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Short communication

The strategy of signal amplification for ultrasensitive detection of hIgE based on aptamer-modified poly(di-acetylene) supramolecules

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ABSTRACT

Herein, we demonstrate three strategies of signal amplification for ultrasensitive detection of human immunoglobulin E (hIgE) based on poly(di-acetylene) supramolecules. To fabricate the ultrasensitive PDA biosensor, ethylenediamine as an interlinker and aptamer as a receptor were introduced into the chip fabrication process. Using the prepared PDA liposome biosensor, the hIgE could be detected up to below 1.0 ng/ml by a primary response. In order to accomplish more ultrasensitive detection of protein on a PDA biosensor, polyclonal hIgE antibody was employed as an external mechanical force for the inducement of a secondary response. As a result, a PDA liposome biosensor sensitivity as high as 0.01 ng/ml for the target hIgE was obtained, with a sensitivity which is one hundred times of that of the method without signal amplification. These results indicate that the proposed strategies were capable of ultrasensitive quantitative and qualitative analyses of biomolecules without non-specific binding of non-target proteins.

1. Introduction

Currently, aptamers are used as alternative receptors. Aptamers are oligonucleotides that can bind to a wide range of target molecules, such as drugs, proteins, and other organic or inorganic molecules, with high affinity and specificity. The systematic evolution of ligands by the exponential enrichment (SELEX) method has been used to isolate ligands with high affinities for IgE, D17.4, which competitively inhibits the IgE/Fcε RI interaction (Liss et al., 2002). The application of aptamers as bio-components in biosensors offers many advantages over classical affinity sensing methods primarily based on antibodies. Since the aptamers can easily be engineered *in vitro*, their mass production is relatively cost-effective. Moreover, while antibodies are sensitive to temperature and denature easily upon contact with surfaces, leading to limited shelf lives, aptamers are stable for long-term storage (You et al., 2003).

As biosensors using conjugated polymers, poly(di-acetylenes) (PDAs) biosensors have been intensively investigated for the detection of chemical and biological molecules (Yang and Swager, 1998; Ho et al., 2002; Kim and Bunz, 2006). PDAs are polymers of diacetylene monomers, a class of single chain lipid molecules characterized by alternating triple bonds. Polymerized PDAs such as liposomes or layered films show extreme color which is easily distinguishable by the naked eye (Charych et al., 1993; Reicher et al., 1995; Ma et al., 1998; Wei et al., 2005; Lee et al., 2007; Choi et al., 2008; Yu et al., 2008; Zhang et al., 2009). Most PDA-based assemblies exhibit rapid blue-red colorimetric transitions due to a wide range of stimuli, such as temperature, pH, mechanical perturbations, solvents, and interfacial ligand–receptor binding. (Okada et al., 1998; Nallicheri and Rubner, 1991; Mino et al., 1992; Chance et al., 1977; Chance, 1980; Charych et al., 1993; Ahn et al., 2003). When the polymerized PDA liposomes undergo color shift from blue to red, strong fluorescence (excitation: 530 nm; emission: 590 nm) is indicated by a red phase (Carpick et al., 2000) that is detectable using a fluorescence microscope or microarray reader with proper filters.

In this paper, we report the strategy of fluorescent signal amplification by an interlinker, an aptamer, and a polyclonal antibody on the PDA liposome biosensor for ultrasensitive detection of human hIgE. First, experiments in which the PDA liposomes were stably immobilized onto a glass substrate by an 'ethylenediamine' interlinker were performed. Moreover, after introduction of the anti-hIgE aptamer in lieu of the hIgE antibody as the receptor on the PDA liposome biosensors, the aptamer-modified PDA liposomes were compared with antibody-modified PDA liposomes. Finally, the 'sandwich method' using polyclonal hIgE antibody was introduced onto the PDA liposome biosensor in order to enhance the fluorescent signal of PDA liposomes. Then, the response of the PDA liposome spots were monitored after introduction of target hIgE at various concentrations (1.0 pg/ml–10 μg/ml).

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2. Materials and methods

2.1. Materials

Monoclonal human IgE antigen used in the PDA liposome biosensor was purchased from DIATEC.com (Norway). Anti-IgE DNA aptamer (D17.4ext) with 3′-biotinylation was custom-synthesized by Bioneer Co. (Korea), and its base sequence was as follows:

D17.4ext (45 mer): 5′-GCG CGG GGC ACG TTT ATC CGT CCC TCC TAG TGG CGT GCC CCG CGC–NH_2-3′

10,12-pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals (OH, USA). 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), monoclonal human IgE antibody, polyclonal human IgE antibody, bovine serum albumin (BSA), fibrinogen from human plasma, human immunoglobulin G (hIgG), *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*-(3-diethylaminopropyl) carbodiimide (EDC), dimethyl sulfoxide (DMSO), ethylenediamine, and absolute ethanol were purchased from Sigma-Aldrich (USA). HPLC-grade chloroform was purchased from Fluka. Amine-coated glass was prepared by Nuricell (Korea). Deionized water (DI, resistance of water was 18.2 MΩ cm), obtained from a water purification system (Human Corporation, Korea), was used for preparation of the washing and buffer solutions.

2.2. Immobilization of PDA liposomes on amine-coated glass surface (Fig. 1(A))

Chloroform solutions of PCDA and DMPC were prepared separately in amber glass vials at −4 °C. Solutions of the two lipid monomers were mixed at 4:1 molar ratios (PCDA:DMPC) for a final lipid concentration of 1.0 mM. After chloroform was removed by N_2 gas, the remaining dry film of mixed diacetylenes was resuspended in 1.0 ml of 10 mM PBS buffer (pH 7.4) by heating in a circulating water bath set at 80 °C with gentle stirring about 15 min. The prepared solution was extruded through prefilter–100 nm membrane–prefilter complex repeatedly. The extruder system was kept at 85 °C for PCDA lipid formation, on the dry bath. Then, the vesicles solution was cooled to room temperature (25 °C) for 20 min. NHS and EDC were separately dissolved in PBS buffer to a total concentration of 200 mM. Then prepared ethylenediamine (total concentration of 1 mM) and an NHS/EDC (equal volume ratios) solution were added to the vesicle solution (Park et al., 2009). The PDA liposome solution, including the interlinker and NHS/EDC, was arrayed by an automated liquid handling system (Aurora biomed, Korea) at room temperature at 9 spots/well, with the 8 wells/amine-coated glass (Nuricell, Korea). The spot out of 8 wells was designated as 'control areas'. The PDA liposome-arrayed glass was then incubated in a chamber at a constant temperature of 4 °C for 2 h. Interlinking and immobilization of the liposome occurred during incubation, after which the PDA liposome-arrayed glass was repeatedly washed with deionized water and 0.1% Tween-20 in water, followed by soft drying under a stream of pure N_2. The arrayed glass was then covered with a multiple frame-seal incubation chamber, forming the final microwell for the multiple chip system.

2.3. Conjugation of biomolecules onto the PDA liposome chip (Fig. 1(B))

After PDA liposomes immobilization, the PDA liposome spots were polymerized under 254-nm UV light at a 1.0 mW/cm^2 intensity for 10 min. After NHS/EDC was dissolved in the PBS buffer, the amine-functionalized anti-hIgE aptamers were added to the solution. These mixed solutions were spotted by an automated liquid handling system on all PDA liposome spots. After spotting the anti-hIgE aptamers, the PDA liposomes-arrayed glass was incubated in a constant-temperature chamber at 4 °C for 3 h, then washed gently with deionized water and dried with pure N_2 gas. The analytes were injected onto each well (from 1 to 8) and the PDA liposome biosensor was incubated at 37 °C for 30 min. Then, the color change was analyzed by fluorescent microscopy after 30 min.

2.4. Detection of human IgE by signal amplification using polyclonal hIgE antibody

Amplification of the fluorescent signal was investigated using a sandwich immunoassay. After reaction between the target human IgE and anti-hIgE aptamer, the polyclonal hIgE antibody (100 ng/ml in PBS buffer) was subsequently injected into the spots and incubated at 37 °C for 30 min during the enhancement step. The subtracted fluorescent signals (a.u.) were corrected by subtracting the control (only PBS buffer) values of the PDA liposome, obtained by flowing polyclonal hIgE antibody over the surface without target hIgE, from the enhanced fluorescent signal values.

2.5. Specificity and selectivity test of target hIgE on aptamer-modified PDA liposome biosensors

Using a PDA liposome biosensor treated with anti-hIgE aptamer, the specificity and selectivity of the target proteins were tested on a prepared PDA liposome biosensor. First, the hIgE, hIgG, fibrinogen, and BSA were diluted to 1.0 μg/ml concentrations. Then, each protein was dropped onto the wells of the PDA liposome biosensors and incubated at 37 °C for 30 min. The PBS buffer was then added onto the aptamer-modified PDA liposome spots as a baseline.

2.6. Fluorescence analysis for hIgE detection

The fluorescent microscope consisted of four major parts: a microscope; a fluorescence unit (composed of a mercury arc lamp as a light source, fluorescent attachment and Nikon G2A filter, suitable for analysis of red phase PDA fluorescence); a digital camera unit (Infinity, USA); image analysis software (i-Solution, Korea). Fluorescence signals from the PDA liposome spots were visualized with this system and digital micrographs taken and spot intensities calculated with the image analysis software. Intensity denotes average intensity value of each pixel.

3. Results and discussion

3.1. Introduction of the interlinker for stable immobilization among liposomes on the PDA liposome biosensors

PDA biosensor chips had a serious problem involving loss of PDA liposomes due to weak immobilization when the PDA liposomes were washed out on the sensing spot during the fabrication process of the PDA biosensor chip. At that time, prior PDA liposome-based solid biosensors showed weak signal and sensitivity of immobilized the PDA spots on PDA liposome chips. To solve this serious problem, the interlinker 'ethylenediamine' was introduced into the fabrication process of the PDA biosensor chip. Using ethylenediamine (1.0 mM) brought strong cross-linking between liposomes through covalent bonding (Park et al., 2009).

To investigate this phenomenon in more detail, fluorescence and electron microscopy was employed to observe the morphology of the PDA layers on glass substrates. Fig. 2(A) shows fluorescence and SEM images of immobilized PDA liposomes both without (i) and with an interlinker (ii), after serial reaction of the aptamer and target hIgE (1.0 μg/ml). The effect of the interlinker is clearly shown in Fig. 2(A) as the PDA liposome was bound more to the surface