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Amperometric immunosensor for detection of antibodies of *Salmonella typhi* in patient serum

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Abstract

An amperometric sensor for detection of antibodies to *Salmonella typhi* in the serum of patients was developed. This involved usage of screen-printed electrodes and recombinant flagellin fusion protein. An indirect enzyme-linked immunosorbant assay was used for detection of antibodies to *S. typhi* in the patient serum. The screen-printed electrodes were made using polystyrene and graphite. These electrodes were tested for their ability to detect 1-naphthol, which is the product formed due to the hydrolysis of the substrate 1-naphthyl phosphate by the enzyme alkaline phosphatase. These electrodes were coated with recombinant flagellin fusion protein made by recombinant DNA technology and blocked with bovine serum albumin (BSA). Further they were incubated with patient serum and goat anti-human alkaline phosphatase conjugate. The immunosensing was performed by using amperometric method. Pooled human serum samples from apparently healthy individuals were used as control. Both the pooled healthy human serum samples and patient sera were subjected to Widal agglutination test and amperometric method. A 100% correlation was found between the Widal test and amperometric method. The time taken for the detection by electrochemical method is 1 h and 15 min, while the time taken by Widal test is 18 h.
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Keywords: Immunosensor; Typhoid; Screen-printed electrode; Amperometry; *Salmonella typhi*; Serum

1. Introduction

The typhoid disease caused by *Salmonella typhi* is more common in developing countries than in the developed ones. It is possible to diagnose this disease either by culture isolation from clinical specimens or by the detection of antibodies in the patient's serum. The culture isolation and further characterisation of biochemical tests take very long time, hence the detection was done by detecting the antibodies of *S. typhi*, produced in the patient serum. Several reports appeared for detection of these antibodies by using various methods, which include Widal agglutination test [1], bentonite flocculation test [2], antiglobulin haemagglutination test [3], indirect haemagglutination test [4], latex agglutination test [5], counter immunoelectrophoresis [6] and enzyme-linked immunosorbant assay (ELISA) [7–10]. However, no immunosensor was reported for detection of antibodies of *S. typhi* in patient sera.

The detection of antibodies of *S. typhi* is done by well-known Widal agglutination technique [1]. It takes nearly 18 h and has several disadvantages such as lack of specificity and sensitivity [11]. In our present studies, we used a highly specific recombinant flagellin fusion protein to detect typhoid antibodies in the patient sera samples. The recombinant technology allows production of antigens and antibodies with good reproducibility [12]. The antigens or antibodies produced by recombinant technology were used in developing immunosensors [13–15].

The electrochemical methods in immunosensing have become very popular recently [16–28]. Various types of electrodes such as glassy carbon electrode [23], screen-printed electrodes [24–27] and carbon paste electrodes were reported. We used screen-printed electrodes (SPEs), in these studies

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and the immunosensing is achieved by using amperometric method. The advantages with SPEs are that they are cheap and can be mass-produced. Few reports [28–30] are available for the direct detection of some of the *Salmonella* species by employing immunosensors. But reports are not available for detection of either *S. typhi* or its antibodies. The detection of antibodies against *Salmonella enteritidis* and *Salmonella typhimurium* in chicken serum by surface plasmon resonance [31] was reported. Compared to this technique, the electrochemical techniques allow a much cheaper method of detection. In this paper, we present our results on immunosensing by amperometric method, which was found to be very fast compared to Widal agglutination test. The details of SPEs and optimisation of various parameters for detection have been presented, in this communication.

2. Experimental

2.1. Apparatus

The electrochemical experiments were performed with the help of PARC 370 system comprising Model 273A Potentiostat/Galvanostat and PARC software. For amperometric experiments, Philips recorder Model No. PM 8043 (Germany) was used. The homemade screen-printed electrodes served as working electrodes. Saturated Ag/AgCl electrode and a platinum wire were used as reference and counter electrodes, respectively. The stirrer model no. TH100 supplied by Spectralab (India) was used.

2.2. Reagents

Polystyrene was obtained from local sources. Eltecks (Bangalore, India) supplied graphite powder, and the dielectric ink. 1-Naphthyl phosphate, purified grade was obtained from Lancaster, UK. 1-naphthol was obtained from SD-Fine Chemicals (India) and was used without further purification. Goat anti-human polyvalent alkaline phosphatase conjugate (Sigma catalog No. A50) was received from Sigma, (St. Louis, Mo, USA). Ethanoldiamine (EDA) was obtained from Acros Organics and Tris–HCl buffer (analytical-reagent grade) was obtained from HiMedia (India). Magnesium chloride of G.R. grade was obtained from Sarabhai M Co.

2.3. Preparation of flagellin of Salmonella typhi by recombinant DNA technology

S. typhi specific flagellin fusion protein was used as antigen to detect the typhoid antibodies in the patient and normal control serum samples. Flagellin protein was prepared using recombinant DNA technology [32]. The procedure for preparation of the antigen is described briefly here. *S. typhi* flagellin gene sequence was amplified by polymerase chain reaction and ligated into the pVNLGST env 185A 1140 cloning and expression vector. This vector allowed production of recombinant protein as fusion with glutathione-S-transferase (GST). *Escherichia coli* strains DH5α and BL21 (λDE3) were used for the transformation and expression of GST fusion protein, respectively. The expressed flagellin was purified using GST-sepharose column (Amersham Pharmacia Biotech, USA) as per the protocol of the manufacturer and the protein content was determined by Bradford method [33] and found to be 900 μg/ml.

2.4. Collection of patient samples

Serum specimens used in this study were obtained from 15 clinically suspected patients of typhoid fever during admission in the G.R. Medical College and Hospital, Gwalior, India. Pooled serum samples from four apparently healthy individuals with no previous history of typhoid fever were included as control. All the serum samples were subjected to Widal agglutination test to find out the typhoid antibodies using Span typhoid tube agglutination test kit (code no. 17412A-17417-OH, Span Diagnostics Private Ltd., Udhana, Gujarat, India). The same samples were subjected to immunosensing using screen-printed electrodes.

2.5. Preparation of screen-printed electrodes

The screen-printed electrodes were prepared with the help of a manual screen printer as reported in literature [34]. Ink, prepared by mixing polystyrene dissolved in mesitylene and graphite was used for screen-printing the electrodes. After screen-printing, the electrodes were dried at 80 °C for 2 h, in an oven. Various compositions of polystyrene and graphite were tested It was found that an ink containing 60% graphite and 40% polystyrene was found to be suitable. The size of these electrodes was 30 mm × 5 mm. A layer of dielectric ink was screen printed over the electrode so that an area of 7 mm^2 is exposed. The resistance of the electrodes measured at a distance of 1 cm apart was found to be approximately 500 Ω.

2.6. Preparation of the blocking, washing solutions and electrolyte

Three percent BSA was prepared in Tris–HCl buffer (0.2 M, pH 7.2). Washings were performed using Tris–HCl buffer (pH 7.2). Ethanoldiamine (EDA) buffer (pH 9.8) containing 0.1 M of ethanoldiamine, 0.1 M sodium chloride and 1 mM magnesium chloride was used as electrolyte in the amperometric experiments.

2.7. Steps involved in immunosensing

The immobilisation of antigen was performed by placing 100 ng of antigen dissolved in 5 μl of Tris–HCl buffer (pH 7.2) on the electrode for 15 min. This was followed by washing with Tris–HCl buffer. Later the electrode was blocked with 3% BSA for 15 min. It was washed and then 5 μl of

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