Available online at www.sciencedirect.com
ScienceDirect
Biosensors and Bioelectronics 22 (2007) 2371–2376
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Short communication

Detection of SEB gene by bilayer lipid membranes nucleic acid biosensor supported by modified patch-clamp pipette electrode

Nan Liu, Zhixian Gao*, HuanYing Zhou, Mingxiang Yue

Institute of Hygiene and Environmental Medicine, Academy of Military Medical Science, Tianjin 300050, China

Received 20 April 2006; received in revised form 15 September 2006; accepted 27 September 2006
Available online 7 November 2006

Abstract

This work reports a kind of novel bilayer lipid membranes (BLMs) nucleic acid biosensor supported by modified patch-clamp pipette electrode was developed to detect staphylococcus enterotoxins B (SEB) gene. BLMs were formed within 15 min and able to be operated at least 24 h. Hydrophobic dodecane tail (C_{12}) modified 18 bp single-stranded DNA (ssDNA) probe was immobilized on BLMs. The electrochemical currents versus the different concentration of ssDNA probe immobilized on BLMs indicated linearly correlation. The BLMs nucleic acid biosensor was fabricated by selecting the ssDNA probe as the signal sensing element with the concentration of 273.65 ng/mL. The electrochemical performance of the biosensor for the detection of SEB was investigated. The result showed that linear relationship was found between the current and ln(concentration) from 20 to 5000 ng/mL and the detection limit was 20 ng/mL. In addition, the biosensor was specific response to SEB gene and showed no significant current alteration in electrolyte which containing no SEB gene. Finally, Atom Force Microscope (AFM) images could be observed and used to evaluate the superficial microstructure of BLMs, ssDNA immobilized on BLMs and BLMs after hybridization. The BLMs nucleic acid biosensor supported by modified patch-clamp pipette electrode will become a highly sensitive, rapid, selective analytical tool for detection of *Staphylococcus aureus*, which produce SEB.

Keywords: Biosensor; Bilayer lipid membrane; Patch-clamp pipette; SEB gene; Atom Force Microscope

1. Introduction

Food poisoning accidents and food-borne diseases have adverse effects on public health. *Staphylococcus aureus* and its enterotoxins (SEs) are major causes of bacterial food poisoning outbreaks, toxigenic syndromes, one of the hospital infections, etc. It is the second most frequent cause of food-borne disease outbreaks in France after *Salmonella* (Rosec and Gigaud, 2002). From June 3rd to 10th, 2004, there were more than 200 children poisoned by SEs-polluted milk powder in Guiyang and Anshun City, Guizhou Province, China.

SEs resulted in food poisoning are a group of low molecular weight and dissolvable proteins. In term of their antigenicity, SEs have been divided into several serotypes, such as A, B, C_1, C_2, C_3, D, E and H. SEB encoded by entB gene (i.e. SEB gene) is an important enterotoxin produced by various strains of *S. aureus* and also categorized as one of the biological warfare agents. Such an enterotoxin was considered as one of the potential threats to national security. There are many traditional and mature immunological technologies available to identify SEB, including passive hemagglutination (PHA) (Johnson et al., 1967), radioimmunoassay (RIA) (Holečková et al., 2002), enzyme-linked immunosorbent assay (ELISA) (Brien et al., 2000), etc. However, the disadvantages such as intensive labor, rigorous experimental conditions and high false positive rate are commonly encountered. SEB gene detection is an alternative for the special *S. aureus*, which produce SEB. SEB gene can be detected by DNA probe or (and) DNA probe labeling technique and polymerase chain reaction (PCR) (Notermans et al., 1988; Neill et al., 1990).

Biomembrane plays an important role in biological physiology, such as nervous impulse, molecular transduction, signal processing and energy transduction. BLM system is by far the most biomembrane-like, lipid flowing, self-assembling and is capable of the ligand-receptor recognition (Tien and Ottova, 1999). Due to the liquid crystal property, the bilayer

* Corresponding author. Fax: +86 22 84655403.
E-mail address: gaozhx@163.com (Z. Gao).

0956-5663/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2006.09.037

lipid molecules are characterized in sequencing and mobility, which functional substances can be modified by embedding in them. Supported BLMs(s-BLMs) as model membranes can be deposited on the end or surface of metallic wires, SnO_2 glass, bare carbon electrode, piezoelectric quartz crystals, agar or gel substrates and on microchips (Ottova et al., 1994). BLMs are characterized as a relatively biocompatible structure for the development of new types of electrochemical biosensors with fast response and high sensitivity and could potentially be employed in many fields, such as continuous monitoring or rapid screening of a wide range of chemicals of environmental, pharmaceutical and clinical interest (Wang et al., 1996; Nikolelis et al., 2002; Zhang et al., 2001).

The development of patch-clamp technique has brought a revolution of life science research especially the detection of transmembrane electrical signals transduction. Patch-clamp pipette is an extremely sensitive ionic channel recording apparatus via recording the ion current, resistance and capacitance to reflect the movements of single or multi ion channel molecules in cellular membrane. With the negative pressure suction to the biomembrane (ca. one or several square microns) by the tip of the tiny patch-clamp electrode, micro electric current through ionic channel can be dynamic or static observed with little leakage and low background noise. Since the structure and properties of BLMs were similar to biomembranes, the generation of electronic signals in BLMs also can be captured by patch-clamp pipette system.

In this paper, we present here a novel BLMs nucleic acid biosensor supported by modified patch-clamp pipette electrode for the detection of SEB gene. The whole process was simple and fast. We also employed Atom Force Microscope (AFM) to observe the superficial structure of blank BLM, BLM immobilized by ssDNA probe, BLM that after hybridization and purging.

2. Experimental

2.1. Materials and apparatus

Agar was purchased from Bio Basic Inc., Canada. Egg phosphatidylcholine (EPC), cholesterol (CH) and hexadecylamine (HDA) were supplied by Sigma Inc., USA. *n*-Decane was purchased from Fluka Inc., USA. A 18 bp ssDNA probe which specific to SEB gene terminated at 5′ end with C_{12} and the PCR primers were synthesized by Sino-American biotechnology company, Beijing, China with the following sequences: ssDNA probe: 5′-C_{12}-ATGCATCTACACAAACCT-3′ and PCR primers (5′–3′): F: -CGGAATTCATGGAGAGTCAACC- AGA, R: AGGGATCCTCACTTTTTCTTTGTCG.

Teflon tubes (inner diameter: 0.8 mm, outer diameter: 0.9 mm) were made by Tianjin Scientific Apparatus Company (Tianjin, China). High pure silver wires (diameter: 0.6 mm, purity: 99.99%) were made by Yinhai Science and Technology Inc., Tianjin, China.

Axopatch200B patch-clamp pipette amplifier with its matching analytical microcomputer system (Axon Instrument Inc., USA) was used for recording, storing and analyzing the signal of electrochemical current. All electrochemical cells and the patch-clamp pipette were isolated in a grounded Faraday case in order to prevent the outer disturbance of electromagnetic waves. PCR reaction was carried out with PTC-200 DNA Engine Cycler (MJ Research, USA). Nanoscope IIIα Multimode Atom Force Microscope (Digital Instrument Inc., USA) and NSG20 AFM probe (NT-MDT Inc., USA) was used for the observation of the BLMs surface in different states. All other chemicals were of analytical grade. Deionized water was purified by ASD-II high pure water apparatus and had a minimum resistivity of 18 MΩ cm. All experiments were performed at the temperature of 25 ± 2 °C.

2.2. Experimental bacteria stains

Two standard staphylococcal strains which produce SEB, 10 strains of that do not produce SEB, 2 strains of *Escherichia coli*, 2 strains of *Salmonella typhimurium* and 2 strains of *Shigella flexneri* were gifted by Dr. Li.

2.3. Preparation of modified patch-clamp pipette electrode supported BLMs

Membrane forming solution was prepared by EPC, HDA and CH (EPC:HDA:CH = 4:2:1) dissolving in *n*-decane as a solvent at the concentration of 20 mg/mL and stored in a nitrogen atmosphere at the temperature of −4 °C. Firstly, the inner wall of teflon tubes (the length was ca. 6 cm) was cleaned with deionized water and the silver wires (the length was ca. 4 cm) was shined with soft sand paper before inserting the length of 3 cm part into the teflon tube. Secondly, 1 mol/L KCl solution mixed with 2% hot melting agar was effused to the teflon tube. After agar cooling and solidification, a nascent agar surface was cut by a scalpel and immediately immersed into the membrane forming solution for ca. 3–5 min. And then, the end of the teflon tube coated with lipid solution was immersed into a 0.1 mol/L KCl aqueous solution to form BLMs. Thirdly, the other end of the teflon tube was plugged into the patch-clamp pipette electrode by silver wire's connection. Axopatch 200B patch-clamp pipette amplifier was then employed to measure the ion currents through BLMs and the electrolytic cells were isolated in Faraday case. After zero adjustment of the liquid junction potential and the background current reached to a steady condition, external command was prepared to clamp down on BLMs by switching to V-Clamp mode.

2.4. Data recording of C_{12}-ssDNA immobilization on modified patch-clamp pipette electrode supported BLMs

A 50 mV constant voltage was clamped down on BLMs of the biosensor which being immobilized by C_{12}-ssDNA with different concentrations of 0, 10, 20, 40, 80, 160 and 320 ng/mL in 0.1 mol/L KCl electrolyte solution in electrochemical cells. Data were recorded by software Clampex 8.1. Optimal concentration of ssDNA probe, which immobilized on BLMs was selected. Then, the BLMs nucleic acid biosensor was able to be prepared to detect SEB gene. To check up the performance of the biosen-