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ScienceDirect
Talanta 72 (2007) 859–862
Talanta
www.elsevier.com/locate/talanta

Short communication

A surface plasmon resonance biosensor for detecting *Pseudomonas aeruginosa* cells with self-assembled chitosan-alginate multilayers

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Received 13 October 2006; received in revised form 11 December 2006; accepted 11 December 2006
Available online 20 December 2006

Abstract

A self-assembled multilayer (SAMu) including the alginate layer was prepared for detecting *Pseudomonas aeruginosa* cells in a solution and its potential was evaluated with a BIAcore system. After layer-by-layer formation, the refractive units (RU) values monitored with the biosensor increased by the interaction between the layers. The responses by the binding of *P. aeruginosa* cells to the alginate-immobilized SAMu were visualized immediately upon injection of the cell suspension. The RU values after injection of the cells were measured with approximately 1152, 656 and 173 for 1×10^9, 1×10^8 and 1×10^7 CFU/ml. This result suggests that the alginate-immobilized SAMu will have useful application for detecting *P. aeruginosa* cells in a biosensor analysis.

Keywords: Biosensor; Chitosan; Alginate; *Pseudomonas aeruginosa*; Self-assembled multilayers; Pathogen detection

1. Introduction

Rapid detection of *Pseudomonas aeruginosa* emerged as one of the most problematic Gram-negative pathogenic bacteria is an essential mission due to their clinical importance [1,2]. *P. aeruginosa* is the major causative bacteria in food putrefaction and leads cause of pseudomonal infections to burn patients, mechanically ventilated patients, cystic fibrosis patients, etc.

Recently, to analyze the pathogen several detectable methods including polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA), bioluminescence and florescent labeling analytic techniques have been employed [3]. However, the detection techniques may require relatively long time and many process including isolation, biochemical testing and morphological examination. In some other way, a technology based on surface plasmon resonance (SPR) offers rapid and real-time measurement for monitoring antigen–antibody interactions. Its good points is also label-free and a specific analysis.

In the present study, the potential of a self-assembled multilayer (SAMu) applied to a SPR sensor for detecting *P. aeruginosa* was investigated. The SAMu was constructed using polyelectrolytes formed between positively-charged chitosan and alginate bearing negatively-charged sites. Alginate immobilized on the end of the SAMu is an exopolysaccharide produced by *P. aeruginosa* that has repeating units of mannuronic acid and glucuronic acid. Alginate acts as an adhesion molecule to anchor *P. aeruginosa* to the surface of the colonized respiratory epithelium [4,5] and *P. aeruginosa* increases alginate synthesis to protect oneself from phagocytosis, antibiotics and even attenuates the host response [6,7]. Alginate layer attached on the surface of a sensor chip may show strong interaction with *P. aeruginosa* and the SAMu bearing alginate layer can used as the sensor chip to detect *P. aeruginosa*.

The aim of this study was to prepare the SAMu immobilizing alginate on the end of the sensor chip, which is applied as

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doi:10.1016/j.talanta.2006.12.021

biosensor for detecting *P. aeruginosa*. The interactions between the SAMu bearing alginate and *P. aeruginosa* were monitored by SPR based estimation using a BIAcore instrument.

2. Materials and methods

2.1. Microorganism

P. aeruginosa (KCTC No. 1928) obtained from Korean Collection for Type Cultures (KCTC) was used in this study. *P. aeruginosa* was grown for 18 h at 37 °C in Luria-Bertani (LB) broth.

2.2. Reagents

Alginate (Medium viscosity, 3500 cps at 2% concentration), glutaraldehyde and cysteamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Chitosan (Mw 50–80 K) with degree of 85% deacetylation was obtained from Chitoful Co. (Yeosu, Republic of Korea). All other chemicals were of regent grade and used without further purification.

2.3. Preparation of self-assembled chitosan-alginate multilayer

Alginate-attached SAMu was prepared with the method described by Deng et al. [8]. In brief, the gold surface of the sensor chip was treated with 'piranha' solution, which is consisted of sulfuric acid and NaOH solution and then immersed in 0.2% (w/v) aqueous solution of cysteamine for 2 h. The resultant gold surface was rinsed with distilled water and dried with nitrogen. After immersing the gold surface in 2.5% of glutaraldehyde solution for 1 h, the modified surface was then treated with 0.4% (w/v) of chitosan dissolved in 2% acetic acid for 1 h, washed with distilled water to remove the adhesive chitosan on the surface of chip, and then dried with nitrogen at the room temperature. Alginate solution (1% (w/v) in distilled water) was dropped on the chitosan layer to form the alginate-chitosan multilayer by ionic interaction, rinsed with distilled water and then dried at room temperature.

The SAMu formed on the gold surface was characterized through measurement of SPR responses at interval of each preparation steps. After each procedure, the changed refractive units (RU) values were investigated with BIAcore®J system (BIAcore® AB, Uppsala, Sweden).

2.4. Evaluation of P. aeruginosa binding to the SAMu of the sensor chip surface

P. aeruginosa cells were harvested by centrifugation after incubation in LB medium for 18 h. The cells were washed once and re-suspended to ~1×10^9 CFU/ml with 0.5 M sodium acetate buffer. The prepared whole cells suspension was injected in the BIAcore system fitted with the SAMu chip at a flow rate of 17 μl/min for 7 min. The specific interaction between *P. aeruginosa* cell and the SAMu of the sensor chip surface was determined by monitoring changes of RU values using. To investigate specific interaction of *P. aeruginosa* cell on the SAMu of the sensor chip, *P. fluorescens* cell and *Seratia marcenscens* cell were employed as contrast groups. The signal changes produced by the interaction of the cells on the SAMu of the sensor chip were monitored by measuring the shift of resonance angle in SPREETA™ system (Texas Instruments Inc., USA).

Table 1
The changes of RU and mass value after the ligand immobilization

Type	ΔRU
Cysteamine	1367
Glutaraldehyde	245
Chitosan	307
Alginate	280

ΔRU is individual differences of RU values between the former step and the latter step.

3. Results and discussion

The SAMu on the surface of sensor chip for detecting *P. aeruginosa* was prepared by modifying a gold chip and the interaction between the SAMu and *P. aeruginosa* was monitored with a BIAcore system. The modification of a chip surface by ligand immobilization was characterized by measuring the changed RU values after the immobilization steps [9,10]. Table 1 shows the RU and mass values measured by a BIAcore system after layer-by-layer formation. After cysteamine binding to the chip surface, the RU value increased about 1367 and then individual differences of RU values between the former step and the latter step was about 250–300 after subsequent events. The increment of RU and mass values in the steps means that the SAMu by the layer-by-layer formation was prepared on the chip surface. It is concluded that alginate as a ligand to detect *P. aeruginosa* could be immobilized on the chip surface by ionic interaction with chitosan layer. Liu et al. [11] proposed the self-assembly chitosan/glutaraldehyde/cysteamine (CGC) on the gold surface for an assay using a piezoelectric quartz crystal (PQC) sensor. From their results, it is confirmed that chitosan layer can be formed by interaction with glutaraldehyde/cysteamine layers (Fig. 1).

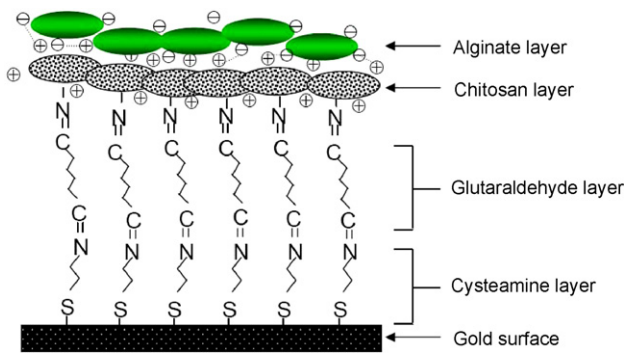

Fig. 1. Illustration of the self-assembled chitosan-alginate multilayers. Cysteamine molecules were conjugated by multipoint electrostatic bonding between their sulfur groups and gold surface. To cross-link chitosan layer glutaraldehyde was bonded to the cysteamine layer. Alginate layer formed with ionic interaction between chitosan layer can interact with *P. aeruginosa* cells.

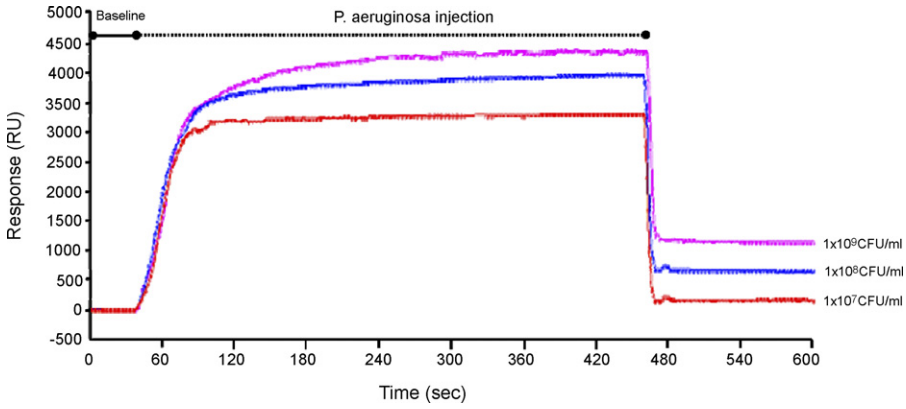

Fig. 2. Binding sensorgram of *P. aeruginosa* to the alginate-immobilized SAMu. After injection of a suspension of *P. aeruginosa* cells ($\sim 1 \times 10^9$ CFU/ml), the responses were typically showed by the interaction of the SAMu and the cells.

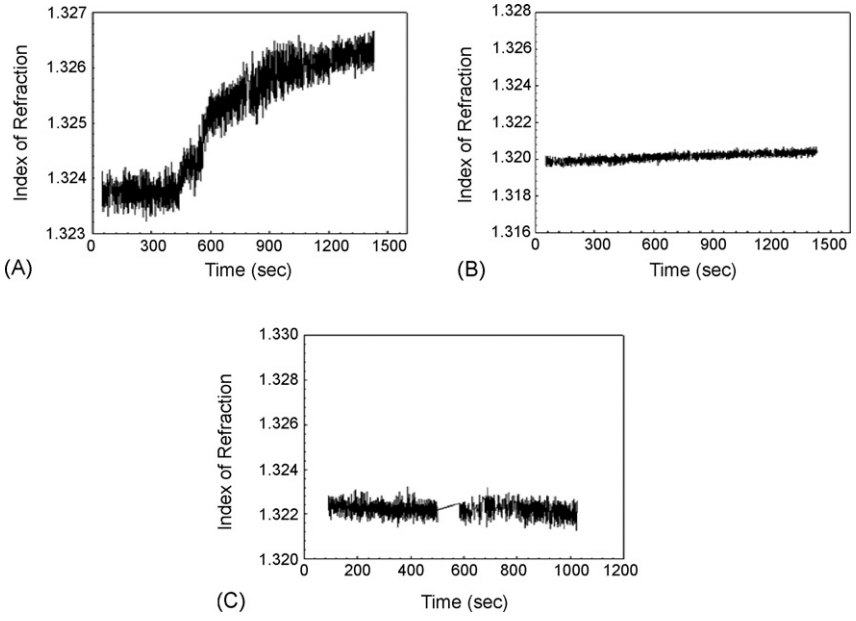

Fig. 3. Refractive index change by the interaction of (A) *P. aeruginosa*, (B) *P. fluorescens* and (C) *S. marcenscens* to the SAMu on the sensor chip. The number of the cells was 1×10^8 CFU/ml for all the groups.

The binding of *P. aeruginosa* cells using the SAMu based on alginate was evaluated with a BIAcore system. The sensogram showing the binding of *P. aeruginosa* on the SAMu of the chip surface was presented in Fig. 2. The responses by the binding of the cells were rapid and visualized immediately upon injection of the *P. aeruginosa* suspension. In the binding analyzes of the cells, the RU values reached approximately 1152, 656 and 173 for 1×10^9, 1×10^8 and 1×10^7 CFU/ml. The responses were dependent on the concentration of the cells administered for detecting. *P. aeruginosa* produces a polysaccharide, alginate, to anchor on the surface of the colonized respiratory epithelium. It may be concluded that an interaction between *P. aeruginosa* and alginate exists and the SAMu including alginate can be used to detect *P. aeruginosa* cells. As shown in Fig. 3, it was confirmed that *P. aeruginosa* could be specifically interacted with the SAMu on the sensor chip compared with *P. fluorescens* and *S. marcenscens*. In the interaction test between *P. aeruginosa* and the SAMu, the index of refraction was clearly changed by the specific binding to the surface of the sensor chip. On the other hand, the change of the index of refraction weakly happened in the interaction test with *P. fluorescens* and the refractive index change did not happen in the test with *S. marcenscens*.

In summary, the SAMu immobilizing alginate to analyze *P. aeruginosa* cells in a solution showed the useful results in this study. The formation of the alginate-immobilized SAMu was confirmed by measuring the changed RU in the BIAcore system. In the study for the detecting *P. aeruginosa* cells, the RU values by the specific interaction with the SAMu increased, depending on the concentration of *P. aeruginosa* cells. Therefore, it is concluded that the alginate-immobilized SAMu may has potential applications for detecting *P. aeruginosa* cells in a solution.

Acknowledgement

This work was supported by grant No. RTI-04-03-03 from the Regional Technology Innovation Program of the Ministry of Commerce, Industry and Energy (MOCIE).

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