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Surface plasmon resonance characterization of monoclonal and polyclonal antibodies of malaria for biosensor applications

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ABSTRACT

Surface plasmon resonance (SPR) screening of monoclonal and polyclonal antibodies of *Plasmodium falciparum* (Moab*Pf* and Poab*Pf*) for recombinant Histidine rich protein-II antigen (Ag) of P*f* (rHRP-II Ag) was conducted in a real-time and label-free manner to select an appropriate antibody (Ab) for biosensor applications. In this study 4-mercaptobenzoic acid (4-MBA) modified gold SPR chip was used for immobilizing the Ag and then Ab was interacted. SEM image showed modification of SPR chip with 4-MBA and EDAX confirmed the presence of 4-MBA on the SPR chip. Equilibrium constant (K_D) and maximum binding capacity of analyte (B_{max}) values for the interaction of Moab*Pf* or Poab*Pf* with the immobilized rHRP-II Ag were calculated and found to be 0.517 nM and 48.61m° for Moab*Pf* and 2.288 nM and 46.80m° for Poab*Pf*, respectively. In addition, thermodynamic parameters such as ΔG, ΔH and ΔS were determined for the interaction between rHRP-II Ag and Moab*Pf* or Poab*Pf* and the values revealed that the interaction is spontaneous, exothermic and driven by entropy. The kinetics and thermodymanic results of this study revealed that the interaction between Moab*Pf* and rHRP-II Ag is more effective than that of Poab*Pf* due to the fact that Moab*Pf* was derived from a single epitope (single clone) whereas the Poab*Pf* was from the mixture of a number of epitopes (polyclones). Finally, SPR methodology was developed for the sensing of malarial antibodies. The limit of detection was found to be 5.6 pg with Moab*Pf* which was found to be the best in our study.

1. Introduction

Malaria is an infectious disease and this is due to the parasite *Plasmodium falciparum* (*Pf*). The world malaria fact sheet report (World Health Organization [WHO] report) of 2011 reveals data received from 104 malaria-endemic nations and territories for the year 2011. The data from 99 of these countries indicates about the on-going nature of malaria transmission. Moreover, the data indicates about the prevention of 1.1 million malaria deaths between the years 2000 and 2010 due to prior interventions. The world malaria report 2012 released by WHO (2012) indicates about an estimated number of 247 million human malarial infections (98% in Africa with 70% being in the age of 5 years or younger). In addition, in the year 2010 the WHO estimate reveals about 219 million cases of malaria and also about 0.66 million deaths. Africa is the most affected continent: about 90% of all malaria deaths occur there. Malaria is more prevalent in sub-Saharan Africa when compared to other regions of the world; in most African countries, more than 75% of cases were due to *Pf*, whereas in other countries the malaria transmission is predominantly due to less virulent plasmodial species. Almost every malarial death is caused by *Pf* (WHO world malaria report 2012 and www.unicef.org).

Pf is one of the species of *Plasmodium* and it is a protozoan parasite which is responsible for malaria in humans. *Pf* is transmitted by the female Anopheles mosquitos. Malaria induced by *Pf* (also called malignant or falciparum malaria) is the most dangerous form of malaria with the highest rates of complications and mortality (Rich et al., 2009; Perkins et al., 2011). More than 120 species of the parasite genus *Plasmodium* are available; however, only four of them infect humans to cause malaria (*P. falciparum, P. vivax, P. ovale and P. malariae*) and among these only *Pf* can cause severe (life-threatening) malaria when compared to the other three species (Coppel et al., 1986). *Pf* entry to the human blood cell leads to the change of shape of red blood cell within 48 h of asexual blood stage cycle; the mature forms change the surface properties of infected red blood cells, and make them stick on blood vessels. This induces the obstruction of microcirculation and results in dysfunction of multiple organs, including the brain in cerebral malaria (Perlmann and Troye-Blomberg, 2000;

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Dondorp et al., 2004). Histidine-rich protein-II (HRP-II) is a naturally occurring histidine and alanine rich protein and is localized in several cell compartments, including the cytoplasm of *Pf*, and it is closely associated with the development and proliferation of the parasite and therefore is perfectly suited to reflect growth inhibition as a measure of drug susceptibility (Harald et al., 2002). Five malaria proteins (HRP1, HRP2, EMP1, EMP2, and EMP3) have been identified on the surface or in association with the cytoskeleton of erythrocytes infected with *Pf* (Rock et al., 1987). HRP-II was identified in every *Pf* parasites regardless of knob phenotype, and was recovered from culture supernatants as a secreted water-soluble protein (Panton et al., 1989). HRP-II may also participate in parasite mature stages evasion of the immune system and their subsequent destruction in the spleen (Rock et al., 1987). HRP-II is produced and secreted by the parasite during its growth and development (Howard et al., 1986). There is evidence for an intracellular route of transport for the malarial protein from the parasite through several membranes and the host cell cytoplasm (Howard et al., 1986; Magowan, 2000).

In view of the above, timely detection of antibodies of HRP-II Ag to know about the malarial outbreak is very vital to save the life of humans for taking appropriate medical measures. Many methods are currently available in the literature for the diagnosis of malaria by detection of HRP-II Ag with dipstick antigen-capture assay or enzyme-linked immunosorbent assay (ELISA) (Beadle et al., 1994; Gaye et al., 1998). Moreover a number of methods are also developed for the detection of *Pf*, including various biological assays like immune fluorescence microscopy (IFA) (Matile and Pink, 1990), fluorescence microscopy (Lenz et al., 2011), western blot analysis (Parra et al., 1991), ELISA (Wirtz et al., 1989), competitive ELISA (Bualombai et al., 1990), dot blot analysis (Lee et al., 2006), and also a method based on isothermal titration calorimetry (ITC) (Pierce et al., 1999), polymerase chain reaction (PCR) (Patsoula et al., 2003; Pieroni et al., 1998) and surface plasmon resonance (Helg et al., 2003) using merozoite surface protein-1. Though many methods are reported for malaria detection, still methods development and improvisation are vital to avoid labeling which is required with enzyme substrate, much volume of samples requirement, time consuming nature and also non-availability of kinetic and thermodynamic data for most of the malaria antigen (Ag)–antibody (Ab) interaction to know about the nature of reaction. In view of the above, there is a need for the development of immunosensors which are highly specific for Ag–Ab interaction for identification and quantification of specific analytes. SPR immunosensors have recently attracted a lot of attention due to their high sensitivity, real time and label-free monitoring capability of biological interactions (Toyama et al., 1998).

In SPR the interaction of a biomolecule immobilized on the SPR chip surface with its counterpart in solution is monitored without any labeling of the biomolecules by using the interfacial refractive index changes associated with the affinity binding interactions (Myszka, 1999). The important parameters that can be obtained with the help of SPR include protein binding (Ahmad et al., 2003), association/dissociation kinetics (Nordin et al., 2005), and affinity constants (Babol et al., 2005) and these contributed a role of SPR in large application areas such as molecular engineering (Calender, 2006), food analysis (Sternesjo et al., 1995), clinical diagnosis (Inamori et al., 2005), proteomics (Natsume et al., 2002), environmental monitoring (Dillon et al., 2003), bacteriology (Mader et al., 2004), virology (Athmaram et al., 2014), cell biology (Quinn et al., 2000), drug discovery (Cimitan et al., 2005) and warfare agent detection (Gupta et al., 2011a).

In line with our earlier studies on the development of SPR detection methodologies for biological warfare agents (BWAs) such as *Brucella abortus, Salmonella typhi* and *Staphylococcal enterotoxin B* (Gupta et al., 2011b, 2012, Singh et al., 2010), in the present work we employed SPR for the characterization of Moab*Pf* and Poab*Pf* and also for the direct detection of monoclonal and polyclonal antibodies (Moab*Pf* and Poab*Pf*) of recombinant histidine rich protein antigen (rHRP-II Ag) in buffer, using rHRP-II Ag immobilized on a 4-mercaptobenzoic acid (4-MBA) modified gold surface as (to our knowledge) no SPR based detection of rHRP-II Ag antibodies is available in the literature. The main advantage of using 4-MBA for modification of SPR chip is to get a lesser thickness and faster electron transfer on the chip when compared to conventionally used dextarn modified SPR chip as it is well known that SPR response depends on the thickness of the modification on the SPR chip. (Schasfoort and Tudos, 2008: Mendesa et al., 2004). Moreover, parameters affecting the response of SPR were optimized and finally affinity constant (K_D) and maximum binding capacity of analyte (B_{max}) were calculated; in addition, thermodynamic parameters such as change in Gibb's free energy (ΔG), change in enthalpy (ΔH) and change in entropy (ΔS) involved in the interaction between rHRP-II Moab or rHRP-II Poab with rHRP-II Ag of *Pf* were also deduced in this study.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS), sodium acetate, ethanolamine and hydrochloric acid (HCl) were of Fluka grade and obtained from Sigma-Aldrich, Bangalore, Karnataka, India. Moreover, glacial acetic acid, glycine, sodium hydroxide (NaOH) and methanol (MeOH) were supplied by Sigma-Aldrich, Bangalore, Karnataka, India. 4-MBA Aldrich purchased from Sigma-Aldrich, Bangalore, Karnataka, India, was used for the modification of the SPR gold chip (Xantech Bioanalytics GmbH, Metrowingerplatz, Germany). Moab*Pf*, Poab*Pf* and rHRP-II Ag of *Pf* were developed in house by the trained biologists. All chemicals and reagents used in this work were of analytical grade and purification was done wherever necessary before use.

In order to perform SPR measurements, 4-MBA modified SPR gold disc was used. Different buffer solutions were used in this study depending on pH [acetate buffer (pH 4.0–5.5), PBS (pH 6.0–7.5) and glycine–NaOH buffer (pH 8.0–9.0)] for the optimization of pH. All solutions were prepared using water from a Milli-Q system (Millipore India, Bangalore, Karnataka, India) throughout the experiment.

2.2. Instruments

The biomolecular interactions were investigated using a two channels cuvette based electrochemical surface plasmon resonance system (Autolab ESPRIT, Ecochemie B.V., Utrecht, The Netherlands) and a diode laser was a source with a fixed wavelength of 670 nm in conjunction with a scanning mirror to modulate the plane polarized light beam on the SPR substrate. The outcome of the SPR measurement was monitored using a PC with data acquisition software version 4.3.1.

2.3. Preparation of 4-MBA modified gold chip

Bare gold SPR chip was modified with 0.01 M 4-MBA using a spin coater (Autolab Spin coater, Ecochemie B.V., Utrecht, The Netherlands). First 75 µl of 4-MBA was dispensed on bare gold chip at 100 rpm. After this, spin speed was increased up to 2500 rpm and kept for 5 min in order to spread the liquid on