Sensors and Actuators B 252 (2017) 813–821

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Screening and evaluation of aptamers against somatostatin, and sandwich-like monitoring of somatostatin based on atomic force microscopy

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ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form 29 May 2017
Accepted 4 June 2017
Available online 16 June 2017

Keywords:
Aptamer
Aptasensor
Systematic evolution of ligands by exponential enrichment
Atomic force microscopy
Peptides
Somatostatin

ABSTRACT

A sensing system was constructed to monitor the target peptide via two aptamer-based sensors pinching. First, aptamers against somatostatin (SST) were selected via the systematic evolution of ligands by exponential enrichment, and four aptamers were selected from a single-stranded DNA library. Their specificities to SST were evaluated via surface plasmon resonance and atomic force microscopy (AFM). Next, two aptamers with higher specificities to SST were used as aptamer-based sensors; one aptamer was modified with a chip, and the other was modified with a probe. Based on AFM system, the probe was surveyed on the chip in SST solutions, simultaneously measuring an interactive force. The label-free SST could be detected, and then the change in its concentration could be monitored at levels that ranged from 2 to 2000 nM. The interactive force of a single pair was approximately 45 pN, and the molecule number was associated with the interactive force. Therefore, we could firstly select the aptamers against somatostatin, and the sandwich-like monitoring system can be used to promote peptide sensor or monitoring system using an aptamer-based sensor.

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1. Introduction

Detecting and monitoring peptides is required for evaluation or as an assurance of quality in various research fields such as food handling [1] and therapeutic biological evaluation [2]. Peptides generally contain approximately 50, or fewer, amino acids, and some peptides serve as hormones that exert control over biological functions [3–7]. For example, somatostatin (SST) regulates the endocrine system and inhibits the release of growth hormones [4,6], and angiotensin (AGT) causes an increase in blood pressure via the renin-angiotensin-aldosterone system [5,7]. Although their functions are important for a living body, few tools have been developed for their detection and monitoring *in vitro*. For example, liquid chromatography/mass spectrometry (HPLC/MS) has been mainly used for the sensing or monitoring of peptides [8,9]. The HPLC/MS method requires multiple analysis steps, and a bio-analytical method such as the use of biosensors is highly desirable [10,11].

Aptamer technology has been applied in the use of biosensors and in drug development. Aptamers are single-stranded nucleic acids that contain between 30 and 70 nucleotides, and they can generally interact with high specificity to a wide variety of target molecules [12]. The most effective way to *in vitro* select a highly specific aptamer is referred to as the Systematic Evolution of Ligands by EXponential enrichment (SELEX) [13–16]. SELEX consists of an iterative process of screening from an oligonucleotide pool followed by an amplification of the screened aptamers. Once they are selected, aptamers can be used as biotechnological tools in processes such as cancer chemotherapy [17,18] and as electronic sensors [19,20]. For example, an aptamer-based sensor (referred to aptasensor) has been used to detect chiral peptides via an electrochemical method [20]. However, an aptasensor for peptides is still in the early stages of development, and is used mainly as an electrochemical sensor. The various uses of these sensors for evaluation will require novel methodologies.

Aptasensors have been applied to molecular sensing via atomic force microscopy (AFM). AFM has progressed as a method for topographical imaging via a probe (referred to as a cantilever) [21], and has also been used to measure interactive force via probes modified with biomolecules [22–24]. Previously, AFM sensing systems have

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http://dx.doi.org/10.1016/j.snb.2017.06.019
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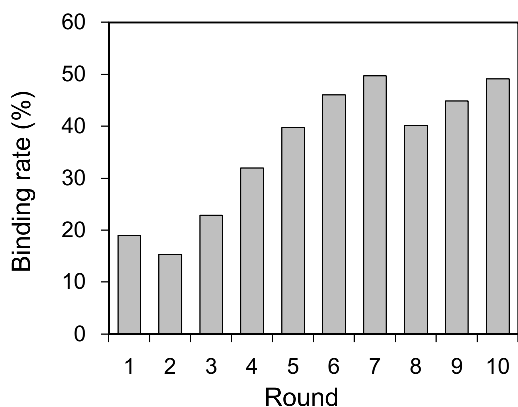

Fig. 1. Binding rate in each of the SELEX rounds. Binding rate is the ratio of the column bound ssDNA in the total added ssDNA as an index of a concentration.

combined an aptamer-modified cantilever with a target protein-labeled chip [22]. Based on this reported methodology, a novel AFM sensing method has been proposed to detect and monitor a label-free peptide.

The methodology proposed in the present study was focused on targeting SST as a model peptide. An aptamer against SST was selected from a single-stranded DNA (ssDNA) library via SELEX, and then the specificities of the aptamers were evaluated via surface plasmon resonance (SPR) and AFM. Then, either the cantilever or the chip was modified with two types of aptamers that were highly specific to SST. As the aptasensors, these aptamers were modified with the cantilever or the chip. Interactive force measurement could be performed in a SST solution. Then, the interactive forces can be indexed for monitoring the change in SST concentration. The overall average interactive force was correlated with the level of SST concentration, and statistical fitting analysis enabled an estimation of the SST number. Therefore, the sandwich-like monitoring system based on AFM should enhance peptide sensing or monitoring.

2. Material and methods

2.1. Reagents, buffers, and the cantilever

Dithiobis[sulfosuccinimidylpropionate] (DTSSP) and SM-$(PEG)_{12}$ were purchased from Pierce (Missouri, USA). *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from GE Healthcare Japan, Ltd. (Tokyo, Japan). The peptides, SST and AGT, and streptavidin from *Streptomyces avidinii* were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

Folding buffer (50 mM Tris-HCl, 300 mM NaCl, 30 mM KCl, 5 mM $MgCl_2$, pH 7.6), Tris-EDTA buffer (TE; 10 mM Tris-HCl, 1 mM EDTA), phosphate buffered saline (PBS; 100 mM phosphate, 150 mM NaCl, pH = 7.5), and Tris-borate-EDTA buffer (TBE; 10.8 g/L Tris, 0.002 M EDTA, 5.5 g/L Boric acid) were used as either a wash or a measurement solution.

A gold-coated silicon cantilever (Olympus Co. Ltd., Tokyo, Japan, BL-RC150VB-C1, spring constant: 0.006 N/m) was used in all experiments.

2.2. Selection of the aptamers against SST

The aptamers against SST were selected based on previously reported methods [25,26]. A double-stranded DNA (dsDNA) library was prepared as follows. In brief, a synthetic DNA oligonucleotide (95 mer) library with 50 random nucleotide (N50) sequences was amplified over 20 cycles of PCR (95 °C, 15 s; 55 °C, 15 s; 68 °C, 30 s) using primers of P1 and P2 (Table 1). The dsDNA library used in the 1st round was prepared via the purification of DNA oligonucleotides of 80–110 bp.

Table 1
Sequences of the primers that were used.

Primer	Sequence
N50	5′-CAGCTCAGAAGCTTGATCCTGTG-N50-GACCTCGAAGTCGTGCAT-3′
P1	5′-CAGCTCAGAAGCTTGATCCTGTG-3′
P2	5′-TGCAGATGCACGACTTCGAGTC-3′
P3	5′-biotin-TGCAGATGCACGACTTCGAGTC-3′

A biotinylated ssDNA library was prepared as follows. The dsDNA library was amplified over 20 cycles of PCR (95 °C, 15 s; 55 °C, 15 s; 68 °C, 30 s) using primers of P1 and P3 (Table 1). Then, the amplified dsDNA library was purified as follows. An avidine-modified column was prepared via the addition of 100 μL of streptavidine sepharose to a microspin column (GE Healthcare Japan Ltd.). The amplified dsDNA library was reacted in the avidine-modified column for 30 min. The column was then washed four times with a folding buffer followed by reacting 200 μL of 0.15 M NaOH for 5 min. The ssDNAs in the column were eluted and the biotinylated ssDNA library was neutralized via 200 μL of 0.15 M HCl. Finally, the concentration of biotinylated ssDNAs was adjusted to 2 μM.

An aptamer that would specifically bind to SST was selected via a SST-modified gel, as follows. First, the SST-modified gel was prepared by adding NHS gel to a chromatography column (GE Healthcare Japan Ltd.). The column was then washed three times with 10 mL of 1 M HCl. Then, 1.5 mL of 1 M HCl was left in the column, and 500 μL of a 2.5 mg/mL SST solution in PBS was added to the column. After reaction, the solution was poured out, and 1 M Tris-HCl was reacted to block the unreacted NHS. The column was washed with 50 mL of the folding buffer, and then 2 mL of the folding buffer was added. The aptamer that specifically interacted with SST could then be selected via SELEX. The SST-modified gel was added into the microspin column (GE Healthcare Japan Ltd.) and this column was washed with 400 μL of the folding buffer. The solution of biotinylated ssDNA library was then heat-shocked and allowed to slowly cool at room temperature. Then, 150 μL of the biotinylated ssDNA library was added into the microspin column with the SST-modified gel, which was allowed to react for 30 min. The reacted column was washed four times with 400 μL of the folding buffer, and then 0.5 g/L of SST solution was reacted for 1 h in the folding buffer. After the reaction, 20 μL of chloroform was added into the eluted solution to establish structural disorder in the SST. The solution was separated via centrifuge (condition: 190 g, 10 min), and then 3 μL of glycogen was added. The eluted ssDNA library was dissolved into 30 μL of TE after the ethanol precipitation. Then, the eluted ssDNA library was amplified over 20 cycles of PCR (95 °C, 15 s; 55 °C, 15 s; 68 °C, 30 s) using primers of the P1 and P2 (Table 1). The 1st round of the dsDNA library was prepared to extract and purify the DNA to a level that fell between 80 and 110 bp. In each of the SELEX rounds, both the preparation of the biotinylated ssDNA library and selection via the SST-modified gel was performed. Non-specific ssDNAs were removed from the 7th round for use as a chromatography column without SST modification, before selection via the SST-modified gel.

After the 10th round, the dsDNAs were cloned into *Escherichia coli* using a TA cloning kit for DNA sequencing. DNA sequencing was carried out using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, CA, USA) and a BigDye-terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's protocol. The secondary structure was evaluated via the

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