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Surface plasmon resonance detection of biological warfare agent Staphylococcal enterotoxin B using high affinity monoclonal antibody

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ABSTRACT

A novel sensitive method was developed for the detection as well as quantification of Staphylococcal enterotoxin B (SEB) using surface plasmon resonance (SPR). It is well known that the amount of SEB needed to cause the intoxication to human beings is very less and this concentration (0.02 μg/kg) is highly dangerous, hence, it is used as biological warfare agent. Thus, the need to develop a reliable and potential detection system against SEB is warranted. In the present work, SEB antibody was immobilized on carboxymethyldextran modified gold chip. The immobilization of SEB antibody and interaction of antigen with immobilized antibody were *in-situ* characterized by SPR and electrochemical impedance spectroscopy. A sample solution containing SEB antigen was injected in a working channel and the results revealed linearity in the concentration from 2.0 to 32.0 pM with a detection limit of 1.0 pM. By using kinetic evaluation software, K_D (equilibrium constant) and Bmax (maximum binding capacity of analyte) values were calculated and found to be 13 pM and 424.23, respectively. Moreover, the thermodynamic parameter, change in Gibb's free energy was deduced and found to be −62.08 kJ/mol and this value shows the spontaneous interaction between SEB antigen and SEB antibody. In order to optimize the detection method, temperature and pH variation studies were also performed. Interference study was conducted to know the selectivity for the antigen–antibody interaction of SEB. The selectivity efficiency of SEB, SEC, SEA and SED were 100, 27.15, 20.01 and 12.05%, respectively towards SEB antibody.

1. Introduction

Staphylococcus aureus is a major human pathogen that produces numerous toxins including the pyrogenic toxin Staphylococcal enterotoxin (SEs). SEs are responsible for causing gastroenteritis in human beings [1,2]. SEB is from a family of nine major serological types of emetic enterotoxins. SEB is the most heat stable of this group and it is also resistant to the proteolytic enzymes of the gastrointestinal tract in addition to its stability in low pH. SEB can cause nausea, vomiting, diarrhea and anaphylactic shock if it is ingested. After aerosol exposure, symptoms experienced are sudden onset of fever, chills, headache and cough. The fever may last for several days and the cough may persist for up to four weeks. Very high exposure levels may also lead to pulmonary edema. SEB is an incapacitating toxin, but it is rarely lethal and the amount of SEB required to cause the intoxication is very less and this concentration is highly dangerous, hence, it is used as biological warfare agent (BWA). The estimated 50% lethal dose (LD_{50}) in human is 0.02 μg/kg of SEB by aerosolized exposure [3]. Thus, developing newer detection methodologies for SEB detection is unavoidable in addition to the existing immunological testings such as ELISA [4–10], piezocrystal immunosensors [11,12], time resolved fluorescence assay [13], gold nanoparticle based immunoassays [14,15], commercially available kits [16,17], PCR [18–20], electrochemical immunosensors [21,22] and magnetoelastic immunosensor [23]. However, most of the above immunological methods are having limitations such as relatively slow response, labelling, non-specificity and cross reactivity and these parameters make uncertainties in the definite identification of SEB. Moreover, other methods based on mass spectrometry [24], chromatography [25], CNT [26] and real time label free detection methodologies based on SPR were also reported in the literature [27–29]. However, in the earlier reported SPR based detection methods for SEB the authors did not characterize the modification and antigen–antibody interactions using other techniques. Moreover, they did not conduct interference studies with other types of SE toxins and also not report about kinetic and thermodynamic parameters related to SEB antigen–antibody interactions. In this study, we have calculated kinetic and thermodynamic parameters and also conducted interference study with other types of SE toxins. In addition, we have also conducted electrochemical impedance characterization of the modification and interaction of SEB antigen with immobilized SEB antibody.

Our establishment is working for the defences against biological toxicants and warfare agents [30–37], hence, in the present study we report a label free real time SPR sensing optical method for the

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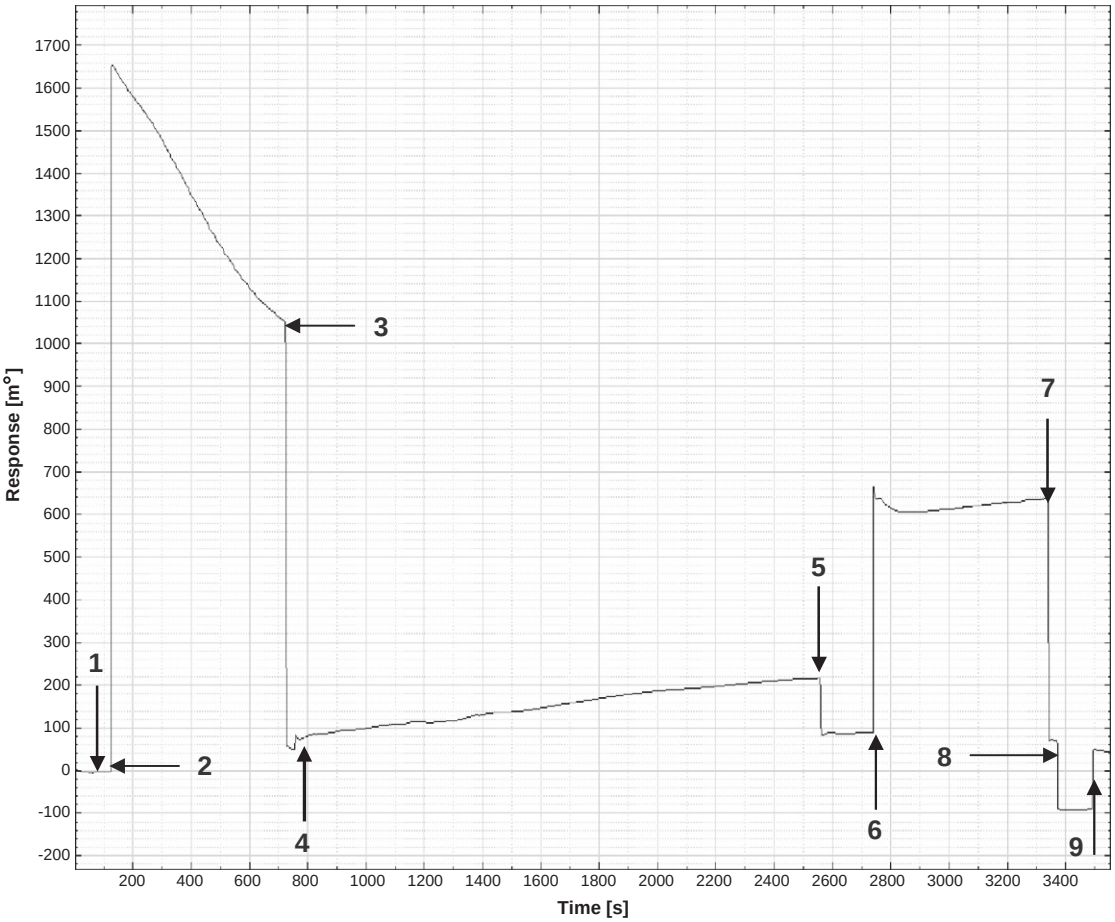

Fig. 1. Sensorgram showing different steps [(1) Baseline, (2) EDC-NHS activation, (3) Washing, (4) Antibody coupling, (5) Washing, (6) Deactivation, (7) Washing, (8) Regeneration and (9) Back to baseline] involved in the immobilization of SEB antibody (1 ng/ml) on the carboxymethyldextran modified gold chip.

detection of BWA SEB by using a carboxymethyldextran modified gold chip. The experimental parameters that affect SPR angle change such as temperature and pH were also optimized. Finally, K_D, ΔG and Bmax values were also deduced using kinetic evaluation software.

2. Experimental

2.1. Chemicals and reagents

N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS), bovine serum albumin (BSA) and hydrochloric acid (HCl) were procured from Fluka. Citric acid, boric acid, tween-20, sodium hydroxide, dipotassium hydrogen phosphate and potassium dihydrogen phosphate were supplied by Sigma-Aldrich. 5, 5′-Diethylbarbituric acid was received from Merck. All chemicals and reagents used were of analytical grade and purification was performed wherever necessary before use. 0.05 M phosphate buffer (pH 8.0) was used as coupling buffer in the experiments and dilution of SEB antibody was also carried out using this buffer.

SEB (M. wt. 30 kDa), SEA (M. wt. 29 kDa), SEC (M. wt. 29.7 kDa) and SED (M. wt. 26.2 kDa) antigens were prepared by cloning and expression of *seb*, *sea, sec* and *sed* genes, respectively [38] and the monoclonal SEB antibody was raised by developing hybridoma [39] by the trained biologists in our establishment. Carboxymethyldextran modified gold chip with a thickness of 20 nm used for SPR measurements was purchased from Xantec Bioanalytics (Germany). Universal buffer (a mixture consisting of citric acid, boric acid, potassium dihydrogen phosphate and 5, 5′-diethylbarbituric acid) of pH from 4.0 to 9.0 (at 25 °C) was used in this study for the optimization of pH. All solutions were prepared using water from a Milli-Q system throughout the experiment.

2.2. Instruments

The biomolecular interactions were conducted using a two channel cuvette based electrochemical surface plasmon resonance system (Autolab ESPRIT, Ecochemie B.V., The Netherlands). The outcome of the SPR measurement was automatically monitored using a PC with data acquisition using the SPR software version 4.3.1 and all kinetic data were obtained using the SPR kinetic evaluation software version 5.0 (Ecochemie B.V.). Electrochemical studies were performed in the ESPR cuvette cell coupled to a three electrode system. The gold chip was the working electrode, a Ag/AgCl (saturated KCl) was the reference electrode and a platinum was the counter electrode. For EIS studies, a FRA II module with FRA software 4.9 (Ecochemie B.V., The Netherlands) was used. The pH of the buffers was measured with a EUTECH instrument pH meter (pH-1500, Singapore). All experiments were carried out at 25 °C unless otherwise specified and the temperature of cuvette was controlled by a Julabo HE-4 (Germany) water bath.

2.3. Immobilization of antibody on the carboxymethyldextran modified gold SPR sensor chip

Prior to the immobilization of antibody on the carboxymethyldextran modified gold chip, 50 μL of 0.05 M phosphate buffer (pH 8.0) was passed every 120 s interval for 600 s in order to get a stable