Talanta 128 (2014) 505–510

Contents lists available at ScienceDirect

Talanta

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

A thiophene-modified screen printed electrode for detection of dengue virus NS1 protein

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ARTICLE INFO

Article history:
Received 15 February 2014
Received in revised form
7 June 2014
Accepted 8 June 2014
Available online 14 June 2014

Keywords:
Screen printed electrode
Thiophene
Dengue virus
Immunosensor

ABSTRACT

A thiophene-modified screen printed electrode (SPE) for detection of the Dengue virus non-structural protein 1 (NS1), an important marker for acute phase diagnosis, is described. A sulfur-containing heterocyclic compound, the thiophene was incorporated to a carbon ink to prepare reproducible screen printed electrodes. After cured, the thiophene SPE was coated by gold nanoparticles conjugated to Protein A to form a nanostrutured surface. The Anti-NS1 antibodies immobilized via their Fc portions via Protein A, leaving their antigen specific sites free circumventing the problem of a random antibodies immobilization. Amperometric responses to the NS1 protein of dengue virus were obtained by cyclic voltammetries performed in presence of ferrocyanide/ferricyanide as redox probe. The calibration curve of immunosensor showed a linear response from 0.04 μg mL^{-1} to 0.6 μg mL^{-1} of NS1 with a good linear correlation ($r=0.991$, $p<0.05$). The detection limit (0.015 μg mL^{-1} NS1) was lower than conventional analytical methods. In this work, thiophene monomers incorporated in the carbon ink enhanced the electroanalytical properties of the SPEs, increasing their reproducibility and sensitivity. This point-of-care testing represents a great potential for use in epidemic situations, facilitating the early diagnosis in acute phase of dengue virus.

1. Introduction

Dengue is a significant public health threat, with estimates of 50 to 100 million cases per year and around 3 billion people at risk of infection, mainly in tropical and subtropical regions [1]. Infection can result in a broad spectrum of disease syndromes ranging from an asymptomatic or mild infection, classical dengue fever (DF), to the potentially fatal dengue haemorrhagic fever (DHF) and dengue shock syndrome [2]. So far, there is no effective anti-viral therapeutic on the market and supportive therapy such as fluid replacement is the only treatment for severe forms of the disease. An early and accurate laboratory diagnosis of dengue could assist clinical management [3]. An ideal blood test for diagnostic should be affordable and easy to use with high performance and sensitivity to distinguish the acute-phase of dengue [4]. Additionally, it should not be costly and not require several steps, being adaptable at laboratory or at a point-of-care diagnostic without compromising its accuracy [5].

The most important development in dengue diagnostics in recent years is the advent of the specific detection of dengue virus NS1 antigen [6]. Enzyme-linked immunosorbent assays (ELISA) for detecting the NS1 were developed and demonstrated excellent sensitivity and specificity in detection of dengue infections [6–8]. NS1 glycoprotein is circulating mostly from days 1–6 after the onset of clinical symptoms, with the peak NS1 antigen detection occurring between days 3 and 5, in both primary and secondary infections, and hence is an excellent diagnostic target for acute dengue diagnosis [4,8]. Although the classical techniques are very powerful for monitoring, they are time consuming and are not adaptable for in situ and real time detection, beyond require skilled personnel [9,10]. Alternatively, rapid diagnostic test (RDT) for NS1 detection based on immunochromatography was proposed [11]. However, even if RDTs can provide opportunities for point-of-care, they have limitations regarding detectability, once their results are limited to a qualitative analyses (yes/no), becoming difficult the diagnostic of the acute-phase of dengue that is correlated with NS1 levels. Contrary, biosensors can supplier quantitative responses through a transducer that converts biochemical reactions in a measurable electric signal [12].

Electrochemical biosensors employing screen printed electrodes have emerged as adequate tools for point-of-care testings. They have

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http://dx.doi.org/10.1016/j.talanta.2014.06.009
0039-9140/

innumerous advantages, such as ease of mass production and versatility [13]. Screen printed electrodes (SPEs) can combine good electrochemical proprieties and portability with simple and inexpensive fabrication techniques, thus being a good strategy to accomplish safety, disposable and quantitative immunonsensors [14]. In the fabrication of SPEs are used inks containing different chemical compounds, polymers or functional linking that can printed onto diverse type of plastic or ceramic substrates. The incorporation of compounds in the inks used for printing on the electrodes is a determinant factor for their selectivity and sensitivity required for each analysis [15].

Thiophene monomer derivatives have been pointed as attractive compounds to prepare electrochemical sensor, because they increase the conductivity, reduce the redox potential and improve the thermal and electrochemical stability [16]. Herein, thiophene monomers were incorporated into the carbon ink to form a homogenous and conductive composite, supplying a suitable signal amplification strategy to improve the electrochemical characteristics of the SPE increasing the sensitivity due to higher current densities and charge transfer across the interface electrode–electrolyte. Furthermore, sensitivity of immunosensors can be improved by increasing the amount of antibody immobilized on the electrode surface. Variety of nanostructures materials, with similar dimensions to biomolecules (antibodies, enzymes, DNA) owning different sizes, shapes and exceptional properties; such as metal nanoparticles (NP), quantum dots, carbon nanotubes and nanowires have employed for improvement of electrochemical biosensors. Nevertheless, NP which has capability for in situ synthesis onto the various composite films for antibody immobilization can improve the electrochemical signal and adsorption capacity of antibodies, and consequently enhance detection sensitivity. Therefore, the use of NPs represents a promising integration of electrochemical methods with new nanomaterials and electroactive complexes for electrochemical immunosensing [17].

It is well-known that way as antibodies are immobilized on the electrode surface affects the performance of an immunosensor. Fab portions of antibodies should be free for recognizing and binding to the epitopes of antigens. The Protein A extracted from Staphylococcus aureus has high affinity to the Fc portion of immunoglobulins from a variety of species, being widely used to promote an oriented antibody immobilization [18]. When the Protein A was used in a chromatographic assay, it was capable of binding antigen at over 80% of their theoretical capacity, because of the increased strength of the couple between the antibody Fc portion and protein A [19]. Stable and oriented immobilization of antibodies combined with the electrochemical advantages of thiophene as chemical modifying compound allowed a accurate detection of NS1. No labels were necessary when the antigen–antibody interactions were registered. The method described herein involves one-step preparation process and represents an advance in the production of SPEs for point-of-care testing.

2. Experimental

2.1. Materials and reagents

2.1.1. Chemical reagents and materials

Electrodag PF-407 C carbon ink with a density of 1.13 kg cm^{-3} was acquired from Acheson Henkel Corporation (Port Huron, MI, USA). Thiophene, protein A–conjugated gold nanoparticles (PtnA–AuNP) with approximately 20 nm (P6855), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$) and glycine, were acquired from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer saline (PBS) (10 mmol L^{-1}, pH 7.4) used in all experiments was prepared by dissolving 0.2 g KCl, 8.0 g NaCl, 0.24 g KH_2PO_4 and 1.44 g Na_2HPO_4, in 1000 mL of deionized Milli-Q water from Millipore units (Bedford, MA, USA). All chemicals were of analytical grade.

2.1.2. Biological reagents

Mouse monoclonal antibodies against the NS1 glycoprotein of dengue virus (ab 138696) used to electrodes preparation and the dengue virus NS1 recombinant full-length protein (ab 64456) were purchased from Abcam (Cambridge, MA, USA). NS1 native protein was obtained from DENV-3 (strain 101.905/BR-PE/03) culture supernatant collected on the 5th day after inoculation in C6/36 cell monolayers, maintained in Leibovitz L-15 medium (GIBCO, Invitrogen, Grand Island, NY) containing 2% fetal calf serum. DENV-3 was detected and identified by RT-PCR [20]. In house ELISA, using anti-NS1 monoclonal antibodies, confirmed the presence of NS1 native protein in virus culture supernatant. As control was used supernatant from C6/36 cell culture (without virus) collected in the same conditions. Both supernatants were cleared by centrifugation for 10 min at 1.500 rpm (400 g).

2.2. Preparation of the thiophene-SPE

The electrodes were manufactured by squeezing a mixture containing carbon ink and thiophene onto a polyethylene terephtalate support to form a think conductive film. Four different concentrations of thiophene in relation to carbon ink were tested: 0.5% (w/V); 1% (w/V); 2.5% (w/V) and 10% (w/V). Prior printing, a plastic mold was used onto the PET rectangular surface (0.4 × 1.0 cm) to ensure electrodes with equal areas. After manufacturing, the electrodes were cured at 60 °C for 20 min. The manufactured thiophene-SPE consisted of with a circular area (Ø=4 mm) joined to a rectangular area (1 mm × 15 mm) used to electrical contact. After ready, the area of the electrode was delimited using a tape for galvanoplasty.

Prior to use, the thiophene-SPEs were pretreated by cyclic voltammetry (CV), scanning 30 cycles with a potential ranging from −2.0 V to 2.0 V, at a scan rate of 0.1 V s^{-1} and step potential of 2.44 mV, using 0.1 mol L^{-1} of KCl solution as the supporting electrolyte [21].

2.3. Apparatus

All the electrochemical experiments were performed in an Ivium Compact Stat potentiostat/galvanostat from Ivium Technologies (Eindhoven, The Netherlands) interfaced with a microcomputer and controlled by Ivium Soft software. A three-electrode system was used, which consisted of a thiophene-SPE as the working electrode (4 mm diameter), an Ag/AgCl electrode as the reference electrode and a helical platinum wire as the counter electrode. The electrodes were set up in a glassy electrochemical cell with 5 mL volume.

Experiments for characterizing the assembling of the thiophene-SPE were performed by CV in presence of 5 mmol L^{-1} of K3Fe(CN)6/K4Fe(CN)6 prepared in 0.1 mol L^{-1} of KCl solution, with a potential ranging from −0.6 V to 1.0 V, at 50 mV s^{-1} scan rate. Antigen–antibody interactions at the interface of the thiophene-SPE were also monitored by differential pulse voltammetry (DPV). DPV measurements were recorded from 0 V to 0.8 V, with pulse amplitude of 0.025 V, width of 0.05 s, and step potential of 0.05 V. The current signals were registered at a fixed potential (0.25 V) and the analytical response to NS1 was obtained taking into account the difference between the peak current (ΔI) of the thiophene-SPE with NS1 and the blank.

Fourier transform infrared (FTIR) spectra of samples were recorded by using a Bruker IFS 66 model FTIR spectrometer in