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Aptamer biosensor for lable-free detection of human immunoglobulin E based on surface plasmon resonance

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ABSTRACT

Aptasensor, an aptamer-based sensor for non-labeled detection of human immunoglobulin E (IgE), was developed using surface plasmon resonance (SPR). Various oligo(ethylene glycol) mixtures of different molar ratios of EG_6-COOH and EG_3-OH were used for the construction of self-assembled monolayers (SAM). Direct assays and modified sandwich assays over a 1.0 nM anti-IgE, aptamer-immobilized, gold chip, consisting of a 1:3 molar ratio of EG_6-COOH:EG_3-OH SAM, were investigated for real-time IgE detection. The linear ranges for both were 1.0–1000 ng/mL and the lowest amounts of detectable analytes in the samples were 3.44 and 2.07 ng/mL for direct and modified sandwich assays, respectively. These results for IgE detection suggest that the aptamer offers the possibility of a simple and realistic biosensor platform based on a SAM-fabricated SPR system. Furthermore, as an optic-biosensor, an SPR aptasensor could be used for the diagnosis of allergies.

1. Introduction

Allergies are chronic diseases caused by the exposure of the skin to chemicals, the respiratory system to dust or pollen, or the digestion system to particular foods. In 1967, human immunoglobulin E (IgE) was identified as being closely involved in mediating such hypersensitivity. In all individuals, when present on the surface membrane of basophils and mast cells, IgE highly sensitizes these cells towards stimulation by IgE antibody–allergen interactions [1,2], a key reaction to IgE-mediated allergies [3]. Compared to human immunoglobulin G (IgG), whose concentrations near 10.0 mg/mL, IgE is a trace serum protein [4]. In allergic patients, the serum total IgE levels correspond to the severity of the allergic disease. Therefore, a total IgE serum level is widely reported as a marker of atopic diseases, where patient levels reside above 290 ng/mL (120 IU/mL) [5,6].

Most allergy tests are based on the IgE antibody with the commercially available quantitative test kits making use of the enzyme-linked immunosorbent assay (ELISA) method. The minimum detectable concentration of such immunoassays has been reported to be 12.0 ng/mL [7], where the linear range of IgE detection using a surface plasmon resonance (SPR) sensor was from 12 to 720 ng/mL and the limit of detection of a blood sample was 0.09 ng/mL using a carbon electrode-based sensor [8,9].

Currently, aptamers are used as an alternative receptor. 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/FcεRI interaction [10]. In addition, detection using D17.4ext was achieved by a quartz crystal microbalance (QCM). A similar format has been applied to a microarray, differential pulse voltammetry (DPV), carbon nanotube field-effect transistors (CNT-FETs), and ELISA-type assays [4,11–13]. The initial findings suggest that aptamers can replace antibodies as a prospective receptor for various applications.

In this study, SPR-based sensor systems were investigated with an anti-IgE aptamer for the detection of IgE as a marker of allergies. SPR-based sensor systems for biological applications have been widely used in studies on biomolecular interactions [14,15], with the change in SPR signals is proportional to the refractive index close to the chip surface and is therefore related to the amount of bound analyte. Use of such aptamers have some strong points as DNA–protein interactions are not immune reactions, thus there is little concern for the temperature, and the storage of DNA is easier than that of protein, therefore the shelf life of an aptamer-based biosensor chip base is increased [16–19]. Furthermore, the anti-IgE aptamer has a higher binding affinity than the anti-IgE antibody [2,9]. These systems were able to detect normal and abnormal levels of IgE using high sensitivity and affinity sensors for the allergy test.

2. Experimental

2.1. Materials

The monoclonal immunoglobulin E (IgE) used in this SPR procedure was purchased from Diatec Monoclonals AS (Oslo, Norway).

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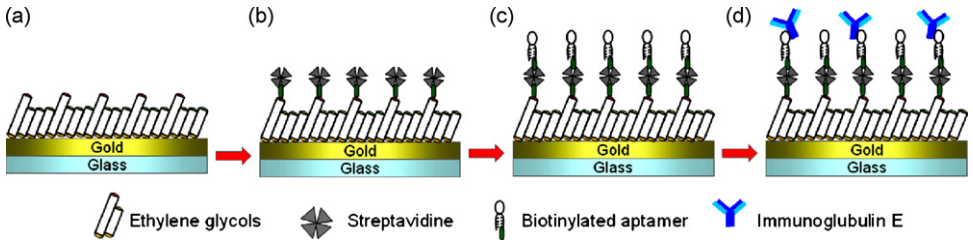

Fig. 1. Schematic diagram of immunoglobulin E (IgE) detection by the anti-IgE aptamer using the surface plasmon resonance (SPR) chip. (a) Carboxylic- and hydroxyl-terminated SAM containing ethylene glycols. (b) Streptavidin immobilization onto the SAM layer. (c) Aptamer immobilization onto the streptavidin layer. (d) Detection of IgE on the aptamer layer.

The 3′-biotinylated anti-IgE DNA aptamer (D17.4ext) was synthesized by Bioneer Co. (Daejeon, Korea), and its base sequence is as follows:

D17.4ext (45-mer): 5′-GCGCGGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCCGCGC-biotin-3′ (1-mercapto-11-undecyl) tri(ethylene glycol) [$HS(CH_2)_{11}(OCH_2CH_2)_3OH$, ($EG_3$-OH)] and 32-mercapto-3,6,9,12,15,18,21-heptaoxadotriacontanoic acid [$HS(CH_2)_{11}(OCH_2CH_2)_6OCH_2COOH$, ($EG_6$-COOH)] were purchased from Cos Biotech (Daejeon, Korea). Monoclonal anti-IgE antibody, bovine serum albumin (BSA), fibrinogen from human plasma, human immunoglobulin G (IgG), Tris–EDTA buffer, biotin hydrazide, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*-(3-diethylaminopropyl) carbodiimide (EDC), dimethyl sulfoxide (DMSO), streptavidin, absolute ethanol, and MES buffer (2-morpholino ethanesulfonic acid, pH 4.7–5.5) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HBS buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3.0 mM EDTA, 0.005% Surfactant P20) and the bare gold surface (SIA Kit Au®) were obtained from BIAcore AB (Uppsala, Sweden).

2.2. Fabrication of SAMs on the bare gold surface

The gold-coated chip was first modified with a mixture of ethylene glycol alkanethiols (EG_6-COOH and EG_3-OH) to form different mixed SAM surfaces. The clean, bare gold chips were separately immersed into absolute ethanol solutions containing EG_6-COOH:EG_3-OH at molar ratios of 1:2, 1:3, and 1:9 for 24 h, followed by a sequential rinsing with ultrapure water and absolute ethanol. The carboxyl groups of the SAM were then activated by adding 100 mg/mL EDC, and then conjugated with 50 mM biotin hydrazide. After 12 h at room temperature with gentle shaking, the chip was cleaned several times with ultrapure water and dried under a pure N_2 stream.

2.3. SPR measurement

SPR measurements were performed on a BIAcore 2000 apparatus (Pharmacia Biosensor AB, Uppsala, Sweden) using the BIAcore 2000 control software. The biotinylated chip was mounted onto the instrument with the working temperature kept at 25 °C and the flow rates of all solutions were maintained at 7.0 μL/min. Streptavidin was immobilized twice by injecting a 20 μg/mL of streptavidin solution for 10 min. After the streptavidin chip was prepared, it was washed with buffer at a flow rate of 20 μL/min and 2-min injections of 50 mM NaOH in 1.0 M NaCl to remove the unbound streptavidin. The biotinylated anti-IgE aptamer was then injected, following which the unbound aptamer was washed away under high buffer flow rates. To investigate the non-specific protein binding of the chip before the determination of the anti-IgE aptamer, control experiments were performed by washing different concentrations of BSA, IgG, and fibrinogen (10, 100, and 1000 ng/mL) over the immobilized surface at 7.0 μL/min for 2 min. IgE was diluted with HBS buffer to yield concentrations from 1.0 ng/mL (5.4 pM) to 10,000 ng/mL (54 nM). The analyte was washed over the immobilized surface for 10 min. After measuring the response, the binding reaction between aptamer and protein, the sensor chip was regenerated by injecting 50 mM NaOH in 1.0 M NaCl for 2 min. The data were evaluated using BIAevaluation 3.2.

3. Results and discussion

3.1. Immobilization of streptavidin and biotinylated anti-IgE aptamer onto the ethylene glycol–SAM surfaces

Mixed SAMs consisting of EG_6-COOH and EG_3-OH were constructed on the gold surface as the basal layer for fabrication of the IgE sensor chip. The EG_6-COOH linker containing the ethylene glycol units was helpful in preventing non-specific binding of proteins by imparting hydrophilic properties to the linker chain (Fig. 1(a)). The carboxylic group of EG_6-COOH was selectively biotinylated in the mixed SAMs by the EDC/biotin hydrazide treatment followed by the immobilization of streptavidin onto the biotinylated mixed SAMs (Fig. 1(b)). The biotinylated anti-IgE aptamer was then injected onto the streptavidin surfaces (Fig. 1(c)). Using this SPR sensor chip, human IgE was detected with SPR (Fig. 1(d)).

When the biotinylated anti-human IgE aptamer was injected onto the streptavidin surface, the SPR signals increased by 194 ± 86.15, 433 ± 17.34, and 404 ± 30.76 RU for 1:2, 1:3, and 1:9

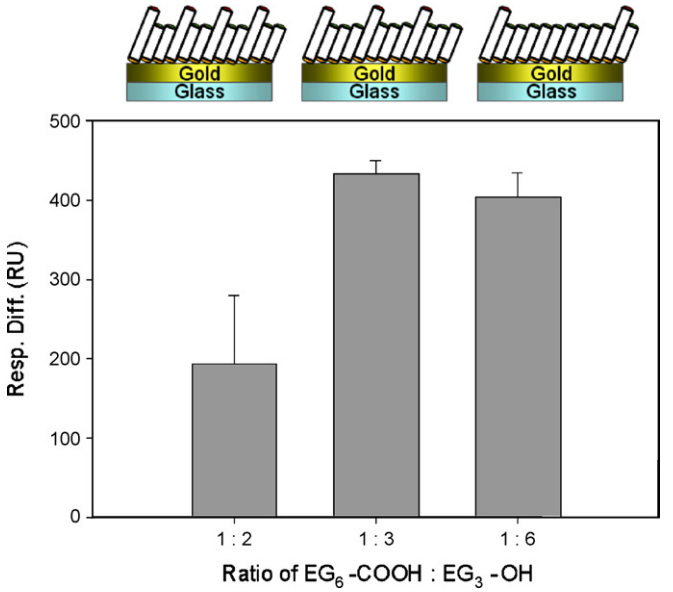

Fig. 2. Immobilization of biotinylated anti-immunoglobulin E (anti-IgE) aptamer onto the streptavidin-immobilized SAM surfaces. The middle of the three bars shows the most stable and highest responses among the three kinds of EG_6-COOH:EG_3-OH surfaces. The error bars illustrate the relative standard deviation (RSD) for three replicates.