Real-time Detection of α-Thrombin Binding to Single-Strand DNA Aptamers by a Highly Sensitive Si-Based Waveguide SPR Biosensor

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Abstract

In this paper, real-time characterization of α-thrombin binding to single-strand DNA (ssDNA) aptamers by novel Si-based waveguide SPR biosensors has been investigated. The gold nanoparticles (AuNPs) modified with anti-thrombin antibodies were employed to bind with α-thrombin via strong antibody/antigen affinity for SPR signal amplification. The detection limit of 1 pM for α-thrombin detection was achieved. .
Keywords: SPR, surface plasmon resonance, sensor

1. Introduction

Surface plasmon resonance (SPR) phenomenon is a charge-density oscillation that exists at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric [1]. SPR biosensor is a label-free optical sensor that measures molecular binding events on a metal surface in real time by detecting the thickness or effective refractive index change of the immobilized biomolecular layer with high throughput. In this paper, a novel antiresonant reflecting optical waveguide of type B (ARROW-B) is proposed to support the low-loss TM-polarized transmission required to excite the SPW at the interface between metal and dielectric layers [2]. The ARROW-B SPR biosensor has the following advantages: realatively large core size which is suitable for the efficient connection to a single-mode fiber, flexible design rules for choosing optical materials and the thickness, and compatible with standard semiconductor fabrication process.

In this research, the binding characteristics of α-thrombins to ssDNA aptamers are chosen as the primary analytes for the following biomedical assays. α-Thrombin is a serine protease with multiple functions in both procoagulant and anticoagulant abilities, and it is the only protein capable of catalyzing the cleavage of fibrinogen to produce fibrin clot [3], which can facilitate coagulation process and prevent hemorrhage. Unfortunately, the malfunction of α-thrombin can lead to hemorrhage, and an excessive coagulation function results in dissemination of the clot in undamaged tissues and results in thrombosis. Therefore, to specifically inhibit α-thrombins in vivo with synthetic compounds is an important goal in the prevention of thrombosis. There are several α-thrombin-binding DNA aptamers discovered over the past decade. The most extensively studied one is a 15-mer single-stranded DNA (AP15) with sequence of 5'-GGTTGGTGTGGTTGG-3' to form an intermolecular quadruplex structure and binds to thrombin at the fibrinogen recognition site (FRE) [4].

2. Experiments

2-1. Materials

Phosphate buffered saline (PBS) consisting of 2.7 mM KCl, 1.5 mM KH_2PO_4, 140 mM NaCl, and 8.1 mM Na_2HPO_4 at pH 7.2 was used. PBS is isotonic and non-toxic to cells, and thus its primary function is to dilute the biomolecular reagents. Glutaraldehyde (GA), $CHO(CH_2)_3CHO$, with concentration of 2.5 % solved in the phosphate buffered saline (PBS) was employed to tightly immobilize on the Au surface via the covalent binding between an aldehyde functional group and gold. Streptavidin (SA), with concentration of 0.1 mg/ml was solved in the PBS buffer. The biotinylated AP15, with concentration of 1 μM solved in PBS buffer, served as primary probes specifically recognizing α-thrombins with great affinity and accuracy. A 25-mer DNA spacers were introduced into the 15-mer probes to increase the accessibility of the AP15. Glycine with concentration of 1 M served to block the remaining active aldehyde groups of GA.. The molecular weight (M.W.) of α-Thrombin is 37423 Da. Fig. 1 shows

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the schematic presentation for the procedure of the bioassay experiment on real-time detection of α-thrombin binding to the AP15. First, the Au surface was flushed by the PBS buffer to reach a baseline as shown in Fig. 1 (a). GA was then injected into the circulating-flow system and strongly immobilized on the Au surface Fig. 1 (b). PBS buffer was injected into the flow system without circulation between two adjacent sample injections to remove the unbound biomolecules. Later, SA was injected to bind to the immobilized GA as shown in Fig. 1 (c). The biotinylated AP15 were injected to bind to SA to form a GA-SA-biotinylated AP15 sandwich complex as shown in Fig. 1 (d). Glycine was then injected to block the active aldehyde groups of GA as shown in Fig. 1 (e). α-Thrombins were injected and captured by immobilized AP15 as shown in Fig. 1 (f). Finally, AuNPs modified with anti-thrombin antibodies were applied to bind with the α-thrombins via high antibody-antigen affinity as shown in Fig. 1 (g).

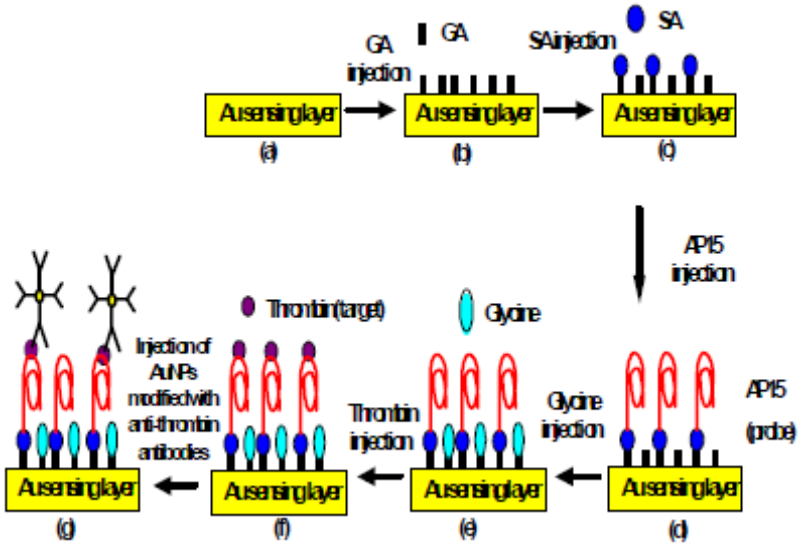

Fig. 1: Schematic presentation of the procedure for the bioassay experiment on real-time detection of α-thrombin binding to AP15.

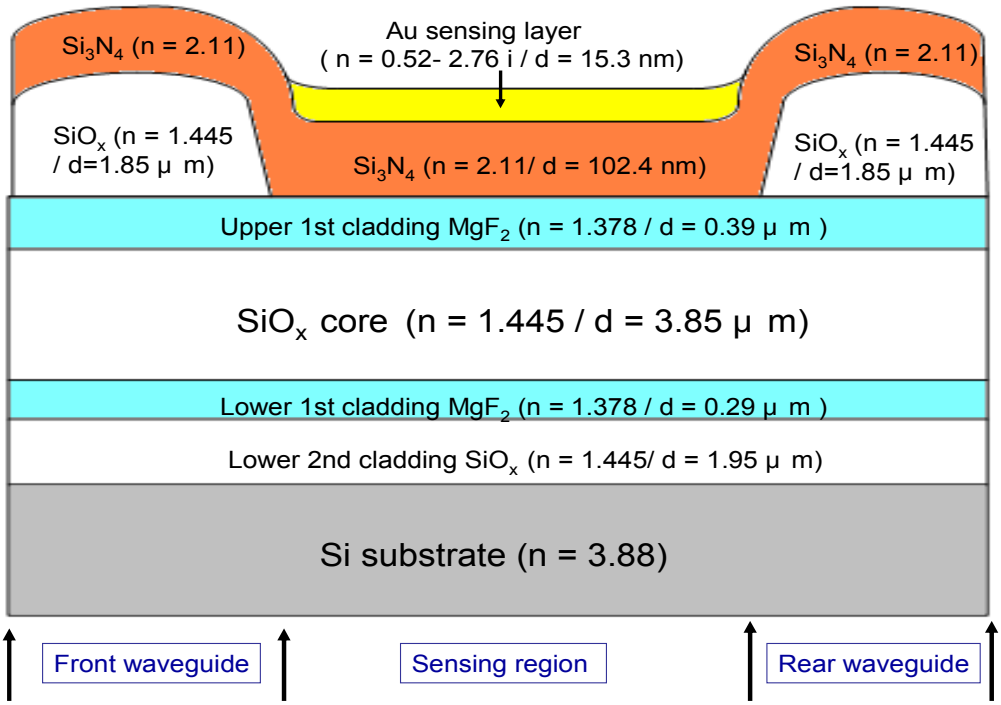

Fig. 2:Cross-section of Si-based ARROW-B SPR sensor..

2-2. Design and fabrication of Si-Based Waveguide SPR biosensors

The ARROW-B is an optical waveguide which consists of one core and two lower cladding layers. The refractive indices of the core and second cladding layers are both higher than that of the first cladding layer. Light propagates in the core by frustrated total internal reflection. A Fabry-Perot cavity is designed within the second cladding layer as a reflector. In this study, a symmetrical ARROW-B structure was introduced to greatly reduce the propagation loss in this waveguide region resulting from the change of the outermost environment and the scattering loss caused by the fabrication imperfections at the core/superstrate boundary. The cross-section of our Si-based ARROW-B sensor is shown in Fig. 2 [2]. The front and rear waveguides with upper cladding layers and a Si_3N_4 adjusting layer can support low-loss propagation of light even in these waveguide regions when a high-index O-ring is applied on the sensor chip during biomedical assay. Our device were fabricated with available standard semiconductor prosessing.

2-3. Optical measurement system

An optical measurement system were applied to perform the given bioassay experiments on real-time detection of α-thrombin binding characteristics to AP15. For the optical measurement system, a He-Ne laser of wavelength 633.2 nm was applied as the light source. The TM-polarized light was focused on the input channel of the waveguide by an objective lens (10x). At the output end, the beam was focused by an objective lens (20x). Furthermore, an iris diaphragm was used to filter out the light noise. The output light signal was collected and measured by a photodetector connected to a powermeter and the data were transmitted to a personal computer with a LabVIEW software to record and store the data digitally. The circulating-flow system consisted of one sample bottle, two sample tubes with 1 mm inner diameter connected to a micro-pump with a flow rate range of 10-200 μl/min, and a sample loop of volume of 1.50 ml. To construct an seamless liquid flow channel on the sensor chip, an O-ring was added on the chip and sandwiched by two plastic slides.

3. Results and discussions

Fig. 3 shows the typical SPR response curve of the real-time detection of the α-thrombin binding to the AP15 by an ARROW-B SPR biosensor. The flow rates of all sample solutions were controlled at the speed of 0.17 ml/min. First, the Au surface was flushed by the PBS buffer for 5 minutes to reach a baseline. Later, GA was injected into the circulating-flow system for 10 minutes. The output power increased significantly at t = 300 s due to strong

immobilization of GA on the Au surface. PBS buffer was injected into the flow system without circulation between two adjacent sample injections to remove the unbound biomolecules and clean the sample tubes. Therefore, all the output power decreased slightly due to the decrease in thickness and effective refractive index of the biomolecular layer upon Au sensing layer. Later, SA was injected into the circulating-flow system and bound to the immobilized GA via the other aldehyde group of GA. The output power decreased at t = 1300 s due to the refractive index change of the biomolecular layer upon Au sensing layer induced by effective binding between GA and SA. Next, the biotinylated AP15 (probe) were injected into the circulating-flow system and bound to the immobilized SA via the strong SA-biotin affinity. The decrease of output power at t = 2130 s resulted from the refractive index change of the biomolecular layer induced by the formation of a GA-SA-AP15 sandwich complex. Glycine was subsequently injected into the circulating-flow system to block the active aldehyde groups of GA at t = 2960 s. The output power increased substantially as a result of the great filling ability of the small-sized glycines in the voids of the sandwiched GA-SA-AP15 complex upon Au sensing layer. α-Thrombins were then injected into the circulating-flow system and captured by the immobilized AP15 at t = 3650 s. Thus, the output power decreased due to the refractive index change of the biomolecular layer induced by the binding of AP15 to FRE site on the α-thrombin surface. Finally, as surface-modified AuNPs were injected into the circulating-flow system to bind to the α-thrombins via antibody/antigen affinity, the refractive index of the biomolecular layer changed accordingly. Consequently, the output power decreased slightly at t = 4650 s.

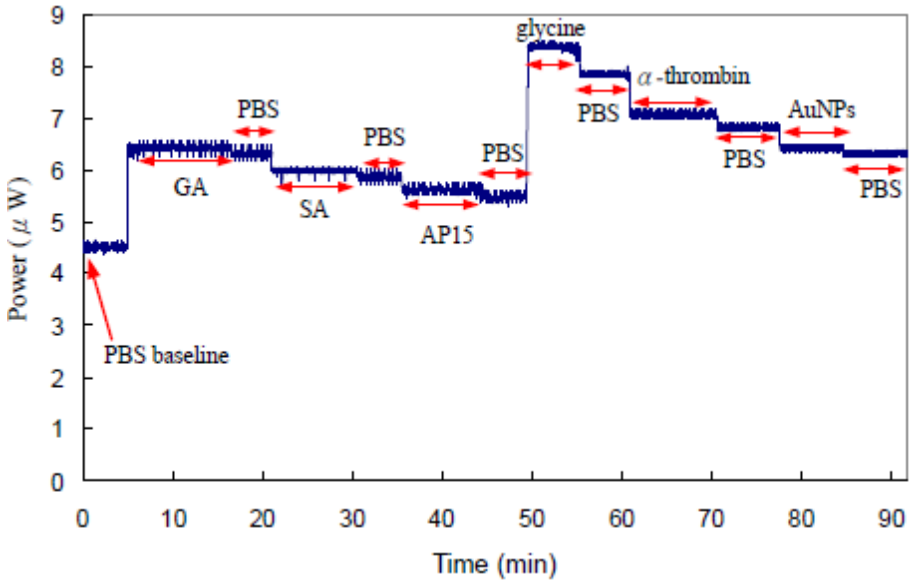

Fig. 3: Real-time detection of α-thrombin binding to AP15 by an ARROW-B SPR biosensor. The Au sensing layer surface was exposed to several sample solutions in the following order: GA (2.5%), SA (0.05 mg/ml), biotinylated ssDNA aptamer AP15 (1 μM), glycine (1 M), α-thrombin (16.2 nM) binding to AP15, and finally AuNPs (1 μg/ml) binding to α-thrombin. Between two adjacent injections, PBS buffer was applied to rinse the Au surface for 5 minutes.

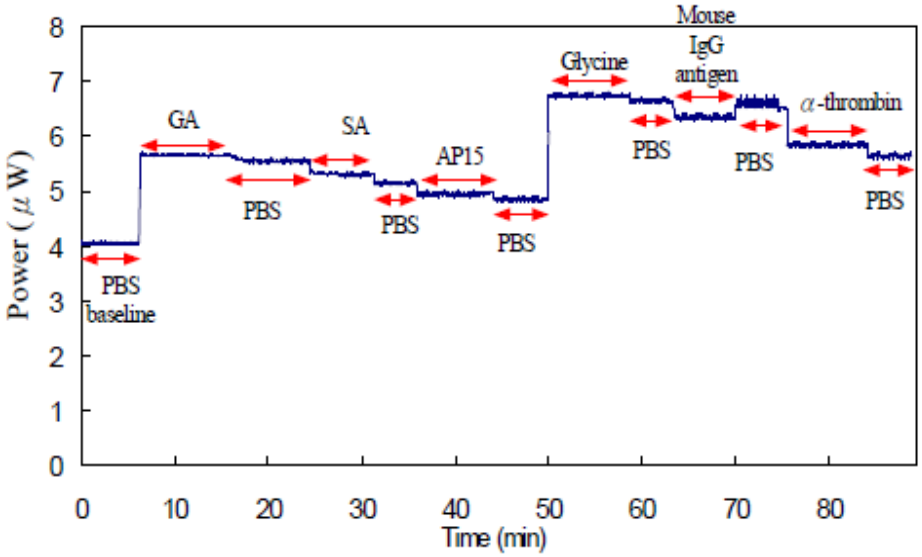

Fig. 4: The influence of non-specific binding between mouse IgG antigens and AP15 on the SPR signals. It can be observed that the output power almost returned to the value prior to the injection of non-specific mouse IgG antigens after the PBS rinse. Finally, SPR signal induced by the specific binding between the α -thrombin and AP15 can be observed as the α -thrombin was injected into the circulating-flow system.

To investigate the influence of non-specific molecular binding to AP15 on the SPR signals, a control experiment was performed by injecting the mouse IgG antigens into the circulating-flow system as shown in Fig. 4. The Au sensing layer was exposed to several sample solutions in the following order: GA (2.5%), SA (0.05 mg/ml), biotinylated AP15 (1 μM), glycine (1 M), mouse IgG antigen (0.1 μg/ml), and finally α-thrombin (16.2 nM). Between two adjacent injections, PBS buffer was applied to rinse the Au surface for 5 mins. The flow rates of all sample solutions were controlled at the speed of 0.17 ml/min. It can be observed that the output power almost returned to the value prior to the injection of non-specific mouse IgG antigens after the PBS rinse. The possible cause of the small shift from the original value can be attributed to the very weak interaction between AP15 and mouse IgG antigens. Finally, SPR signal induced by the specific binding between the α-thrombin and AP15 can be seen as α-thrombin was injected into the circulating-flow system. Therefore, we can confirm that the effect of non-specific binding between the mouse IgG antigens and the AP15 on the SPR signals was insignificant.

4. Summary

A novel Si-baesARROW-B SPR biosensor operating in the aqueous environment has been investigated. The real-time detection of α-thrombins binding characteristics has shown the detection limit of this ARROW-B SPR biosensor to α-thrombin was at 1 pM.

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