Detection of Protein A Produced by Staphylococcus aureus with a Fiber-optic-based Biosensor

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Staphylococcus aureus is a pathogen important in causing human infections and intoxication. A sensitive fiber-optic that produces evanescent waves was developed for the detection of protein A, a product secreted only by S. aureus. In the immunosensor, a 40-mV argon-ion laser that generated laser light at 488 nm was used together with plastic optical fiber and antibodies to protein A were physically adsorbed onto the fiber. The principle of the detection involved a sandwich immunoassay with fluorescein isothiocyanate conjugated with anti-(protein A) immunoglobulin G to produce signals of the antigen-antibody reaction. The detection limit was 1 ng of protein A per milliliter. The fiber-optic immunosensor could be used for rapid and specific detection of S. aureus in clinical specimens and foods.

Key words: biosensor; fiber optic; protein A; Staphylococcus aureus

Staphylococcus aureus is the causative microorganism of a spectrum of pathologic conditions, including circumscriptive suppurations, bloodstream invasion, and a variety of toxic syndromes. In addition, S. aureus is one of several bacteria causing foodborne outbreaks around the world. It can produce several types (A, B, C, D, and E) of enterotoxins that cause gastroenteritis. Therefore, the presence of the bacterium in processed food is a health hazard if the food is held at a temperature that allows growth, which permits synthesis of enterotoxins.

Not surprisingly, the bacterium has several virulence factors including cytoxic hemolysins, leukocidins, enterotoxins, teichoic acid, and protein A. Protein A is produced by S. aureus and can be used as an indicator of the presence of the microorganism in clinical or food specimens. The protein is a cell wall constituent and is covalently linked to the peptidoglycan structure of the bacterium. Some 99% of S. aureus strains have the protein on the cell wall, but few other bacterial species can produce it, and only in small amounts. Although most protein A is bound to the bacterial cell wall, some 80 to 30% of it is secreted during the exponential growth phase, and the extracellular protein can be detected by immunoassay. Recently, an enzyme-linked immunosorbent assay (ELISA) was developed for the detection of protein A with a detection limit of about 0.1 to 1 ng/ml. However, repeated washing and incubation are required during the ELISA, and generally 3 to 4 h are needed for analysis.

Immunodiagnostic sensors permit sensitive measurement of a variety of analytes of all sizes. Such sensors monitor antigen-antibody reactions directly, so quantitative results are available within seconds to minutes after sample introduction. The recent trend has been for immunodiagnostics to be done outside of centralized clinical laboratories, and the particular requirements in such circumstances are being met by the development of optic-fiber sensors that use the electromagnetic field of evanescent waves. The energy field of evanescent waves is limited, so only molecules in the immediate vicinity of the fiber surface will be detected in immunoassay; unbound probe molecules in regions distant from the fibers will not be detected. This kind of detection eliminates the extensive washing needed in conventional immunoassay such as ELISA.

Fiber-optic immunosensors that utilize evanescent waves have been used to detect acetylcholine (a neurotransmitter), the highly toxic bacterial toxin A produced by Clostridium botulinum, the residues of Atrazine (a herbicide), and cocaine. The purpose of this study was to develop a rapid, sensitive, and easy-to-use fiber-optic biosensor for the detection of protein A, which is an indicator of the presence of S. aureus.

Materials and Methods

Production of anti-protein A antibodies. Commercial preparations of protein A (Pharmacia, Uppsala, Sweden) usually contain minor impurities, and need to be purified before their use in immunization. Protein A was purified to homogeneity by preparative SDS-PAGE as described previously. After electrophoresis, the protein bands were stained by shaking the gel in 4 mM sodium acetate for 10 to 20 min. The band corresponding to protein A (molecular weight, 42,000) was cut out from the SDS gel, crushed to fine particles with a 5-ml hand homogenizer, and used to immunize New Zealand White rabbits. After several booster immunizations with 3-week intervals, blood was collected from an ear artery of the rabbits. The IgG fraction was purified from the serum by ion-exchange chromatography and used to coat plastic optical fibers.

Preparation of fluorescein-conjugated antibodies. The procedure used to prepare fluorescent antibody was essentially as described by Hudson and Hay. Briefly, 2 ml of a 10 mg/ml IgG solution in 0.25 mM carbonate buffer, pH 9.0, was mixed with fluorescein isothiocyanate (FITC) (protein: FITC = 20:1 w/w) and incubated at 4°C for 18-24 h. The reaction mixture was then passed through a Sephadex G-25 (Pharmacia) column (0.6 x 12 cm) and eluted with 10 mM phosphate-buffered saline (PBS; pH 7.2) to purify the IgG-FITC conjugate in the void volume. The FITC-labeled anti-(protein A) IgG had a fluorescein protein ratio of about 3.3:1.

Abbreviations: FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; BSA, bovine serum albumin; IgG-FITC, conjugate of IgG and FITC; ELISA, enzyme-linked immunosorbent assay.
Coating of fibers with anti-protein A IgG Plastic fibers (1 mm in diameter, Mitsubishi, Japan) were cut into 50-cm lengths. A 5-cm portion of each fiber was decladded by being immersed in acetone. The fibers were then washed with PBS, air-dried, polished at both ends, and coated with antibodies by incubation of the decladded portions with anti-(protein A) IgG (10 μg/ml) in PBS for 1 h at 37°C. The fibers were washed with PBS and incubated with 2% bovine serum albumin (BSA) at 37°C for 1 h to block the unoccupied sites on the fiber surface. The antibody-coated fibers were stored in PBS at 4°C until use.

Assembling of the fiber-optic immunosensor. The experimental setup used for the evanescent sensor is shown in Fig. 1. An air-cooled argon laser (ion Laser Technology, Salt Lake City, UT) having a 40-mW output power and a single-frequency of 488 nm was used in measurement. To assure that the beam wavelength would be steady, another 488-nm optical filter was used to decrease the purity of the laser beam coupled to the fiber. A 50-cm long plastic fiber was used as the fiber sensor. Both ends of the fiber were polished to increase the coupling efficiency of the laser beam into the fiber. On the fiber sensor, the sensing region was a decladded 5-cm-long segment held in a 15-ml cylindrical reaction chamber of our own design. A 20× microscope objective was used to couple the laser beam into the optical fiber. The emission (wavelength, 520 nm) from the bound IgG-FITC molecules within the field of evanesence was collected by a photomultiplier (Oriel Instruments, Stratford, CT) placed aside the opening of the reaction chamber. A 520-nm interference filter (Edmund Scientific Co., Barrington, NJ) was set between the chamber and the photomultiplier to increase the signal-to-noise ratio. In addition, a power meter was placed at the far end of the fiber to monitor the intensity fluctuation of the incident laser beam.

Assay procedure. Fifteen microliters of protein A (1 to 100 ng/ml in PBS containing 2% BSA) was introduced into the reaction chamber and incubated at room temperature for 10 min. After being washed with PBS, the fiber was placed in the reaction chamber and the background signal was monitored by the power meter. A constant signal was achieved by adjustment of the position of the collart at the near end of the optical fiber. This adjustment was to ensure that every fiber was illuminated under the same conditions to facilitate fiber-to-fiber comparison. Fifteen microliters of IgG-FITC (2 μg/ml) was then introduced into the chamber and the signal from the photomultiplier tube was read at 5, 30, and 60 s and at 1-min intervals after the introduction of the labeled antibody.

Results and Discussion
In our sandwich assay of protein A, anti-(protein A) antibodies are immobilized on optical fibers to capture the antigen, and FITC-labeled anti-(protein A) IgG is used for signal generation. Figure 2 shows the response of the evanescent-wave detection system to different concentrations of protein A. With increasing concentrations of protein A, the signal increased, as is usual for this kind of assay. The gradual increase in the signals with incubation time after sample introduction, similar to that of a saturation curve, reflected the gradual increase in the binding of the IgG–FITC molecules to the captured antigen on the fiber surface. The evanescent wave allowed the sensor to monitor the antigen-antibody reactions in real time.

The detection limit of the immunosensor was 1 ng/ml protein A (Fig. 2). At this concentration, the signal-to-noise ratio was 1.3 when the results were read at 4 min after the introduction of the sample; this ratio increased to 1.7

**Fig. 1.** Fiber-optic Immunosensor.
Diagram of the components of the instrument, the path of laser light, and the fluorescence detection system (see Methods and Methods for details).

**Fig. 2.** Dose Response Curves of Fiber-optic Immunosensor to Different Concentrations of Protein A.
For each concentration of protein A (shown on the right), a different fiber was used. Each fiber was placed in a protein A solution for 10 min before being placed in a solution of anti-(protein A) IgG labeled with FITC. The signal was monitored within a period of 15 min.
if the results were read at 7 min. The assay sensitivity was close to that obtained by ELISA (0.1–1 ng/ml). However, ELISA is done on a 96-well microtiter plate, which is not suitable for analysis of a few samples. The sensitivity of the immunosensor was higher than that (5 ng/ml) described by Ogert et al., who constructed a fiber optic-based biosensor that also utilized evanescent waves to excite rhodamine-labeled antibody for the detection of C. botulinum toxin A. The detection sensitivity of protein A was also higher than that of the fiber-optic immunosensor developed by Tune.

He reported a sensitivity of 3 ng/ml for the detection of creatin kinase (the muscle and brain hybrid dimer; MB), which is a cardiac-specific enzyme; the enzyme can be used as index of tissue damage in the heart after acute myocardial infarction.

The sensitivity of the fiber-optic sensor for protein A might be high partly because of the use of anti-(protein A) IgG rather than normal serum IgG for the coating of the fibers. The advantage of the use of anti-(protein A) IgG is that the Fab fragment and Fc portion of the IgG molecule bind protein A with high affinity and specificity. Compared with when normal serum IgG is used, the sensitivity with anti-(protein A) IgG is 10 times greater.

Figure 2 shows that, irrespective of the protein A concentration, the longer the incubation, the higher the fluorescence signal. However, the increase in the fluorescence signal approached a plateau at 7 min after introduction of the samples for lower concentrations of protein A (1, 5, and 10 ng/ml). For this reason, a calibration curve relating the concentration of protein A and the fluorescence intensity at 7 min was constructed (Fig. 3). The line of regression \( y = 0.692 + 0.089x \) had a correlation coefficient of 0.993. The range suitable for protein A assay was from <5 to 100 ng/ml; this range is close to that for ELISA.

For isolation and identification of S. aureus in clinical specimens, 2 to 3 days are needed. Some hospital infections such as bacteremia caused by S. aureus are dangerous and may be fatal. The incidence of bacteremia and fungemia has been reported to be 3.4 to 28 per 1000 hospital admissions and was estimated to average 10 per 1000 admission (1%) in the United States. Therefore, the development of rapid, simple, and real-time methods to detect the microorganism is of importance. Several latex agglutination tests have been developed for the rapid identification of S. aureus with high sensitivity and specificity. However, isolated pure cultures are usually needed for agglutination tests. Under some circumstances, therefore, subculture from a liquid to a solid medium, which takes overnight incubation, is necessary before the agglutination test can be done. The immunosensor developed in this study perhaps can directly detect protein A, a specific marker of S. aureus, in blood culture bottles that show growth of Gram-positive cocci.

Compared with the conventional culture methods, this method is simple, sensitive, specific, and fast (less than 30 min). Appropriate antimicrobial treatment can thus be started at least 24 h before results by other available methods, and speed of treatment may help reduce the mortality from bacteremia caused by S. aureus.

The growth of this organism in foods is another health hazard. Staphylococcal intoxication is one of the most common cause of foodborne illness in Taiwan and in North America, affecting hundreds of people per year. However, the methods used by the US Food and Drug Administration for the detection of the bacterium are tedious, and 5 to 7 days are needed for analysis. After overnight enrichment of food samples, it would be possible to detect protein A secreted into the medium with this fiber-optic sensor. The assay time for S. aureus in foods can be shortened to 24 h.
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