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Direct and rapid electrochemical biosensing of the human interleukin-2 DNA in unpurified polymerase chain reaction (PCR)-amplified real samples

Mohammad Hossein Pournaghi-Azar[a,*], Esmaeel Alipour[a], Sepideh Zununi[b,c], Haleh Froohandeh[b,c], Mohammad Saeid Hejazi[b,d,**]

[a] Electroanalytical Chemistry Laboratory, Faculty of Chemistry, University of Tabriz, Tabriz, Azarbadjan, Iran
[b] Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Electrochemical detection of polymerase chain reaction (PCR)-amplified human interleukin-2 (IL-2) coding DNA sample (399 bp size) without any purification and pre-treatment is described. To achieve this goal, a sensor was made by immobilization of a 20-mer oligonucleotide (chIL-2) as the probe on the pencil graphite electrode (PGE). This probe is related to the antisense strand of human interleukin-2 gene. The results showed that the electrode could effectively sense the PCR product of human interleukin-2 DNA by anodic differential pulse voltammetry (ADPV) based on guanine oxidation signal. In order to inhibit PCR components interfering effects and improve biosensing performance, various factors were investigated. We found that the desorption of non-specifically adsorbed components of the unpurified PCR samples from PGE surface is easily achieved by washing of the electrode in washing solution for about 300 s. The effectiveness of this procedure was confirmed using purified PCR samples. The selectivity of the sensor was assessed with negative control PCR sample and seven different non-complementary PCR products corresponding to 16S rDNA (bigger than 1500 bp) of various bacterial genuses. Diagnostic performance of the biosensor is described and the detection limit is found to be 69 pM. The reliability of the electrochemical biosensing results was verified by electrophoresis of the PCR products.

1. Introduction

DNA diagnosis has enormous applications in various fields including molecular biology, clinical diagnostics, agriculture, forensic science, and for the detection of pathogens. Main problem in detection of DNA hybridization at physiological levels is that the amount of DNA to be detected is in the femtomolar or attomolar ranges, very lower than the detection limit of general analytical techniques. There are two alternatives to solve this problem: to amplify the sample or to amplify the signal. Obviously, it is difficult to detect DNA at attomolar level by only amplifying the signal. Thus the amplification of sample by the polymerase chain reaction (PCR) is commonly required for this purpose.

The PCR products are usually identified by gel electrophoresis and ethidium bromide staining (Vollenhofer et al., 1999; Permingeat et al., 2002). Capillary electrophoresis has also been used for faster and automatable separation and detection of PCR products (Garcia-Canas et al., 2002; Obeid et al., 2004). Electrophoretic methods, however, do not provide information about the sequence of the DNA fragments. As a result, non-specific amplification products of similar size may lead to erroneous interpretation. Additionally, in some diagnostic tests further procedures are required to be performed on PCR products so that without these complementary experiments diagnosis will not be achieved. Several authors have reported the coupling of biosensors with a DNA amplification method (e.g. PCR) to obtain reliable measurements of clinical interests. For this purpose, various transduction strategies have been reported. For instances, the mass change on the surface of the transducer can be detected with a piezoelectric device (quartz crystal microbalance) (Mannelli et al., 2003; Minunni et al., 2001; Willner et al., 2002; Patolsky et al., 2000). Alternatively, the surface plasmon resonance (SPR) biosensor detects the change of the refractive index in the vicinity of the sensor's surface as a result of hybrid formation (Mariotti et al., 2002; Wang et al., 2004; Feriotto et al., 2002; Giakoumaki et al., 2003; Liu et al., 2005). However, the electrochemical transducers have attracted more attention

* Corresponding author. Fax: +98 411 3340191.
** Corresponding author. Fax: +98 411 334 4798.
E-mail addresses: pournaghiazar@tabrizu.ac.ir (M.H. Pournaghi-Azar), saeidhejazi@tbzmed.ac.ir (M.S. Hejazi).

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than other methods because they are highly sensitive, inexpensive, easy-to-use, portable and compatible with microfabrication technologies. DNA hybridization detection using electrochemical methods are divided into indirect and direct strategies. Incorporation of an electroactive label makes up the principle of indirect DNA hybridization detection methods. The basis of direct DNA hybridization detection strategy lies on direct transduction of signal induced from the oxidation of guanine or adenine moieties in DNA strands that is also called label-free detection method.

Label-free detection strategy seems to be simple, less time consuming and more applicable. Electrochemical label-free biosensing of DNA based on guanine moiety oxidation signal of probe (Yang et al., 2001) or target using inosine substituted probe (Wang et al., 2003) are reported. We have developed a label-free DNA hybridization biosensor using guanine moiety oxidation signal of both probe (non-inosine substituted) and target DNA (Pournaghi-Azar et al., 2007). The presence of only one guanine and seven cytosine bases in the utilized probe, allowed: (a) producing an electrochemically detectable signal for direct optimizing of the probe immobilization conditions and (b) obtaining a significant enhanced electrochemical signal following hybridization with target DNA possessing multiple guanine bases.

Electrochemical methods were used for monitoring the polymerase chain reaction (PCR)-amplified real samples of lysteria monocytogen, monitoring guanine signal after electrochemical enrichment on plastic composite electrodes (Schulein et al., 2002). The PCR samples were tested to detect factor V leiden mutation with the help of guanine oxidation signal on CPE (Ozkan et al., 2002). A label-free electrochemical assay based on guanine oxidation signal to measure telomerase activity is also reported (Eskiocak et al., 2007). A novel electrochemical DNA biosensor for detection of the common functional polymorphism of catechol-*O*-methyl transferase gene in PCR amplicons is described (Ozkan-Ariksoysal et al., 2008).

Some groups (Steichen et al., 2007; Suye et al., 2005; Kobayashi et al., 2004; Kara et al., 2003, 2004; Ozkan et al., 2002; Meric et al., 2002) have reported electrochemical biosensors for detection of DNA hybridization in PCR-amplified real samples to obtain reliable measurement of clinical interests. In order to improve the selectivity and performance of the electrochemical detection methods some works dealt with the purification of PCR products using the Clean-up DNA Purification kits (e.g. Microcon® PCR kits) or polyacrylamide gel by a classical electroelution procedure (Carpini et al., 2004; Djellouli et al., 2007; Berganza et al., 2007).

Following our published paper about interleukin-2 DNA (an immune system cytokine secreted by activated T cells and promotes immune response against bacteria and viruses) detection studies (Pournaghi-Azar et al., 2006, 2007), and its use in biosensing and discrimination of recombinant plasmid on the basis of interleukin-2 DNA insert (Hejazi et al., 2008), here we describe the electrochemical label-free detection of DNA hybridization event in PCR-amplified samples without any purification or pre-treatment of the samples. It is also intended to apply the method for detection of human interleukin-2 DNA and its discrimination from non-complementary unpurified PCR-amplified samples. To achieve this goal, anodic differential pulse voltammetry (ADPV) was used as the monitoring procedure.

2. Materials and methods

2.1. Materials

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 called chIL-2 (5′-CTA AAT TTA GCA CTT CCT CC-3′) corresponding to antisense strand of human IL-2 encoding DNA was used as the probe. This oligonucleotide was supplied (as lyophilized powder) from MWG-Biotech.

The stock solution of the oligonucleotide (100 μg/ml) was prepared in 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.

2.2. Bacterial strains and plasmid

Lactobacillus rhamnosus strain LQ108, Idiomarina sp., Halomonas salina GSP2, Marinobacter sp., Salicola marensis 7Mb1, Halomonas taeanensis and Basillus halophila were used for amplification of 16S rRNA encoding gene of 16S rDNA. Plasmid pEThIL-2 (Hejazi et al., 2008); a prokaryotic expression vector; was used as the template DNA for amplification of human IL-2 encoding DNA.

2.3. Media and enzyme

MH medium (60.7 g NaCl, 15 g $MgCl_2{\cdot}6H_2O$, 7.4 g $MgSO_4{\cdot}7H_2O$, 0.27 g $CaCl_2$, 1.5 g KCl, 45 mg $NaHCO_3$, 19 mg NaBr, 5 g proteose peptone No.3, 10 g yeast extract, 1 g glucose in 1000 ml distilled water; pH 7.2) was used to grow *Halomonas salina GSP2* and *Marinobacter* sp. cells. Halomonas medium (80 g NaCl, 20 g $MgSO_4{\cdot}7H_2O$, 7.5 g casamino acids (with vitamins), 5 g proteose peptone No. 3, 1 g yeast extract, 3 g Na_3-citrate, 0.5 g K_2HPO_4, 0.05 g $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in 1000 ml distilled water; pH 7.5) was used as culture medium for *Idiomarina* sp., *Salicola marensis 7Mb1*, *Halomonas taeanensis* and *Basillus halophila*. *Lactobacillus rhamnosus* strain LQ108 cells were grown in MRS medium (10 g peptone, 8 g beef extract, 4 g yeast extract, 20 g glucose, 2 g triammonium citrate, 5 g sodium acetate, 0.2 g magnesium sulphate, 0.05 g manganese sulphate, 2 g Di-potassium phosphate in 1000 ml distilled water; pH 6.2 ± 0.2 at 25 °C). *Taq* DNA polymerase and PCR reagents were purchased from Fermentas company.

2.4. Genomic DNA extraction

The protocol described by Corbin and co-workers (1994) was used for genomic DNA extraction with some modifications. Briefly, a single colony was cultured in 50 ml liquid MRS medium over night in shaker incubator at 25 °C. Following separation of bacterial cells from the medium by centrifugation for 3 min at 5000 rpm, the bacterial cells were pulverized in liquid nitrogen, suspended in a solution I (10 mM Tris (pH 7.4), 1 mM EDTA, 0.5% sodium dodecyl sulphate (SDS), 0.1 mg/ml of proteinase K) and lysed by incubation at 37 °C for 1 h. Then the solution II containing 0.8 M NaCl and 1% CTAB was added to the lysates, and incubated at 65 °C for 20 min and extracted with equal volume of chloroform-isoamylalcohol (24:1). DNA was precipitated from the aqueous phase with 0.6 volume of isopropanol and finally purified using ethanol 70%.

2.5. PCR amplification of 16S rRNA and IL-2 encoding DNA fragments

Plasmid pEThIL-2 was used as the template DNA for IL-2 DNA amplification using polymerase chain reaction (PCR) technique with Taq DNA polymerase in the presence of forward (5′-GCA CCT ACT TCA AGT TCT ACA-3′) and reverse (5′-TTA AGT CAG TGT TGA GAT GAT G-3′) primers. PCR amplifications were carried out on a