Journal of Electroanalytical Chemistry 717-718 (2014) 119–124

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Electrochemical immunoassay based on aptamer–protein interaction and functionalized polymer for cancer biomarker detection

Zahra Taleat [a,b], Cecilia Cristea [a], Giovanna Marrazza [c], M. Mazloum-Ardakani [b], Robert Săndulescu [a,*]

[a] Analytical Chemistry Department, Faculty of Pharmacy, IuliuHaţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[b] Chemistry Department, Faculty of Science, Yazd University, Yazd, Iran
[c] Ugo Schiff Chemistry Department, Universita degli studi di Firenze, Italy

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form 17 December 2013
Accepted 10 January 2014
Available online 22 January 2014

Keywords:
Poly(*o*-aminobenzoic acid)
MUC1 protein
Aptamer
Electrochemical immunoassay

ABSTRACT

An electrochemical aptamer-based biosensing assay for MUC1 protein detection by using methylene blue (MB) as electrochemical indicator and modifying the electrode surface using a functionalized conductive polymer was developed. The optimization steps starts with electropolymerization of *o*-aminobenzoic acid (*o*-ABA) onto graphite based screen printed electrodes (SPEs). Immobilization of primary antibody as the capturing probe was performed directly on poly *o*-ABA modified electrodes. Then, a sandwich like structure was fabricated upon MUC1 protein–aptamer complex formation, exploiting aptamer as the detection probe and methylene blue as the electrochemical active marker interacting with the aptamer without previous labeling. The aptamer instead of antibody was successfully used for the electrochemical detection. The recognition of immunoreactions and aptamer binding event was identified via monitoring the interfacial electron transfer resistance with electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). CV and differential pulse voltammetry (DPV) were employed to detect the change of MB oxidization peak current related with the human MUC1 protein concentration. DPV detection showed a reliable and more sensitive quantification of MUC1 with a detection range of 1–12 ppb and a lower detection limit (0.62 ppb).

1. Introduction

Aptamers are artificial nucleic acid ligands showing specific binding affinity for amino acids, drugs, proteins, small molecules [1] and cancer cells and other organic or inorganic molecules [2]. Aptamers have attracted more and more researchers as soon as they are selected from large combinatorial nucleotide libraries by an in vitro evolution process of systematic evolution of ligands by exponential enrichment (SELEX) [3]. Aptamers are more and more applied in biosensors development, thanks to their many advantages as recognition elements in biosensing for a broad range of targets, good thermal stability, and chemical stability, easy to be labeled and high affinity [4]. For protein detection, aptamers rival conventional antibodies with advantages of easy production, excellent controllability and versatility. In most of the aptamer based protein detection methods, aptamer–protein recognition events are measured by detecting conformational change of aptamer [5].

As is well known, aptamers interact with their targets and form special three dimensional conformations. According to the relative spatial positions of targets and aptamers in the target–aptamer complexes, the targets could be divided into two groups: embedded group and outside-binding group. Biomacromolecules, typically protein targets, which possess complicated spatial structures, belong to the outside-binding group [6].

In recent years, aptamers have been shown to be useful for the development of the aptamer-based protein recognition assays. Currently, several nucleic acids aptamers-based biosensors for protein detection have been developed. The signal transduction approaches were mainly based on the quartz crystal microbalance (QCM) [7], surface plasmon resonance (SPR) [8,9], fluorescence approach [10] other optical methods [11] and electrochemical methods [12–15].

Electrochemical immunosensors, which combine specific immunoreactions with electrochemical transduction, have attracted growing attention in recent years [16–25]. Different electrode materials have been used for electrochemical immunosensors, including glassy carbon electrodes (GCEs) [26–28], graphite based screen-printed electrodes (SPEs) [29,30], indium tin oxide [31], and Au [32,33,27].

The modified electrode surface allows highly dense immobilization of biomolecules, long-term stability of attached biomolecules, low non-specific binding, and proper biomolecular orientation to permit simple and rapid specific interactions. Conductive polymers (CPs) and in particular functionalized CPs are useful for this

* Corresponding author. Tel.: +40 264597256/2412; fax: +40 264597257.
E-mail address: rsandulescu@umfcluj.ro (R. Săndulescu).

1572-6657/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jelechem.2014.01.015

purpose. Poly o-aminobenzoic acid (PABA), can be considered as an carboxyl functionalized aniline conductive polymer capable of self-doping is of interest as a soluble derivative of polyaniline [34]. Its carboxylic acid group serves as a functional group that can be used as a matrix material for immobilization onto substrates or for covalent bonds with biomolecules such as proteins and antibodies [9]. Therefore, functionalized polymers are widely used as a platform to immobilize the primary antibody on the electrode surface.

To extend the application of functionalized CPs and aptamer in the detection of protein, we used them to construct a sandwich mode biosensor for the detection of MUC1 cancer biomarker protein. Some protein targets have dual binding sites, which endows them the ability to bind two recognition molecules and form sandwich-like complexes. Since these proteins could specifically bind either aptamers or antibodies, the sandwich structures have three basic formats: aptamer–protein–aptamer, antibody–protein–aptamer and antibody–protein–antibody. The first two formats have been widely employed in the design of aptamer-based biosensors [6].

MUC1 protein used in the present sandwich approach was of great interest because MUC1 plays an important role in the tumor genesis of several cancer types.

MUC1 over-expression is involved in metastasis by inhibiting tumor cell adhesion and in escaping from immune surveillance. MUC1 is a rigid glycoprotein overexpressed in different types of malignancies acting also as docking protein for signaling molecules [35].

Analyzing tissue sections with the antibodies by immunohistochemistry it became clear that although MUC1 is widely expressed by normal glandular epithelial cells, the expression being dramatically increased when the cells became malignant. In other words, in the normal glandular epithelial cell, MUC1 expression is limited to the apical surface bordering a lumen. In cancer cells however, which have lost polarity, the mucin is expressed all over the surface. This has been well documented in breast and ovarian cancer and available data suggest this is also true in some lung, pancreatic and prostate cancers [36].

Methylene blue (MB) was used as an electrochemical indicator for the study of MUC1 protein–aptamer interactions. It is known that MB is reduced to a leucomethylene blue (LB) at an electrode surface by accepting two electrons. This indicator was therefore used for detection of protein–aptamer interactions [37–39].

As well known, MB can specifically bind with G base in ss-DNA [40]. Utilizing these characteristics, this electrochemical active marker has been widely reported to directly interact with single and double strand DNA as an electrochemical active indicator [41–47]. Aptamer, as a kind of ss-DNA with rich of G bases, can undoubtedly absorb lot of MB onto its surface. At the same time, a kind of ss-DNA with special function, aptamer can specifically bind with its target molecules. Based on these facts, it is easy to design an electrochemical biosensor for detecting all kinds of analytes by using aptamer to load MB. MUC1 was selected as the model target analyte. On contrary to the conventional way to obtain the electrochemical signal by modifying the aptamer with appropriate markers, the MB interacted directly with MUC1 aptamer to avoid a previous labeling procedure. Electrochemical measuring of the recognition events possesses plenty of advantages, which is stable, simple, cost-effective, and avoids external modification on the biomolecules. Moreover, as MB could easily interact with DNA aptamer, employing antibody as the capturing probe could reduce the background signal.

The experimental conditions such as antibody concentration and the incubation times for antibody, MUC1 protein and aptamer concentration and dilutions were examined.

2. Materials and methods

2.1. Reagents

o-Aminobenzoic acid (*o*-ABA), 1-Ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from ALFA AESAR (Germany). Bovine serum albumin (BSA), ethanolamine, magnesium chloride, sodium chloride, potassium chloride and poly-oxyethylene sorbitan monolaureate (Tween 20) were purchased from Sigma–Aldrich; MUC1 monoclonal mouse antibody (Ab1) and MUC1 recombinant human protein (MUC1-Ag) were provided by Novus Biological.

Aptamer 5′-GCAGTTGATCCTTTGGATACCCTGGTTTTTTTTTTTTTT T-3′ (purified by HPLC) was purchased from AlphaDNA (Canada). Aptamer was dissolved in 10 mM TRIS buffer containing 1 mM EDTA.

Sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium ferrocyanide ($K_4[Fe(CN)_6]$) potassium ferricyanide ($K_3[Fe(CN)_6]$), sulfuric acid, methylene blue (MB), ethylenediaminetetraacetic acid (EDTA) and Tris (hydroxymethyl)aminomethane (TRIS) were obtained from Merck. All reagents were of analytical grade and used as received.

2.2. Instrumentation and electrochemical measurements

Electrochemical measurements were carried out with an AUTOLAB PGSTAT 30 digital potentiostat/galvanostat (EcoChemie, Netherlands). The electrochemical parameters were controlled with GPES 4.9 software. Screen-printed electrodes (SPEs), type DRP 110 consisting in a graphite working electrode, a graphite counter electrode and a silver pseudo-reference electrode were purchased from Dropsens (Spain). Cyclic voltammetric measurements were applied in different potential windows for electropolymerization and detection measurement in PBS (pH 7.4). Electrochemical impedance measurements of electrodes were performed with the following parameters: frequency range 100 kHz–100 mHz in the presence of 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (in PBS, 0.1 M NaCl, pH 7.4), an alternative voltage of 10 mV in amplitude (peak-to-peak), at the dc potential of 0.13 V.

2.3. Electropolymerization of o-aminobenzoic acid (o-ABA)

The electropolymerization of *o*-aminobenzoic acid (*o*-ABA) at SPEs was achieved by applying potential cycles from 0 to 1.0 V, at a sweep rate of 50 mV s^{-1} in 1 M H_2SO_4, 0.1 M KCl solution containing 50 mM o-aminobenzoic acid for 15 cycles. After polymerization, the electrode was rinsed with 1 M H_2SO_4 and Milli-Q water to remove the excess monomer.

Optimization of *o*-aminobenzoic acid concentration and electropolymerization, as well as the results obtained by CV and EIS techniques were already reported in our previous study [38].

2.4. Fabrication of sandwich assay on poly-ABA/SPE

A schematic diagram of the construction of a poly-ABA-based biosensor and detection of MUC1 protein is shown in Fig. 1. After electropolymerization, the terminal carboxylic groups were activated by 0.4 M EDC and 0.2 M NHS solution prepared in 0.1 M PBS buffer (pH 5.0), in order to immobilize the primary antibody. The NHS/EDC mixture was added for 1 h to activate the carboxylic acid group of poly-ABA to N-hydroxysuccinimide ester. After rinsing with PBS buffer (pH 7.4), 8 μL of 40 ppm Ab1 solution in 0.1 M PBS buffer (pH 7.4) was incubated for 1 h, followed again by rinsing with PBS solution. To avoid any nonspecific binding, 10 mM