Biosensors and Bioelectronics 30 (2011) 261–266

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Aptamer biosensor for label-free square-wave voltammetry detection of angiogenin

Lidong Li*, Hongtao Zhao, Zhengbo Chen, Xiaojiao Mu, Lin Guo*

School of Chemistry & Environment, Beihang University, Beijing 100191, China

ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 7 September 2011
Accepted 19 September 2011
Available online 6 October 2011

Keywords:
Aptamer
Angiogenin
Biosensor
Square-wave voltammetry

ABSTRACT

Angiogenin (Ang), one of the most potent angiogenic factor, is related with the growth and metastasis of numerous tumors. This paper presents a very simple and label-free square-wave voltammetry (SWV) aptasensor to detect angiogenin, in which an anti-angiogenin-aptamer was used as a molecular recognition element, and the couple ferro/ferricyanide as a redox probe. At the bare gold electrode, the redox couple ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$) can be very easily accessed to the electrode surface to give a very strong SWV signal. At the anti-angiogenin/Au electrode surface, when angiogenin was added to the electrochemical cell, the binding of the analyte results in less availability for a redox reaction, which led to smaller SWV current. To quantify the amount of angiogenin, current suppressions of SWV peak were monitored using the redox couple of an $[Fe(CN)_6]^{4-/3-}$ probe. The plot of signal suppression against the logarithm of angiogenin concentration is linear with over the range from 0.01 nM to 30 nM with a detection limit of 1 pM. The aptasensor also showed very good selectivity for angiogenin without being affected by the presence of other proteins in serum. It is the first time to use a very simple method to detect the cancer marker. Such an aptasensor opens a rapid, selective and sensitive route for angiogenin detection and provides a promising strategy for other protein detections.

1. Introduction

Aptamers are nucleic acid-based molecules that can be selected to bind essentially to any molecule of choice (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Compared with antibodies, aptamers possess more advantages over antibodies such as chemical synthesis, selection through the SELEX (systematic evolution of ligands by exponential enrichment) process, easy modification, high stability, target versatility, easy-to-stock, and resistant to denaturation and degradation. These properties make aptamers a promising class of agents for biomolecule detection. Aptasensors for biomolecules have been developed on the basis of different technologies, for example fluorescence (Chang et al., 2010), surface-enhanced Raman spectroscopy (SERS) (Cho et al., 2008), microgravimetric (Minunni et al., 2004), and electrochemistry (Baker et al., 2006; Zuo et al., 2007; Jin et al., 2007; Zayats et al., 2006; Willner and Zayats, 2007). Among these, electrochemical methods have attracted most attention in the development of aptasensors because of their high sensitivity, simple instrumentation, low production cost, fast response, and portability.

Angiogenin (Ang), one of the most potent angiogenic factor, a 14.4-kDa polypeptide, is a homologue of bovine pancreatic ribonuclease A (RNaseA) with a ribonucleolytic activity of four to six orders of magnitude less than that of RNaseA. Angiogenin stimulates endothelial cells to form diacyl–glycrol and to secrete prostacyclin by activating phospholipase C and phospholipase A2 (Fett et al., 1985; Bicknell and Vallee, 1988, 1989). Angiogenin also has a ribonucleolytic activity that appears to be necessary for neovascularization (Shapiro et al., 1986; St Clair et al., 1987). Tumor necrosis factor-a (TNF-(r)) induces the production and secretion of basic fibroblast growth factor in endothelial cells as well as being a chemoattractant for monocytes and a macro-phage activator (Leibovich et al., 1987; Frater-Schröder et al., 1987; Okamura et al., 1991; Beutler and Cerami, 1986). Therefore, Ang is related with the growth and metastasis of numerous tumors, it is very important to be able to assess this molecule at trace level with high sensitivity. Serum concentration of Ang is commonly detected with antibody-based enzyme-linked immunosorbant assay (ELISA) (Katona et al., 2005). In 1998, AL6, a 45 nt DNA aptamers of Ang, was generated by in vitro selection process (Nobile et al., 1998). Afterwards, Yang et al. used a single-fluorophore-modified derivative of this aptamer to detect Ang in serum based on fluorescence anisotropy (Li et al., 2007). Wang et al. developed a method by using a FRET-based aptamer probe for rapid angiogenin detection (Li et al., 2008).

* Corresponding author. Tel.: +86 010 82338162; fax: +86 010 82338162.
E-mail addresses: lilidong@buaa.edu.cn (L. Li), guolin@buaa.edu.cn (L. Guo).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.09.022

Among all the voltammetric techniques, square-wave voltammetry has many advantages, such as the excellent sensitivity and the high speed. This high speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal-to-noise ratio. Compared to both linear sweep and cyclic voltammetry, square-wave voltammetry has a much broader dynamic range and lower limit of detection because of its efficient discrimination of capacitance current. Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels (Parham and Rahbar, 2010; Nezamzadeh et al., 2007). In the square-wave voltammetry, the peak height is directly proportional to the concentration of the electroactive species and direct detection limits of redox species as low as 10 nM are possible. In addition, FIS (Faradaic Impedance Spectrometry) is also a commonly used method for probing protein binding events such as antibody–antigen interactions (Dijksma et al., 2001; Ruan et al., 2002; Bardea et al., 2000). However, FIS can be also associated with nonspecific impedance changes that could be easily mistaken for specific interactions (Bogomolova et al., 2009), that is, inability to discriminate between specific and nonspecific binding (both causing impedance increase). It has been found other factors leading to nonspecific impedance changes, such as: (i) initial electrode contamination; (ii) repetitive measurements; (iii) additional cyclic voltammetry (CV) or differential pulse voltammetry (DPV) measurements; and (iv) additional incubations in the buffer between measurements. Thus, these factors can occasionally cause very unstable signals.

Here we describe a novel label-free bioelectronic strategy for transducing aptamer–angiogenin recognition events based on recognition-induced steric-hindrance between redox probe ($Fe(CN)_6^{4-/3-}$) and the electrode surface for controlling access of redox marker ions used in SWV signal transduction. The formation of such bioaffinity complexes commonly leads to an insulating layer that retards the interfacial electron transfer kinetics between the redox probe and the electrode and increases the electron-transfer resistance. Here, we demonstrate that the aptamer–angiogenin interaction leads to an increase in the electron transfer resistance, repelling the redox marker ions to approach the electrode, leading to a substantial decrease in SWV current. The analytical technique described here only involves the utilization of a single-stranded (ss) DNA aptamer and a redox probe in solution. Thus the proposed SWV aptasensor for angiogenin is very simple, rapid, cost-effective, highly sensitive and reproducible, especially, it is lable-free, and requires no external modification on the biomolecules. Since the proposed SWV method possesses many advantages, this procedure could potentially be applied to the aptamer-based detection of other proteins and small molecules in the future. In addition, it is the first time to use a very simple method to detect the cancer marker. It provides a promising strategy for the cancer diagnosis in the future.

2. Experimental

2.1. Apparatus and reagents

A CHI 660C electrochemical workstation (Chenhua Instruments Co., Shanghai, China) was used for the electrochemical measurements. All experiments were performed using a conventional three-electrode system with a fabricated aptasensor or bare gold (diameter, 2 mm) as the working electrode, Ag/AgCl (sat. KCl) as the reference electrode, and a platinum counter electrode. All potentials were referred to the reference electrode.

The synthetic anti-angiogenin oligonucleotides, ssDNA(3′-(SH)-$(CH_2)_6$-CGG ACG AAT GCT TTG ATG TTG TGC TGG ATC CAG CGT TCA TTC TCA-5′) were obtained from TaKaRa Biotechnology (Dalian, China) Co., Ltd. Angiogenin, thrombin, lysozyme, bovine serum albumin and bovine hemoglobin form lyophilized erythrocytes were purchased from CNS Bioservices Co., Ltd. Tris–(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from the Alfa Aesar (Tianjing) company. Tris-base was purchased from Sigma–Aldrich.

All other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water. Phosphate buffer pH 7.0 containing 1 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ was chosen as the supporting electrolyte for electrode characterization and angiogenin assays.

2.2. DNA preparation

99 μL TCEP immobilization buffer (I-B: 20 mM Tris–HCl/140 mM NaCl/5 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$ at pH 7.41) solution and 1 μL 100 μM anti-angiogenin aptamer were mixed together. Herein, TCEP was a reducer agent, which was intended to disrupt any disulphide bonds and ensure that free –SH groups are ready to react with the gold surface. Afterwards, the mixture was heated to 90 °C and allowed to gradually cool to room temperature in I-B. This heating and cooling step helps to maintain the structural flexibility of the aptamers. Afterwards, the 10 μL droplet of anti-angiogenin aptamer (1 μM in I-B) was placed on the Au electrode surface for later electrochemical analysis.

2.3. Electrode pretreatment

Prior to use, gold substrates were cleaned by immersion in a 3:1 mixture of concentrated H_2SO_4 and 30% H_2O_2 (*piranha*) for 5 min at 70 °C, followed by rinsing with copious amounts of water. *CAUTION: piranha reacts violently with organic solvents, and should be handled with extreme caution*. Afterwards, the electrode was polished with 1.0, 0.3, and 0.05 μm alumina slurry on a polishing pad consecutively. The electrode was then ultrasonically cleaned in ethanol for 1 min and in water for 2 min. The electrode was subsequently voltammetrically cycled in 0.5 M H_2SO_4 with the potential between −0.2 and +1.6 V at 0.1 V s^{-1} until a representative cyclic voltammogram of a clean gold electrode was obtained. Afterwards, the electrode was incubated in 10 μL droplet of anti-angiogenin aptamer (1 μM in I-B) for 12 h at room temperature in 100% humidity. Finally, the gold surface was rinsed again with I-B and then with deionized water, followed by drying under an N_2 stream. For detection of angiogenin, 10 μL droplets of different angiogenin concentrations in I-B were deposited on to the aptasensor and kept for 30 min at 37 °C. Non-binding angiogenin was removed by rinsing with I-B and deionized water. A schematic representation of aptasensor with fabrication steps and performance is displayed in Scheme 1.

2.4. Electrochemical measurements

All electrochemical measurements were performed in an electrochemical cell containing 10 mL of 0.2 M PBS, 1 mM $K_4[Fe(CN)_6]$–1 mM $K_3[Fe(CN)_6]$ (pH 7.0). The electrochemical square-wave voltammetry measurements were carried out under the following conditions: The voltage scanned from 0 V to 0.6 V with a potential incremental of 0.004 V, the amplitude, the frequency and the static time were kept as 0.025 V, 25 Hz, and 2 s, respectively. The Faradic impedance spectroscopy was recorded within the frequency from 100 kHz to 0.1 Hz with a sampling rate of 2 points per decade. The cyclic voltammetric experiments were