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Biosensor based on lectin and lipid membranes for detection of serum glycoproteins in infected patients with dengue

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ABSTRACT

In this work, we developed a biosystem based on Concanavalin A (ConA) and lipid membranes to recognize glycoproteins from the serum of patients contaminated with dengue serotypes 1, 2 and 3 (DENV1, DENV2 and DENV3). The modified gold electrode was characterized using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and atomic force microscopy. Morphological analyses of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), DPPC-ConA, DPPC-ConA-DENV1, DPPC-ConA-DENV2 and DPPC-ConA-DENV3 revealed the existence of a non-uniform covering and large globules. EIS and CV measurements have shown that redox probe reactions on the modified gold electrodes were partially blocked due to the adsorption of lipid-ConA system and reveal the interaction response of the immobilized ConA to the presence of glycoproteins of dengue serum. The biosystem exhibited a wide linear response to different concentrations of sera of dengue serotypes 1, 2 and 3. A higher impedimetric response to glycoproteins present in dengue serotype 3 was observed. Our results demonstrate the applicability of lectin and lipid membranes to the development of biosensors for dengue infections.

1. Introduction

Dengue virus (DENV) is considered a global public health problem and approximately 40% of the world population is at risk of contracting this infection (Organization, 2009). The four serotypes of the dengue virus (DENV1, DENV2, DENV3 and DENV4) are transmitted to humans and cause two clinical syndromes: classical dengue fever (DF) and hemorrhagic fever/dengue shock syndrome (DHF/DSS) (Gubler, 2002; Guzman and Kouri, 2002). The viral particles consist of the three structural proteins (capsid, premembrane and envelope glycoprotein) and the seven nonstructural ones (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) important for dengue virus replication (Lindenbach and Rice, 2001).

Among the techniques available for the diagnosis of dengue infections, reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM antibody capture enzyme immunoassay (MAC-EIA) have been commonly used (Guzman and Kouri, 2004). The RT-PCR rapidly detects the viral RNA of all serotypes from the patient's serum in the viremia phase while MAC-EIA is suitable for distinguishing between primary and secondary dengue infections based on the IgM/IgG ratio, despite not providing a specific diagnosis for the dengue virus, which can be easily confused with other febrile illnesses. The combination of these two approaches is more appropriate for accurately detecting dengue (Poersch et al., 2005). Despite the individual benefits of each method, both have limitations, such as high technological support demands and possible false negative results (Teles, 2011).

Currently, new methods for dengue diagnosis are required to provide specific and rapid detection in real-time, early states of the disease, allied to lower cost. Such methods would enable an appropriate protective management, surveillance and future immunization strategies to restrain the spread of the disease. In addition, the clinical symptoms are often confused with a variety of viral and bacterial diseases, including other flaviviruses infections such as West Nile virus, Japanese encephalitis virus, yellow fever virus, tick-borne encephalitis virus, Murray Valley encephalitis virus and St. Louis encephalitis virus (Gould and Solomon, 2008; Ross, 2010).

In this context, point-of-care biosensors can provide a good performance in diagnosing for high sensitivity and selectivity, handling

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facility and rapid detection (Seah et al., 1995). Electrochemical impedance spectroscopy and cyclic voltammetry are effective techniques to investigate changes in the electrode/electrolyte interface in the development of biosensors for real samples (Mantzila et al., 2007; Prodromidis, 2010). Electrochemical biosensors have also been used in the development of virus biosensors (Caygill et al., 2010), including the dengue virus (Binh et al., 2012; Cavalcanti et al., 2012; Cheng et al., 2012; Fang et al., 2010).

The use of carbohydrate recognition in the development of biosensors is the key point in the identification of various pathologies (Zeng et al., 2012). It is known that in the early stages of dengue virus infection there is an increased expression of specific glycoproteins in the serum (Xu et al., 2006; Dussart et al., 2006). Recently, lectin-based biosensors obtained by us have shown distinct patterns of response for glycoproteins in the serum of patients infected by DENV1, DENV2 and DENV3 (Andrade et al., 2011). In addition, a biosensor for patients with DF and DHF has also been developed by our group (Oliveira et al., 2009).

Concanavalin A (ConA), extracted from jack bean seeds (*Canavalia ensiformis*), is one of the most widely studied lectins. At pH 7.4 it exists as a tetramer with a higher degree of organization, molecular weight of 110 kDa and an overall size of 6.7 nm × 11.3 nm × 12.2 nm (Ballerstadt et al., 2006; Bouckaert et al., 1996). It is known that under neutral conditions each subunit of ConA contains one binding site for glucose- and mannose-specific carbohydrate structures, one for calcium and manganese cations, which activate the binding site of protein for carbohydrates, and a third one for hydrophobic recognition (Liu et al., 2007). Thus, ConA is extensively used in protein-carbohydrate interaction (Pei et al., 2005; Mori et al., 2009; Pedroso et al., 2008), including studies for the diagnosis of dengue (Andrade et al., 2011; Pereira et al., 2008).

In addition, the use of lipid molecules with biosensors reduces the interference of electroactive species, contributes to signal amplification and, furthermore, creates a native environment for protein immobilization and protection from denaturation (Sofou and Thomas, 2003; Ramsden, 1998). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) is one of the phospholipids commonly found as constituents of cells and organelles and is conventionally used in biosensors (Yamamoto et al., 2010; Puu et al., 2000).

In this study, we developed a novel electrochemical biosensor based on ConA lectin and phospholipid membrane association for the specific identification of glycoproteins present in the serum of infected patients with dengue (DENV1, DENV2 and DENV3). The biosensor was characterized by means of cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM).

2. Experimental

2.1. Materials

Concanavalin A lectin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were obtained from Sigma Chemical Co. (St Louis, USA) and used as received. The dengue sera were available in the Laboratory of Virology/FAMERP stocks. In this work we used the sera of patients contaminated with dengue serotypes 1, 2 and 3 (three patients of each serotype) and serum controls (three patients). All sera were previously characterized as patient dengue negative (serum control) and dengue positive (serotypes 1, 2 and 3) using RT-PCR (Mondini et al., 2007). Phosphate buffer saline (PBS) (pH 7.4) containing 0.1 M $CaCl_2$ and 0.1 M $MnCl_2$ was used to prepare the ConA solutions. The ultrapure water was prepared with a purification system (Millipore-Synergy). All chemicals and solvents were of analytical grade and used as received, without further purification.

2.2. Preparation of lipid vesicles

Liposomes were prepared based on a lipid film formed by the evaporation of solvents (Amselen et al., 1990; Andrade et al., 2004). Liposomes were obtained using 1 mM DPPC dissolved in chloroform/methanol solution (3:1, v/v) under magnetic agitation (150 rpm/min, 15 min). Next, the organic solution was submitted to evaporation under reduced pressure (25 min at 40 ± 1 °C) and agitation at 120 rpm to remove solvents. Subsequently, the dried lipid film was hydrated through the addition of 1 mL of 0.2 M PBS (pH 7.4) and kept under magnetic stirring (40 min). Finally, the suspension of liposomes was stored at 4 °C until use.

2.3. Preparation of the biosensor system

The bare gold electrode surface was freshly polished with 0.05 μm α-Al_2O_3 paste, rigorously rinsed with ultrapure water and cleansed ultrasonically in deionized water for 5 min. After this process, the lipid-modified electrode was obtained by dropping liposome solution onto the bare gold electrode surface at 25 °C for 15 min. After the first immobilization, the modified electrode was rinsed with water to remove non-adsorbed lipid, incubated into 1 mL of the ConA (50 μg/mL) in 10 mM PBS (pH 7.4) for 15 min and rinsed with water to remove excess of the ConA. Finally, the lipid-ConA-modified electrode was exposed to negative serum control or serum from patients infected by DENV1, DENV2 and DENV3 diluted in 10 mM PBS solution (pH 7.4) for 20 min at room temperature. The serum dilutions used in this study were 1:10, 1:250, 1:500, 1:1000, 1:1500 and 1:2000.

2.4. Impedance measurements

Electrochemical measurements were performed using a three-electrode system containing an Ag/AgCl as reference electrode, platinum as a counter electrode and a bare gold electrode as the working electrode (ϕ = 2 mm). All the experiments were carried out on a PGSTAT 128 N potentiostat (Autolab, Eco Chemie, The Netherlands), interfaced with an analyzer controlled by a computer. The impedance spectra were then recorded in the frequency range of 100 mHz to 100 kHz. The amplitude of the applied sine wave potential (SWP) was 10 mV, while the direct current (dc) potential was limited at the open circuit potential measured just before the application of the SWP. CV measurements were performed with a potential sweeping between −0.2 to 0.7 V at a scan rate of 50 mV s^{-1}. CV and EIS measurements were carried out at different stages of the preparation of the modified electrode and performed in the presence of 10 mM $K_4[Fe(CN)_6]^{4-}$/$K_3[Fe(CN)_6]^{3-}$ (1:1) solution (used as a redox probe) in PBS (pH 7.4). All electrochemical measurements were performed in triplicate using at least three different sensors at room temperature inside a Faraday cage.

2.5. Atomic force microscopy measurements

Atomic force microscopy (AFM) measurements were performed using a commercial PicoSPM II microscope (Molecular Imaging, USA). Cantilevers with a silicon AFM probe (Multi 75AL, NCHR, resonant frequency = 75 kHz, force constant = 3 N m^{-1}) were used for the noncontact mode AFM in air at room temperature (approximately 25 °C). Lateral resolution was set at 512 × 512 pixels in a scan area of 5 × 5 μm. Images were obtained from at least three macroscopically separated areas to ensure a good distribution and analyzed using AFM Gwyddion software (Necas et al., 2008).