



Aptamer biosensor for label-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 EG₆-COOH and EG₃-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 EG₆-COOH:EG₃-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.

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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 Diatex Monoclonals AS (Oslo, Norway).

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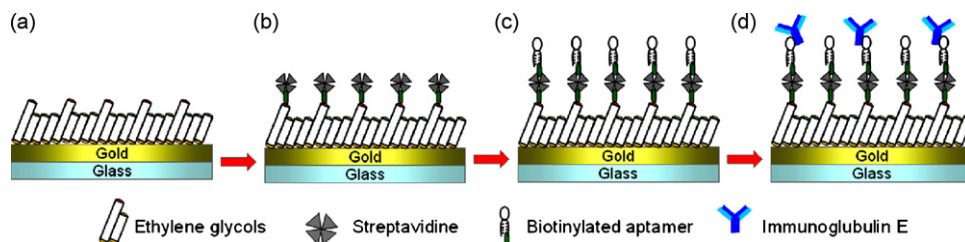


Fig. 1. Schematic diagram of immunoglobulin E (IgE) detection by the anti-IgE aptamer using the surface plasmon resonance (SPR) chip. (a) Carboxylic- and hydroxyl-terminated SAM containing ethylene glycols. (b) Streptavidin immobilization onto the SAM layer. (c) Aptamer immobilization onto the streptavidin 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'-GCGCGGGGCACGTTTATCCGTCCTCCTA-GTGGCGTGCCCCGCGC-biotin-3' (1-mercapto-11-undecyl) tri(ethylene glycol) [HS(CH₂)₁₁(OCH₂CH₂)₃OH, (EG₃-OH)] and 32-mercapto-3,6,9,12,15,18,21-heptaaxadotriacontanoic acid [HS(CH₂)₁₁(OCH₂CH₂)₆OCH₂COOH, (EG₆-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 (EG₆-COOH and EG₃-OH) to form different mixed SAM surfaces. The clean, bare gold chips were separately immersed into absolute ethanol solutions containing EG₆-COOH:EG₃-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 N₂ 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 EG₆-COOH and EG₃-OH were constructed on the gold surface as the basal layer for fabrication of the IgE sensor chip. The EG₆-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 EG₆-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

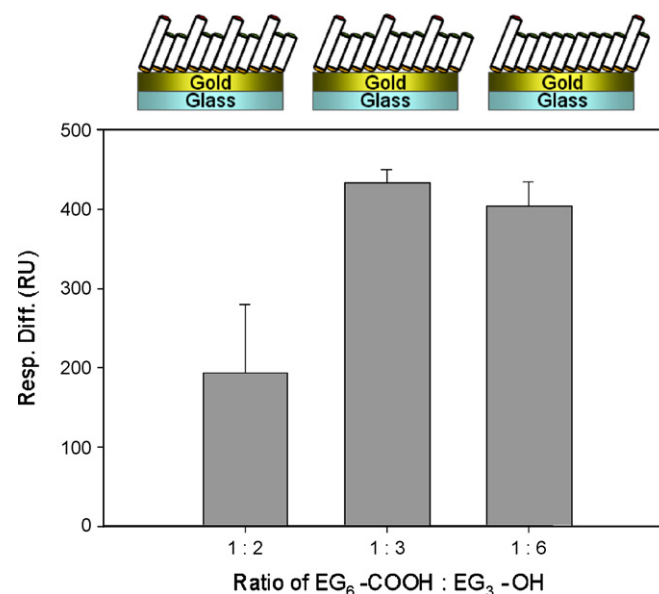


Fig. 2. Immobilization of biotinylated anti-immunoglobulin E (anti-IgE) aptamer onto the streptavidin-immobilized SAM surfaces. The middle of the three bars shows the most stable and highest responses among the three kinds of EG₆-COOH:EG₃-OH surfaces. The error bars illustrate the relative standard deviation (RSD) for three replicates.

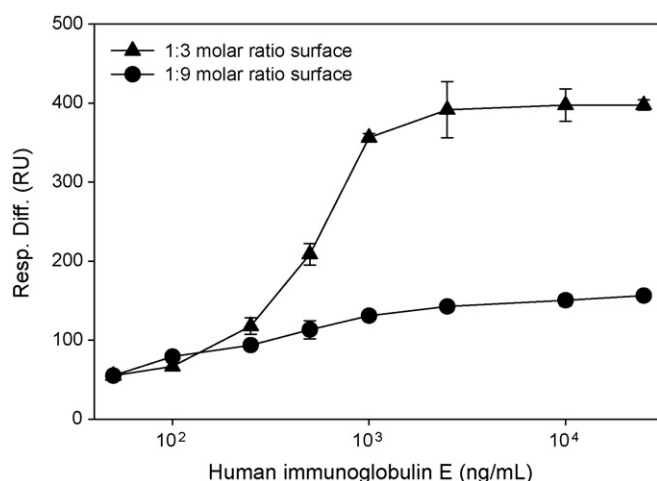


Fig. 3. Comparison of immunoglobulin E (IgE) detection on the SAM surfaces of 1:3 and 1:9 molar ratio of EG₆-COOH:EG₃-OH. The error bars illustrate the relative standard deviation (RSD) for three replicates.

molar ratios of EG₆-COOH:EG₃-OH surfaces, respectively (Fig. 2). The reason for the result is that nominal sizes of proteins and aptamers varied and the anti-IgE aptamer was a single strand nucleotide, 100 times smaller than IgE. In the case of the 1:3 molar ratio surface, the surface could be fully covered with aptamers without steric hindrance or non-specific bindings.

Various concentrations of IgE were detected on two kinds of SAM surfaces, 1:3 and 1:9 molar ratio surfaces, covered with anti-IgE aptamers. Fig. 3 indicates that the 1:3 molar ratio surface was more sensitive than the 1:9 surface because of higher aptamer content. It was also found that the 1:3 molar ratio surface was more suitable for the detection of IgE.

Next, the amount of aptamer was optimized on the 1:3 molar ratio surfaces (Fig. 4). The 1.0 nM anti-IgE aptamer showed the highest and most efficient responses among the tested concentrations of aptamer. Because of limited amounts of carboxylic materials for the SAM surfaces, excess aptamer was easily washed away by running buffer solution above the 1.0 nM anti-IgE aptamer. An anti-IgE aptamer concentration below 1.0 nM was insufficient to cover the -COOH of SAM surfaces.

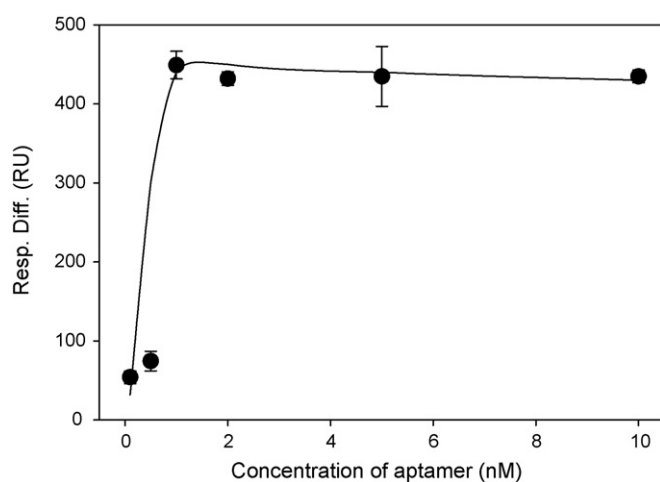


Fig. 4. Immobilization of various concentrations of biotinylated anti-IgE aptamers onto the streptavidin-immobilized 1:3 SAM surfaces. The error bars illustrate the relative standard deviation (RSD) for three replicates.

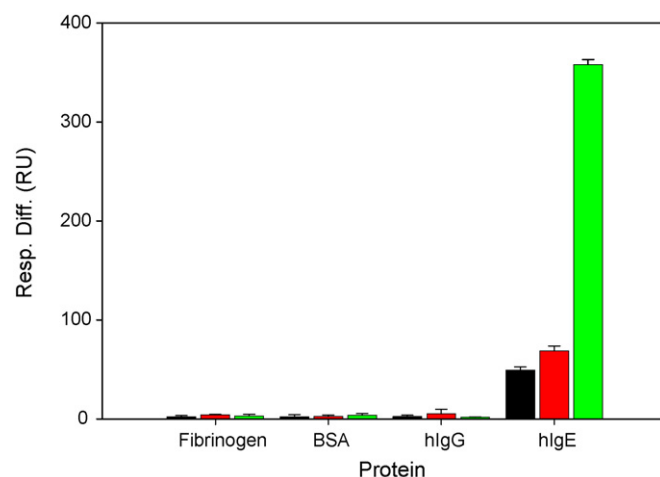


Fig. 5. Non-specific binding of different proteins on the aptamer-immobilized 1:3 surfaces. Each set of three bars indicates the response units generated by injecting concentrations of 10, 100, and 1000 ng/mL, respectively. The adsorption of fibrinogen, bovine serum antigen (BSA), and immunoglobulin G (IgG) was dose-independent and negligible. The error bars illustrate the relative standard deviation (RSD) for three replicates.

3.2. Kinetic analysis of IgE/anti-IgE aptamer interaction based on SPR system

To measure IgE concentration-dependent response changes in the affinity between aptamer and IgE, 1 nM of anti-IgE aptamer was preimmobilized over streptavidin layer, followed by various concentration of IgE (from 0 μ g/ml to 1000 ng/ml) was injected over 1:3 molar ratio SAM surface. To obtain rate constants the injection time was constant (250 s), the association phase and dissociation phase were controlled uniformly [20]. Then, collected sensorgrams were readily fitted to the '1:1 Langmuirian interaction model' using BIAevaluation 3.2 software, suggesting that binding is properly described by the rate equation of $A + B \leftrightarrow AB$ pseudo-first order interaction. Rate constants for association (k_a) and dissociation (k_d) for IgE/anti-IgE aptamer interaction determined by SPR system were $9.61 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $2.21 \times 10^{-6} \text{ s}^{-1}$. This result indicates IgE/anti-IgE aptamer interaction useful to determine quantity of IgE on the SPR system, where anti-IgE aptamer immobilized 1:3 molar ratio SAM surface.

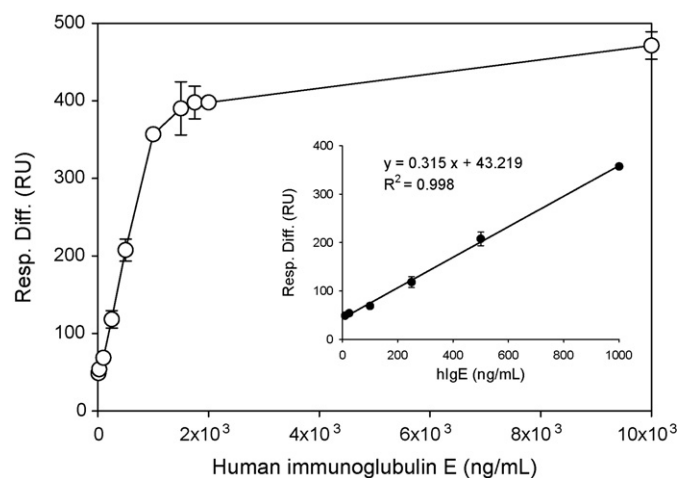


Fig. 6. Calibration curve and zoom of linear range for the detection of IgE obtained from the anti-IgE aptamer-immobilized surface using the BIAcore instrument. The error bars illustrate the relative standard deviation (RSD) for more than three replicates.

3.3. Specificity of anti-IgE aptamer for IgE detection on SAM surfaces

Non-specific binding of different proteins on the aptamer surfaces was investigated. Since human serum includes many kinds of biomolecules, confirmation of specificity is prerequisite. Serum protein such as BSA, fibrinogen, and IgG were added onto the SPR sensor chip at the same concentration. Each protein of 10, 100, and 1000 ng/mL was injected over the anti-IgE aptamer-immobilized 1:3 molar ratio SAM surface. As shown in Fig. 5, each bar indicates the response unit generated by injecting different concentrations of the 10, 100, and 1000 ng/mL, respectively. The data clearly shows that the specific binding of anti-IgE aptamer occurred only for the IgE. Response unit changes of IgE/anti-IgE aptamer interactions were 49.27 ± 3.27 , 68.63 ± 4.99 , and 358 ± 4.98 RU for 10, 100, and 1000 ng/mL, respectively. In the case of other proteins, protein/anti-IgE aptamer interactions were less than 5.0 RUs in diverse concentrations. The adsorptions of fibrinogen, BSA, and IgG were dose-independent and negligible.

3.4. IgE detection by anti-IgE aptamer on the SAM surfaces based on the SPR system

To measure IgE concentration-dependent response changes in the affinity between the anti-IgE aptamer and IgE, 1.0 nM of the anti-IgE aptamer was preimmobilized over a streptavidin-layered 1:3 molar ratio SAM surface, followed by the injection of various concentrations of IgE from 1.0 to 10,000 ng/mL in HBS buffer over an aptamer-preimmobilized surface. Fig. 6 shows a linear range of IgE detection up to 1000 ng/mL, with a minute increment of response units above 1000 ng/mL of IgE.

Since normal individuals have 240–288 ng/mL of IgE, the section of linear range is sufficient for the identification of IgE levels. The linear regression equation for IgE detection by IgE/anti-IgE aptamer interaction was: $y = 0.315x + 43.219$ ($R^2 = 0.998$), where y and x are the response difference units (RU) and analyte (IgE) concentration (ng/mL), respectively. The relative standard deviation (RSD) of the blank sample (zero concentration) was 0.361. The limit of detection (LOD) of the IgE was calculated as three times the standard deviation for the average measurements of blank samples ($\text{LOD} = 3 \times \text{RSD}/\text{slope}$) [21]. Therefore, the LOD was determined to be as low as 3.44 ng/mL (18.5 pM), 3.5-fold lower than that of IgE detection using the anti-human IgE antibody chip [7].

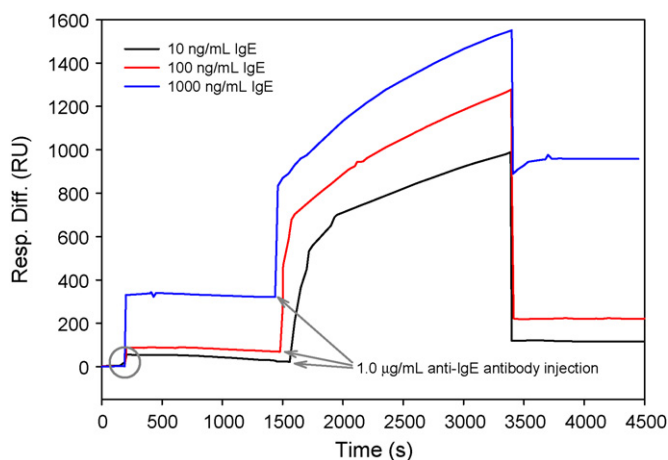


Fig. 7. Signal enhancement of immunoglobulin E (IgE). The circle shows the time when the IgE solutions (10, 100, and 1000 ng/mL) were injected over the anti-IgE aptamer layer; the solid arrows show the time when the 1.0 $\mu\text{g/mL}$ anti-IgE antibody was injected.

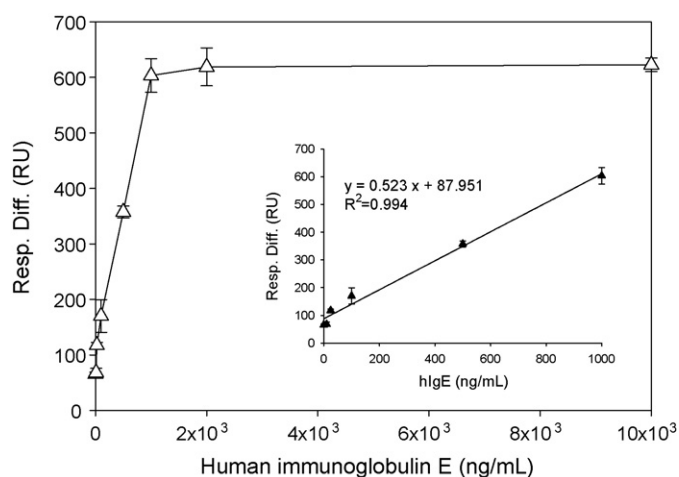


Fig. 8. Calibration curve and zoom of linear range for the detection of IgE obtained from the anti-IgE aptamer and anti-IgE antibody sandwich assay using the BIAcore instrument. The error bars illustrate the relative standard deviation (RSD) for three replicates.

These results show that the SPR aptasensor has broader linear ranges of IgE detection and lower LODs than the SPR sensor based on antibodies. In addition, the aptamers are not proteins, thus the shelf life of an aptasensor chip is longer than that of the immuno-SPR sensor chip.

3.5. Signal enhancement by the modified sandwich method using anti-IgE antibody

To increase the sensitivity of IgE detection, anti-IgE aptamer and anti-IgE antibody were used for the modified sandwich assay. After various concentrations of analytes (IgE) (from 1.0 to 10,000 ng/mL) were added to the anti-IgE aptamer surfaces, 1000 ng/mL of anti-IgE antibody was injected in an IgE-added flow cell.

Fig. 7 shows the real-time sensorgrams of the primary response and enhanced response. The circle in the graph indicates the time when the various concentrations of IgE solutions were injected over anti-IgE aptamer layer and solid arrows show the injection time of 1.0 $\mu\text{g/mL}$ anti-IgE antibody. After injection of anti-IgE antibody, resonance units increased by 67.8 ± 7.12 , 154 ± 24.99 and 600.8 ± 29.98 RU for 10, 100 and 1000 ng/mL of IgE, respectively.

The calibration curves for IgE detection obtained from the anti-IgE aptamer and anti-IgE antibody sandwich assays using the BIAcore SPR instrument system and zoom of linear range are shown in Fig. 8. The linear range of the modified sandwich assay for IgE detection was ~ 1000 ng/mL, the same as the direct assay with the linear regression equation for the IgE/anti-IgE aptamer interaction being: $y = 0.523x + 87.951$ ($R^2 = 0.994$). The LOD of the IgE detection in this SPR sensor with the anti-IgE aptamer and anti-IgE antibody (modified sandwich assay) was 2.07 ng/mL (11.1 pM), about half the LOD of primary IgE detection by the anti-IgE aptamer layer only (direct assay).

4. Conclusions

Given its high affinity, stability, and small size, the anti-IgE aptamer was chosen as the ligand for the SPR system designed for IgE detection. Accordingly, we have effectively demonstrated an aptamer-based sensor (aptasensor) chip for allergy diagnosis by a non-labeled detection of IgE.

Firstly, an SPR-based gold chip was fabricated. In order to make a vehicle for the investigation of specific interactions at the bare gold surface, three kinds of molar ratios of $\text{EG}_6\text{-COOH}:\text{EG}_3\text{-OH}$ SAM

were evaluated. A 1.0 nM anti-IgE aptamer surface, on the 1:3 molar ratio SAM surface, was suitable for high-resolution detection of IgE and showed higher aptamer coverage. With the anti-IgE aptamer system, a wide range of IgE concentrations could be detected. Two kinds of aptamer sensors based on the SPR system for IgE detection were also demonstrated, namely the sensor on the anti-IgE aptamer layer and the anti-IgE aptamer/anti-IgE antibody sandwich assay. Furthermore, the SPR-aptasensor discriminated between IgE and other proteins such as IgG and fibrinogen with high affinity.

Based on the optimum conditions, the direct assay and modified sandwich assay were investigated for real-time IgE detection by the aptamer. The linear ranges for both were 1.0–1000 ng/mL and the lowest amounts of detectable analytes in the sample were 3.44 and 2.07 ng/mL, respectively. These LODs were 3.5- and 6-fold lower than those of the anti-human, IgE-immobilized SPR chip, respectively.

These results enabled diagnosis of allergy disease by the detection of IgE since allergic patients show high total serum IgE (greater than 290 ng/mL). In addition, the aptasensor showed a linear IgE detection range broader than the SPR sensor based on antibodies and offers the possibility of a simple and realistic platform.

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