Biosensors and Bioelectronics 23 (2008) 1819–1824

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A simple and direct electrochemical detection of interferon-γ using its RNA and DNA aptamers

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ARTICLE INFO

Article history:
Received 8 November 2007
Received in revised form 25 February 2008
Accepted 25 February 2008
Available online 2 March 2008

Keywords:
Tuberculosis
Interferon-gamma (IFN-γ)
Electrochemical impedance spectroscopy
RNA aptamer
DNA aptamer

ABSTRACT

Tuberculosis is the most frequent cause of infection-related death worldwide. We constructed a simple and direct electrochemical sensor to detect interferon (IFN)-γ, a selective marker for tuberculosis pleurisy, using its RNA and DNA aptamers. IFN-γ was detected by its 5′-thiol-modified aptamer probe immobilized on the gold electrode. Interaction between IFN-γ and the aptamer was recorded using electrochemical impedance spectroscopy and quartz crystal microbalance (QCM) with high sensitivity. The RNA-aptamer-based sensor showed a low detection limit of 100 fM, and the DNA-aptamer-based sensor detected IFN-γ to 1 pM in sodium phosphate buffer. With QCM analysis, the aptamer immobilized on the electrode and IFN-γ bound to the aptamer probe was quantified. This QCM result shows that IFN-γ exists in multimeric forms to interact with the aptamers, and the RNA aptamer prefers the high multimeric state of IFN-γ. Such a preference may describe the low detection limit of the RNA aptamer shown by impedance analysis. In addition, IFN-γ was detected to 10 pM by the DNA aptamer in fetal bovine serum, a mimicked biological system, which has similar components to pleural fluid.

1. Introduction

Tuberculosis is a common and lethal disease caused by *Mycobacterium tuberculosis* (WHO Report, 2007). Research interest in tuberculosis has increased since it became widely known that it is a major cause of death in AIDS patients. The *M. tuberculosis* direct test (MTD) has been conventionally performed to diagnose tuberculosis, and results are analyzed using PCR amplification for *M. tuberculosis*-specific DNA fragments (Lezama et al., 1997; Signh, 2006). Such a histological approach has significant limitations, such as false-positive results. Recently, it has been reported that some tuberculosis-related cytokines may be biological markers for the diagnosis of tuberculosis. Thus, many cytokines, including interferon (IFN)-γ, have become conspicuous anew for the diagnosis of tuberculosis pleurisy (Villegas et al., 2000; Yamada et al., 2001).

IFN-γ produced by natural killer (NK) cells, dendritic cells, activated $CD4^+$ T cells, and other lymphocytes has many physiological roles in an immune system and inflammatory stimuli (Boehm et al., 1997). In addition, it has been reported that IFN-γ takes part in the development of pleural effusion of tuberculosis, and the protein may be a powerful biological marker with high sensitivity and specificity (Wongtim et al., 1999; Aoe et al., 2003). Detections of IFN-γ were tried by an immunosensing technique that utilizes the antibody (Dijksma et al., 2001; Bart et al., 2005). This technique has a high sensitivity, whereas it is a labor-intensive and time-consuming traditional method. For developing new and expedient methods as a substitute for immunosensor, some attention has been focused on aptamer-based methods to detect biological markers (Navani and Li, 2006). Since the aptamer has high-binding affinity and selectivity for its target molecules like antibodies (Nimjee et al., 2005; Proske et al., 2005; Carothers et al., 2006), it is suitable as a powerful biological diagnostic tool. Also, aptamers have several advantages over antibodies, such as chemical synthesis *in vitro*, easy modification, and low cost. Moreover, aptamers are resistant in various buffer conditions, pH and temperature (Radi et al., 2005; Stadtherr et al., 2005).

Several approaches for the detection of protein–DNA interactions have been identified using electrochemical (Bakker, 2004; Ban et al., 2004; Drummond et al., 2003; Rodriguez et al., 2005), optical (Nie and Zare, 1997), fluorescence (Fang et al., 2001; Li et al., 2007b), and quartz crystal microbalance (QCM) (Williams and Rouzina, 2002; Liss et al., 2002) methods. Of these, electrochemical impedance spectroscopy is an attractive method because it is performed in label-free manner with simple and direct detection, and practicable in more diverse environments than any other method (Darain et al., 2004a,b; Cho et al., 2006). In this method, formation of

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doi:10.1016/j.bios.2008.02.021

the protein–DNA complex can be readily detected by analyzing the change in charge transfer resistance (R_{ct}) at the electrode interface.

In the present study, the immobilized aptamers, RNA (Kubik et al., 1997) and DNA aptamers (Balasubramanian et al., 1998), were used to detect IFN-γ by electrochemical impedance spectroscopy with high sensitivity. It was also crosschecked by QCM. The different detection limit between the RNA and DNA aptamer for IFN-γ was verified by electrophoretic mobility shift assay (EMSA).

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA) was purchased from Promega (USA). Fetal bovine serum (FBS; GIBCO) and SYBR Green I (Molecular Probes) were purchased from Invitrogen (USA). Sodium phosphate monobasic, sodium phosphate dibasic, Tris/HCl, ferricyanide, pentanethiol, β-mercaptoethanol, bis(sulfosuccinimidyl) suberate (BS3) and diethyl pyrocarbonate (DEPC) was purchased from Sigma–Aldrich (USA). RNase-free solution spray was purchased from CLP (USA).

2.2. Aptamers

The RNA aptamer primarily derived in SELEX was modified with a fluorine or amine group at the 2′ OH group of sugar moieties of pyrimidine bases (Kubik et al., 1997). However, we used the unmodified RNA aptamer since it showed a strong binding affinity to IFN-γ with high specificity. To immobilize the aptamers on the gold electrode, the 5′-thiol-modified IFN-γ-binding RNA aptamer (5′-HS-$(CH_2)_{12}$-GGG AGG ACG AUG CGG ACA CCG UUA AUC UGA GGC CCU GUC CUA UUC CUU CAC GCC UCA GA-3′) (Kubik et al., 1997) and DNA aptamer (5′-HS-$(CH_2)_{12}$-GGG GTT GGT TGT GTT GGG TGT TGT GT-3′) (Ramanathan et al., 1994; Balasubramanian et al., 1998) were used in the present study. Both RNA and DNA aptamers, PAGE-purified, were purchased from Samchully Pharm (Korea) and Bioneer (Korea), respectively. The 5′-thiol-modified aptamers were further purified using a reverse-phase chromatography column.

2.3. Expression and purification of IFN-γ

A human IFN-γ gene was cloned with an N-terminal $(His)_6$-tag into a pET-28a–c(+) expression vector with a tobacco etch virus (TEV) protease site. IFN-γ was over-expressed from *Escherichia coli* strain BL21 (DE3) by 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 20 °C. Harvested cells were resuspended in 20 mM Tris/HCl, pH 8.0, 300 mM NaCl, 5% glycerol, 0.05 mM β-mercaptoethanol, 1 μg/ml lysozyme and 200 μM phenylmethanesulfonyl fluoride (PMSF). Cells were sonicated at 4 °C, and the supernatant was purified through the Hi-trap Ni-affinity column (GE Healthcare). TEV protease was added to the eluted IFN-γ sample to remove the tag. The digested protein was further purified using a MonoS column (GE Healthcare). Finally, highly purified IFN-γ (up to 99%) was obtained via the HR75 column (GE Healthcare).

2.4. Immobilization of aptamers on the gold electrode for impedance analysis

All equipment related to the RNA aptamer studies were treated in a solution of 0.1% DEPC. Glassware and plastics were soaked in DEPC solution for 2 h at 37 °C and autoclaved to remove the remaining DEPC. Other equipments that could not stand the sterilizing procedure were treated with RNase-free solution spray and rinsed with DEPC-treated water.

Prior to aptamer-immobilization, the working gold electrode was mechanically polished with 0.05 μm alumina for 30 min, and then sonicated in distilled water for 1 min. An electrochemical cleaning process was conducted by cycling from a potential of −0.8 to +1.3 V versus Ag/AgCl in a 0.1-M H_2SO_4 solution, and then the electrode was dried under blowing nitrogen gas. The working gold electrode was functionalized with 0.5 μM of each aptamer by immersion in 500 μl incubation buffer (20 mM sodium phosphate (pH 8.0), 5 mM $MgCl_2$ and 0.5 mM dithiothreitol (DTT)) with 0.5 μM pentanethiol for 6 h. The aptamer-immobilized electrode was thoroughly rinsed with distilled water and equilibrated with the incubation buffer for 30 min. These processes were carried out at room temperature with gentle shaking at 80 rpm. The 0.1-M β-mercaptoethanol solution was added to the aptamer-immobilized electrode for 1 min.

2.5. Impedance measurement for interaction between IFN-γ and aptamers

An electrochemical cell was organized into a three-electrode configuration: Ag/AgCl reference electrode in saturated KCl solution, platinum counter electrode and oligonucleotide-immobilized gold working electrode. Faradaic impedance spectra were recorded using a PARSTAT 2263 (USA) at an open circuit potential of 10 kHz to 100 MHz. All electrochemical impedance measurements were performed in 10 mM sodium phosphate (pH 10.3) buffer with 5 mM $[Fe(CN)_6]^{3-/4-}$, and impedance spectra were collected as a form of Nyquist plots.Interactions between the aptamers and IFN-γ were detected with an increase of [IFN-γ] in sodium phosphate buffer: 100 fM, 10 pM, 1 nM and 100 nM. Detection of IFN-γ in FBS solution was also carried out. For this experiment, the FBS solution was buffer-exchanged to 10 mM sodium phosphate buffer, pH 10.3 using G25 column. IFN-γ was added to the pretreated FBS buffer at various concentrations: 10 pM, 1 nM and 100 nM.

BSA and FBS were used to examine the selectivity of this aptamer-based IFN-γ sensor. The DNA-aptamer-immobilized electrode was incubated with variable [BSA] for 30 min: 10 pM, 100 pM, 1 nM and 10 nM. The electrode was also dipped into FBS solution to confirm the selectivity for IFN-γ, and impedance signals were measured for every 30 min.

2.6. QCM analysis to confirm the sensitivity of each aptamer

QCM analysis was carried out using an EQCN-1000 L (Biomechatron, Korea), and a 9.0-MHz AT-cut Au/Ti quartz crystal working electrode was used. The working electrode was contacted with 2 ml buffer solution on one face. The incubation buffer (20 mM phosphate pH 8.0, 5 mM $MgCl_2$ and 0.5 mM DTT) was filled into the QCM cells to make steady state before immobilization of each RNA and DNA aptamer on the electrode. After the quilibration with the buffer, each 0.5 μM aptamer solution was added to the cell. After RNA aptamer was bound and frequencies were stabilized, IFN-γ was put into the QCM cell from 100 fM to 100 nM. IFN-γ was also detected by DNA aptamer in concentration order: 1 pM, 100 pM, 10 nM and 1 μM.

2.7. Circular dichroism (CD) analysis

CD spectra were acquired using a Jasco J810 spectropolarimeter (Jasco, Tokyo, Japan) with 10.0-mm path length quartz cuvets. Time constant and sensitivity of the instrument were set at 4 s and 1 millidegree, respectively. IFN-γ and oligonucleotide aptamers were scanned over the wavelength range 200–350 nm to check the conformational change in the secondary structure of the protein and aptamer under high-pH conditions. The detection solutions