the signal sequence present in the template DNA (i.e., precursor IL-2 DNA) was removed in the consequent amplified DNA. PCR amplification was carried out on a gradient master cycler (eppendorf). PCR reactions contained about 100 ng template DNA, 0.1 μM of each primer, 1 mM of dNTPs, *Pfu*Turbo DNA polymerase buffer with final concentration of 1×, and 1 μL (2.5 U) *Pfu*Turbo DNA polymerase in total volume of 50 μL. The PCR program used for amplification of IL-2 DNA was: following the initial denaturation step (96 °C for 5 min), samples were subjected to 35 cycles of PCR consisting of 96 °C for 1 min, 52 °C as annealing temperature for 30 s and 72 °C as extension time for 35 s with a final extension time of 72 °C for 10 min.

2.3. Construction of pEThIL-2 expression plasmid

Amplification of IL-2 encoding DNA was carried out using FhIL-2 and RhIL-2 primers.The forward and reverse primers created SacI and BamHI recognition sites at 5′ and 3′ ends of PCR product respectively, allowing in frame cloning of the amplified DNA inside pET21a(+) plasmid using the same sites in the plasmid.

The amplified fragment and pET21a(+) were subjected to *Sac*I and *Bam*HI restriction digestion and were size fractionated using agarose gel electrophoresis and then the desired bands were cut out of the gel and DNA fragments were extracted using DNA gel extraction kit. The digested amplified DNA and digested plasmid were ligated together in order to clone IL-2 encoding DNA into the pET21a(+) plasmid leading to construction of pEThIL-2 plasmid harboring mature IL-2 encoding DNA. All DNA manipulation procedures were conducted based on common molecular biology protocols (Sambrook and Rucell, 2001). Finally, in order to investigate the presence of any mutation in the amplified DNA, the construction was sequenced by MWG-Biotech, Germany.

2.4. Electrochemical measurements

Electrochemical experiments were performed using AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie., The Netherlands). The utilized three-electrode system was composed of a PGE (surface area of 0.037 cm^2) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode.

2.4.1. Preparation and activation of the pencil graphite electrode (PGE)

The body of pencil lead was tightly coated with Teflon band. Electrical contact with the lead was achieved by soldering a copper wire to the metallic holder of the working electrode. The pencil lead was fixed vertically and immersed in the solution in which the contact was only achieved via cross-section of the electrode. The surface was polished on a weighing paper to a smoothed finish before each use. The electrochemical activation of the surface of polished PGEs was carried out at optimized potential of 1.80 V for 5 min in 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl without stirring (Hejazi et al., 2007).

2.4.2. Immobilization of the Probe on the PGE

Following activation of the PGE, the probe was immobilized on the activated electrode by applying 0.50 V to the electrode for 5 min in 0.50 M acetate buffer solution (pH 4.80) containing 1 μM chIL-2 and 20 mM NaCl with 200 rpm stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl.

2.4.3. Hybridization

The diluted plasmid (28.5 ng/μL) was then denatured by heating in a water bath at 95 °C for 5 min and immersed in an ice bath for 2 min. After immobilization of the probe, the PGE was inverted upside down. About 2.5 μL of the plasmid solution was pipetted directly onto the electrode surface. The hybridization was allowed to proceed for 15 min at room temperature, then the electrode was rinsed with 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl (wash buffer solution) with 200 rpm stirring. The same protocol was applied for the interaction of the probe with non-complementary plasmid.

2.4.4. Voltammetric measurements

The electrochemical behavior of the electrode surface was studied using anodic differential pulse voltammetry (ADPV) in 20 mM Tris–HCl buffer (pH 7.00) solution and scanning the electrode potential between 0.50 V and 1.15 V at a pulse amplitude of 50 mV. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction using a ‘peak width’ of 0.01. Repetitive measurements were carried out following renewing the PGE surface by cutting and polishing of the electrode.

3. Results and discussion

3.1. Construction of recombinant plasmid pEThIL-2

Construction of the recombinant plasmid (pEThIL-2) was carried out by specific amplification of the IL-2 encoding DNA using PCR technique. The PCR product was size fractionated using gel electrophoresis. The presence of a 418 bp DNA band confirmed the amplification of the IL-2 encoding DNA. Plasmid pET21a(+) and amplified DNA fragment were double digested with BamHI and SacI restriction enzymes and ligat in reaction was performed using T4 DNA ligase leading to construction of pEThIL-2 plasmid. The construction procedure of the pEThIL-2 plasmid is illustrated in Fig. 1.

In order to confirm the cloning of IL-2 encoding DNA fragment inside the constructed plasmid, the construct was digested with NdeI and XhoI enzymes placed slightly far upstream and downstream of the insert cloning sites inside the

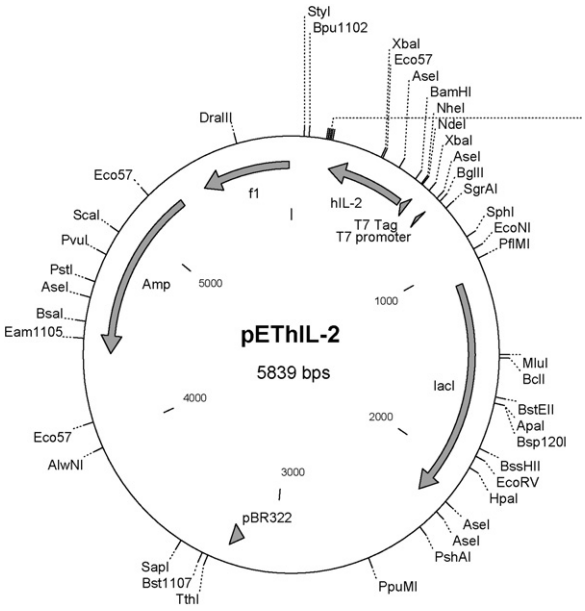

Fig. 1. Construction of pEThIL-2. The amplified mhIL-2 encoding DNA was cloned in pET21a(+) plasmid using *Sac*I and *Bam*HI restriction enzymes leading to construction of pEThIL-2.

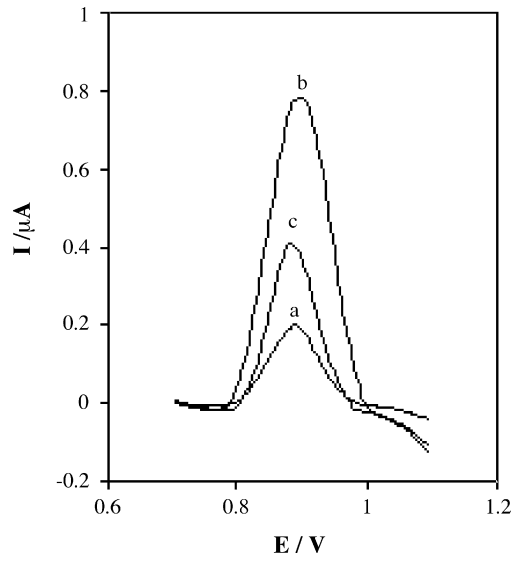

Fig. 3. ADPVs of chIL-2 modified PGE before hybridization (a) and after hybridization with complementary plasmid (pEThIL-2) (b); after interaction with non-complementary plasmid (pET21a(+)) (c). Experimental conditions of electrode activation, probe immobilization and hybrization were as described in Section 2.4. Activation time of electrode is 600 s.

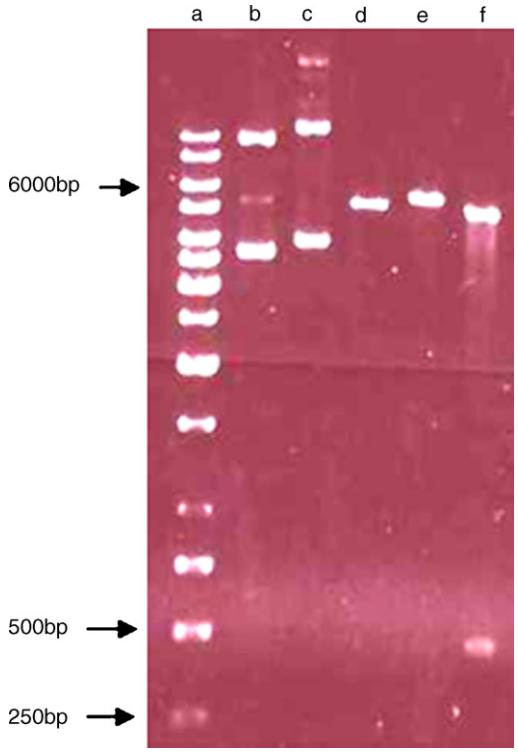

Fig. 2. Digestion of pET21a(+) and recombinant pEThIL-2 plasmids with restriction enzymes to confirm the presence of mhIL-2 coding DNA in pET21a(+). Lane a, 1 kb DNA marker; lane b, undigested pET21a(+); lane c, undigested recombinant pEThIL-2; lane d, pET21a(+) digested with *Nde*I restriction enzyme; lane e, recombinant pEThIL-2 digested with *Nde*I restriction enzyme; lane f, shows the release of mhIL-2 encoding DNA from recombinant pEThIL-2 plasmid following digestion with *Nde*I and *Xho*I restriction enzymes.

plasmid, respectively. The release of a DNA fragment with about 460 bp size confirmed the presence of the IL-2 encoding DNA inside the plasmid (Fig. 2). The constructed plasmid was sequenced and comparison of the sequence of amplified DNA with the original IL-2 encoding sequence from the National Center for Biotechnology Information (NCBI) data base with A14844 accession number, showed that there was no mutation in the amplified IL-2 encoding DNA fragment. The constructed plasmid has a size of 5839 bp from which 5438 bp is 100% identical between pEThIL-2 and pET21a(+) backbone with 5443 bp size.

3.2. Electrochemical sensing of pEThIL-2

3.2.1. Preliminary investigation

Fig. 3 shows the ADP voltammograms for probe-modified activated PGE for 600 s before (curve a) and after hybridization with the complementary plasmid (curve b) as the target DNA. As seen in Fig. 3, a significant increase in the guanine signal (from 194.58 ± 7.73 nA to 800.23 ± 22.4 nA) was observed following hybridization of the target DNA with the probe. This increase in the magnitude of the voltammetric signal represents the extent of the hybridization events at the electrode surface. Having observed the ability of the electrode in detection of target DNA, some experiments with non-complementary plasmid were carried out to assess whether the suggested DNA sensor responds selectively. For this purpose the non-complementary DNA plasmid (pET21a(+)) was subjected. As seen in Fig. 3 (curve c), the interaction between non-complementary plasmid and immobilized probe lead to a significant increase in the guanine oxidation signal (from 194.58 ± 7.73 nA to 420.18 ± 9.37 nA), This increase may be attributed to considerable adsorption of the non-complementary plasmid on some free sites present on

the PGE, or deficient hybridization events between immobilized probe and non-complementary plasmid. In order to remove the adsorbed plasmid from the electrode surface or prevent its non-specific immobilization on the probe-modified electrode some experiments were carried out as follow.

3.2.2. Effect of the electrode washing process

We tried to remove the adsorbed plasmid with vigorous washing of the electrode surface before each measurement. We found that, at the first seconds of electrode washing, guanine oxidation signal decreased slightly, and then remained unchanged for longer washing times even up to 300 s. Therefore, it was concluded that at the experimental conditions (described in Section 3.2.1) washing of the electrode, even for longer times, cannot eliminate the undesired immobilized non-complementary plasmid molecules. It should be mentioned that, the electrode washing step did not show any undesired influence on the stability of immobilized probe as well as on the hybridized complementary plasmid.

3.2.3. Effect of the probe solution concentration

In order to investigate the effect of the probe solution concentration during electrode modification on the selectivity of the biosensor, different concentrations of chIL-2 (0–10 μM) were immobilized on the PGE that activated for 300 s. Then the PGE was allowed to interact with the complementary or non-complementary plasmids for 15 min, and then the electrode was washed with washing buffer solution for 60 s. The results obtained from this experiment showed that with increasing the probe solution concentration and subsequently the probe coverage of the PGE, only nonspecific plasmid adsorption can be decreased, but deficient hybridization with non-complementary plasmid still exists and washing of the electrode surface with wash buffer solution for 60 s is not effective to overcome the problem.

3.2.4. Effect of PGE activation time

We investigated the effect of electrode activation time in order to find an appropriate activation time which is not only suitable for the effective immobilization of the probe on the electrode surface, but also produces so active sites on the electrode surface that direct interaction of them with plasmids will be decreased.

Fig. 4 shows a histogram representing the guanine oxidation signal of immobilized probe before (a) and after hybridization with complementary plasmid (b) and after interaction with non-complementary plasmid (c) in the different electrode activation time and constant washing time of 300 s. As seen in Fig. 4, with shorter activation times (i) the guanine oxidation signal is significantly decreased undesirably for all cases, (ii) there is a favorable decrease in the ratio of non-complementary signal to probe signal (n/p) (n/p = 2.16, 1.82 and 1.4 for 600, 450 and 300 s, respectively); (iii) the ratio of complementary signal to non-complementary signal (c/n) is desirably increased (c/n = 1.90, 2.11 and 2.24 for 600, 450 and 300 s, respectively). Considering that the n/p ratio is decreased with the reduction of electrode activation time, we can conclude that shorter activation times produce a condition that the direct interaction between the active

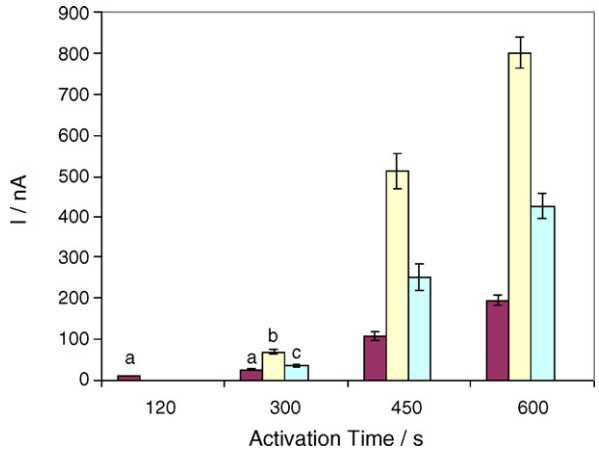

Fig. 4. Histogram representing the guanine oxidation signal of immobilized probe before (a) and after hybridization with complementary plasmid (b) and after interaction with non-complementary plasmid (c) for different electrode activation times. Experiment conditions as described in Fig. 3, and electrode washing time is 60 s.

sites on the electrode surface and plasmids is weakened and thus desorption of nonspecific adsorbed plasmids from the electrode surface can be easily achieved by electrode washing. On the other hand, since nonspecific adsorption of non-complementary plasmid is decreased in shorter activation times, consequently the ratio of c/n is increased in this conditions. As a result, 300 s was selected for electrode activation period for most subsequent works.

3.2.5. Optimization of electrode washing time after 300 s activation

Although selectivity of biosensor was almost improved by 300 s activation time following 60 s electrodes washing in washing buffer solution, the n/p ratio was still rather great. This ratio shows that either some nonspecifically adsorbed non-complementary plasmid may still exist on the electrode surface or some deficient hybridizations between immobilized probe and non-complementary plasmid are probably occurred on the electrode surface. Therefore, in order to further improve the selectivity of the biosensor, the effect of electrode washing time was investigated. Fig. 5A displays the variations of guanine oxidation signal of immobilized probe before (▲) and after hybridization with complementary plasmid (♦) and after interaction with non-complementary plasmid (■) versus different electrode washing times. As shown in Fig. 5A, the variations of guanine oxidation signal of probe before and after interaction with non-complementary plasmid were not considerable between 60 s and 300 s of electrode washing period. While, the guanine oxidation signal of immobilized probe after hybridization with complementary plasmid deceased in first 60 s of electrode washing time, but it unexpectedly showed a noticeable increase between 60 s and 300 s and then slowly decreased for more washing times. This was a surprising result. What does really happen in electrode washing period up to 60 s and 60 s to 300 s? Giving explanation for this question is really difficult. Reduction of signal at first 60 s washing in all cases may be favored desorption of nonspecific and weakly adsorbed

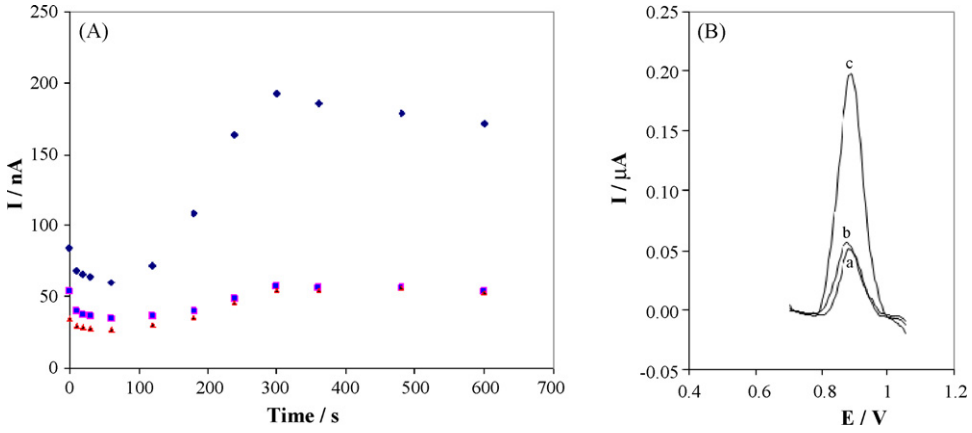

Fig. 5. (A) Variation of guanine oxidation signal of immobilized probe before (▲) and after hybridization with complementary plasmid (♦) and after interaction with non-complementary plasmid (■) vs. different electrode washing times. (B) ADPVs of probe-modified activated PGE before hybridization (a) and after hybridization with complementary plasmid (b) and after interaction with non-complementary plasmid (c) at optimum conditions.

DNA from electrode surface, but unexpected increase of signal especially for probe-target DNA hybrid between 60 s and 300 s maybe due to the rearrangement of probe-DNA hybrids. Indeed, stirring of solution for longer times switches the deficient hybrids towards more complete hybrids, which results in stronger signals due to the fact that complete hybridization leads to incorporating of more guanine nucleotides within the hybrid. The slight reduction of signals for more washing times may be attributed to gradually separation of DNA from electrode surface. As a result, the stirring of electrode for 300 s in washing solution was considered as the optimum time at which the ratio of c/n (2.92) and n/p (1.21) and consequently the selectivity of biosensor is improved.

Fig. 5B shows the typical ADP voltammograms for probe-modified activated PGE before hybridization (curve a) and after hybridization with complementary plasmid (curve b) and after interaction with non-complementary plasmid (curve c) at optimum conditions (cited in legend of Fig. 5). As illustrated in Fig. 5B, a significant increase in the guanine oxidation signal (from 54.19 ± 2.26 nA to 192.03 ± 11.12 nA) observed following hybridization of the complementary plasmid with the probe. Whereas, the interaction between non-complementary plasmid and immobilized probe did not lead to a significant increase in the guanine oxidation signal (from 54.19 ± 2.26 nA to 65.8 ± 4.38 nA). These observations indicate that in selected experiment conditions, only complementary plasmid could form a stable double strand DNA on the probe-modified activated PGE and consequently the selectivity of the biosensor is improved.

3.2.6. Diagnostic performance of the sensor

The difference of guanine oxidation signal of the probe-modified PGE in the absence and presence of complementary plasmid (ΔI) is increased with increasing the plasmid concentration and leveled off at ca. 5000 pg/µL (Fig. 6). Thus, at this concentration the maximum available capacity of the probe immobilized on the electrode surface is involved in hybridization event. As seen in inset of Fig. 6A, the calibration graph is linear between 0 and 500 pg/µL with correlation

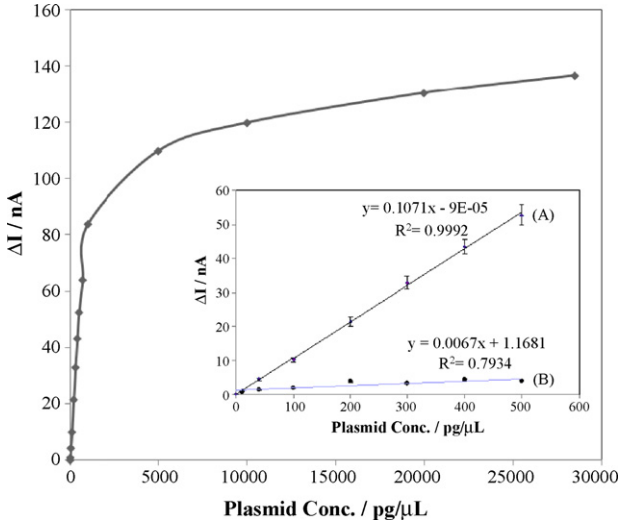

Fig. 6. Plot of ΔI (difference between the guanine oxidation signal of probe-modified PGE in the presence and absence of complementary plasmid) vs. complementary plasmid concentrations. Inset: related calibration graph at concentration range 10–500 pg/µL for complementary plasmid (A) and for non-complementary plasmid (B).

coefficient of 0.9992 for complementary plasmid. The relative standard deviation over three independently probe modified electrodes measured at 200 pg/µL of complementary plasmid was 4.2% indicating a remarkable reproducibility of the detection method. The detection limit was about 10.31 pg/µL. Such detection limit is in comparison with those reported for other label free (guanine-based) electrochemical hybridization detections (Wang et al., 2003; Mascini et al., 2005; Komarova et al., 2005).

The similar hybridization experiments were carried out in the presence of non-complementary plasmid. As seen in inset of Fig. 6B, the increase in guanine signal of probe-modified PGE after interaction with non-complementary plasmid was negligible. These findings clearly confirmed that the present DNA biosensor successfully distinguishes between complementary and non-complementary plasmids.

4. Conclusion

Following construction of recombinant pEThIL-2 plasmid (5839 bp) using pET21a(+) (5443 bp) as the backbone vector with 100% similarity in 5438 bp between two vectors, detection of pEThIL-2 and discrimination of this vector from its backbone was achieved for the first time via a label-free DNA hybridization biosensor using a non-inosine substituted probe on the PGE. The optimization experiments showed that shorter PGE activation times produce a condition in which desorption of non-specifically adsorbed plasmids from the electrode surface can be easily achieved by 300 s washing of the electrode in washing buffer solution that resulted in selective detection and discrimination of the target DNA. The developed biosensor successfully detected pEThIL-2 plasmid in various concentrations starting from 5000 pg/μL to as low as 10.31 pg/μL and discrimination experiments confirmed selectivity of the electrode in detecting pEThIL-2 target DNA.

References

Anderson, G., Urban, O., Fedorca-Cray, P., Newell, A., Nunberg, J., 1987. In: Chanock, R., lemer, R., Brown, F., Ginsberg, H. (Eds.), Vaccines 87: Modern Approaches to New Vaccines. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 22–25.

Boon, E.M., Ceres, D.M., Drummond, T.G., Hill, M.G., Barton, J.K., 2000. Nat. Biotechnol. 18 (10), 1096–1100.

Cai, X., Rivas, G., Farias, P.A.M., Shiraishi, H., Wang, J., Fojta, M., Paleček, E., 1996. Bioelectrochem. Bioenerg. 40 (1), 41–47.

Cerretti, D.P., McKereghan, K., Larsen, A., Cantrell, M., Anderson, D., Gillis, S., Cosman, D., Backer, P.E., 1986. P. Natl. Acad. Sci. U.S.A. 83 (10), 3223–3227.

Devos, R., Plaetinck, G., Cheroutre, H., Simons, G., Degrare, W., Tavenier, J., Remaut, E., Fiers, W., 1983. Nucleic Acids Res. 11, 4307–4313.

Diculescu, V.C., Paquim, A.M.C., Brett, A.M.O., 2005. Sensors 5 (6–10), 377–393.

Hejazi, M.S., Alipour, E., Pournaghi-Azar, M.H., 2007. Talanta 71, 1734–1740.

Jeffrey, S.R., Corthesy, B., Flanagun, W., Crabtree, G.R., 1992. Karger Basel, 266–298.

Kittel, C., Ferko, B., Kurz, M., Voglauer, R., Sereinig, S., Romanova, J., Stiegler, G., Katinger, H., Egorov, A., 2005. J. Virol. 79, 10672–10677.

Komarova, E., Aldissi, M., Bogomolova, A., 2005. Biosens. Bioelect. 21, 182–189.

Liu, J., Tian, S., Tiefenauer, L., Nielsen, P.E., Knoll, W., 2005. Anal. Chem. 77 (9), 2756–2761.

Mascini, M., Carlo, M.D., Minunni, M., Chen, B., Compagnone, D., 2005. Bioelectrochemistry 67, 163–169.

Meuer, S., Dumman, H., Meyer, K., Kohler, H., 1989. Lancet 15.

Palecek, E., Fojta, M., Jelen, F., 2002. Bioelectrochemistry 56 (1–2), 85–90.

Patolsky, F., Lichtenstein, A., Willner, I., 2000. J. Am. Chem. Soc. 122 (2), 418–419.

Perrint, P., Joffret, M.L., Leclerc, C., Oth, D., Sureau, P., Thibodeau, L., 1988. Immunobiology 177, 199–209.

Pournaghi-Azar, M.H., Hejazi, M.S., Alipour, E., 2006. Anal. Chim. Acta 570, 144–150.

Pournaghi-Azar, M.H., Hejazi, M.S., Alipour, E., 2007. Electroanalysis 19 (4), 466–472.

Robb, R.J., 1984. Immunol. Today 5, 203–209.

Robbens, J., Raeymaekers, A., Steidler, L., Fiers, W., Remaut, E., 1995. Prot. Expr. Purif. 6, 481–486.

Sabzi R. E., Sehatnia B., Pournaghi-Azar M.H., Hejazi M.S., 2008. Journal of Iranian Chemical Society, in press.

Sambrook, J., Rucell, D., 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sauer, S., 2006. Clin. Chim. Acta. 363 (1–2), 95–105.

Seow, H.F., Mucha, M.J., Hurst, L., Rothel, J.S., Wood, P.R., 1997. Vet. Immunol. Immunopathol. 56, 107–117.

Wang, J., 2000. Nucleic Acids Res. 28 (16), 3011–3016.

Wang, J., Kawde, A.N., Musameh, M., 2003. Analyst 128, 912–916.

Wang, R., Minunni, M., Tombelli, S., Mascini, M., 2004. Biosens. Bioelectron. 20 (3), 598–605.

Weinberg, A., Kondrad, M., Merigan, T., 1987. J. Virol. 62, 2120–2217.

Weinberg, A., Merigan, T., 1988. J. Immunol. 140, 294–299.

William, J., Qun, Z., 2005. Curr. Gene Ther. 5 (1), 133–142.

Williams, D.P., Regier, D., Akiyoshi, D., Genbouff, F., Murphy, J.R., 1988. Nucleic Acids Res. 16, 10453–10467.

Willner, I., Patolsky, F., Weizmann, Y., Willner, B., 2002. Talanta 56 (5), 847–856.

Wong, E., Goodling, J., 2006. Anal. Chem. 78, 2138–2144.

Xu, Y., Yang, L., Ye, X., He, P., Fang, Y., 2006. Electroanalysis 18, 873–881.

Yang, I.V., Thorp, H.H., 2001. Anal. Chem. 73, 5316–5322.