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Aptamer-Immobilized Surface Plasmon Resonance Biosensor for Rapid and Sensitive Determination of Virulence Determinant

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Shigella sonnei isolate invasion plasmid antigen protein, IpaH, was successfully expressed in recombinant overexpression bacterial system. The soluble expression IpaH was enhanced with molecular chaperon co-expressed environment. Specific aptamer IpaH17 was isolated through the SELEX process and showed fM binding affinity. IpaH17-SPR biosensor platform was involved to verify the binding sensitivity and specificity. The IpaH concentration dependent IpaH17-SPR sensor response was highly linear with a linear regression constant of 99.4% in the range between 0 and 100 ng/mL. In addition, *S. sonnei* revealed the specific RU value and detected in a real-time manner within 1 hour. Our study indicated that IpaH17-SPR sensor can allow for rapid, sensitive and specific determination of *Shigella sonnei* virulent factor.

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1. INTRODUCTION

Shigella species are the causative agent of shigellosis, also known as bacillary dysentery whose symptoms range from dysentery, tenesmus, and abdominal cramps to diarrheoa with bloody and mucous stools.[1] According to the Centers for Disease Control and Prevention (CDC), 165 million people suffer from shigellosis and 600,000 deaths were reported annually worldwide. Most of these deaths occur in developing countries and children under age five. *Shigella* genus comprises of four species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*, *S. flexneri* is the most common species in developing countries whereas *S. sonnei* is the predominant species in industrialized countries.[1] In South Korea, approximately 500 cases of shigellosis by *S. sonnei* infection have reported every year since 2004.[2] Based on the report of Korea Centers for Disease Control and Prevention (KCDC) from 2010 to 2011, over 75% among shigellosis outbreaks were related to an influx of people entering South Korea with a history of international travel.[3–5]

To date, shigellosis tends to spread rapidly in closed and concentrated groups such as army barracks and school by mass feeding.[6] Thus, there is the urgent need for rapid, simple, and specific detection methods for shigellosis. The conventional stool culture methods are time-consuming and can detect only a small fraction of shigellosis because *Shigella* species are fastidious bacteria and can survive poorly outside the human body.[7] To overcome the limitations of traditional method, some studies have developed molecular diagnostic methods like real-time PCR[8,9] and PCR-ELISA.[1] Although molecular detection methods using virulence genes are highly sensitive, they still have limitations as follows: (1) non-specific amplification

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and primer dimerization, (2) requirement of complex lab equipment such as gel-electrophoresis apparatuses and signal read-out machines, (3) low through-put analysis and (4) time-consuming etc.

Many approaches for detecting pathogenic bacteria have been investigated.[6,11–14] Surface Plasmon Resonance (SPR) analysis is one of the representative strategies that offer the advantage of label-free and real-time detection method in pathogen-biosensor platform. Biosensors based on plasmonic nanostructures have become subject of extensive research and have been featured in numerous recent studies.[15–18] By enabling real-time investigation of biomolecular interactions, SPR biosensors have become an important tool in molecular biology. In SPR biosensor, target analytes in assay sample can be flowed over the surface functionalized by the recognizing elements. Aptamers are single-stranded oligonucleotides (DNA or RNA) capable of folding into well-defined structures and motifs that allow them bind to various target molecules with high specificity and affinity. Aptamers have a wide range of advantages over other existing sensors such as stability, design flexibility and cost-effectiveness. Specific aptamers were considered as a recognizing element for the small molecule targets instead of antibody performance in SPR sensor platform. They are predisposed for SPR applications due to their stability and small size relative to antibodies.[13] Our SPR-aptamer sensor offers a convenient and specific platform for diagnosing Shigellosis that could be of great interest in the pathogen detection.

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Shigellosis is a disease caused by the *Shigella* bacteria itself or their toxin molecules.[10,19–21] Upon contact with epithelial cells, *Shigella sonnei* expresses effector (Invasion plasmid antigen; Ipa) proteins that promote invasion and dampen the inflammatory action of host cell via type III secretion system.[22] These virulence determinants of *Shigella* are encoded in the large virulence plasmid. The ipaH genes, one of the effector genes, are found as multiple-copy element on both the plasmid and the chromosome.[23]

We carried out SELEX (Systematic evolution of ligands by exponential enrichment) process to obtain DNA aptamers with high affinity and specificity against IpaH protein. After selection of aptamers, IpaH-binding DNA aptamers were applied to SPR-based biosensor. This IpaH aptamer-immobilized SPR biosensor cause rapid and sensitive determination of *Shigella sonnei* and prevents the spread of shigellosis.

2. RESULTS AND DISCUSSION

2.1. Confirmation of Plasmid pGEX-4T-1-ipaH

The ipaH gene of *S. sonnei* was successfully amplified by PCR using a pair of designed primers and cloned into pGEX-4T-1 prokaryotic expression vector, which enables the expression of GST-IpaH fusion protein. The cloning of ipaH gene was confirmed by restriction digestion assay (Fig. 1). The digested recombinant vector formed two band; ~318 bp ipaH gene and ~4922 bp pGEX-4T-1 vector. The restriction digestion assay indicates that ipaH gene was successfully inserted into the pGEX-4T-1 vector.

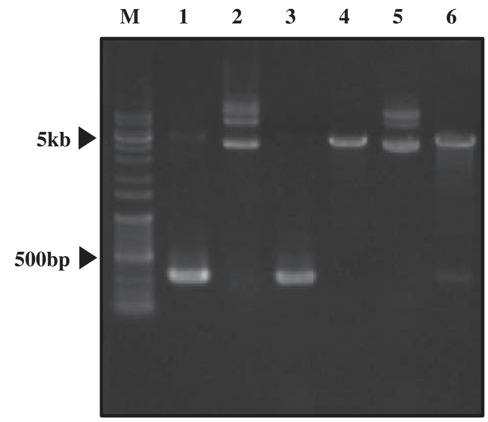

Figure 1. ipaH gene amplification and restriction analysis. Lane 1: PCR product of ipaH gene (321 bp); lane 2: pGEX-4T-1 vector; lane 3: Digested ipaH gene; lane 4: Digested pGEX-4T-1 vector; lane 5: pGEX-4T-1-ipaH recombinant plasmid; lane 6: Digested pGEX-4T-1-ipaH (BamHI and XhoI).

2.2. Increased Soluble Expression of IpaH Protein by pTf16 Co-Expression

The final construct, pGEX-4T-1-ipaH, was transformed into BL21(DE3) harboring pTf16 molecular chaperone vector to increase soluble expression of recombinant IpaH protein. As shown in Figure 2(a), co-expression with pTf16 chaperone vector showed the apparent increased solubility of IpaH in comparison to pGEX-4T-1-ipaH expression. The optimization experiments revealed that 18 °C, 0.5 mM IPTG, pTf16 co-expression reached the highest soluble expression of IpaH protein due to the GroEL-GroES molecular chaperon activity that prevents

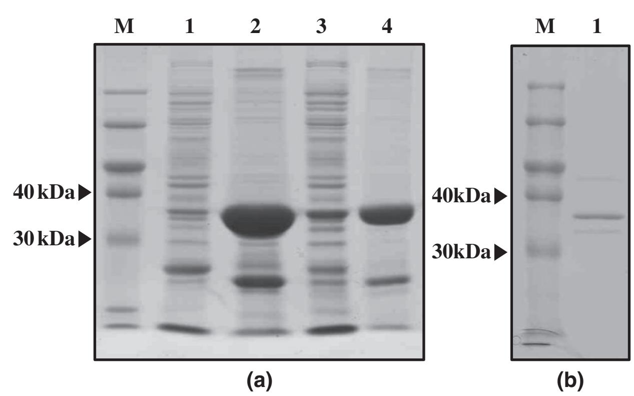

Figure 2. (a) Coexpression of IpaH with molecular chaperon, M: Protein size marker; lane 1: Soluble fraction of pGEX-4T-1-ipaH expression; lane 2: Insoluble fraction of pGEX-4T-1-ipaH expression; lane 3: Soluble fraction of pGEX-4T-1-ipaH co-expression with pTf16; lane 4: Insoluble fraction of pGEX-4T-1-ipaH co-expression with pTf16. (b) Purification of IpaH protein.

aggregation of IpaH protein in *E. coli*.[24] The GST-IpaH fusion protein overexpressed in optimized condition was purified using glutathione sepharose 4B bead. Purified GST-IpaH fusion protein appeared as a single band about 37 kDa on SDS-PAGE after coomassie brilliant blue R250 staining (Fig. 2(b)).

2.3. *In Vitro* Selection of IpaH Binding DNA Aptamers

The SELEX process was carried out to isolate ssDNA aptamers for IpaH protein from random DNA library of approximately 10^{16} diverse sequence. After preparation of single stranded DNA library (Fig. 3(a)), the glutathione sepharose bead was used as a solid-support for partitioning of protein-aptamer complexes. To eliminate support-bound DNA aptamers, we incorporated counter-selection step (negative selection, NS) against glutathione sepharose bead after 7th selection round. Unbound ssDNA in supernatant was then utilized to next round selection. The eluted ssDNA concentrations from each selection round were quantified by nanodrop spectrophotometer (Thermo, USA). As shown in Figure 3(b), the concentration of eluted ssDNA showed the tendency to increase as the selection round progressed until 7th round and then decreased. Since the eluted concentration has reached the highest at 9th selection round after counter-selection, the 9th selection round was selected as the optimal state. To identify aptamer that recognize IpaH protein, the DNA aptamer pool of 8th selection round with the highest IpaH binding affinity was cloned and followed by sequencing of 32 clones. By multiple sequence alignment using ClustalX software program, a total of 19 aptamers with intact random sequence were obtained (see Table I).

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2.4. Affinity Analysis of IpaH Binding Aptamers

The binding affinities of the identified 19 aptamers for IpaH protein were analyzed by Biacore 3000 instrument as described in Materials and Method section. IpaH protein

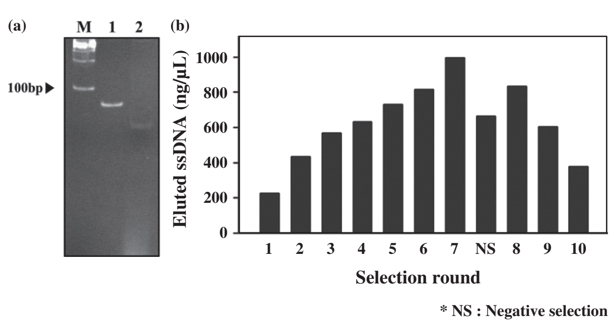

Figure 3. (a) Confirmation of ssDNA using 10% polyacrylamide gel electrophoresis. M: Protein size marker; lane 1: Amplified double stranded DNA; lane 2: Single stranded DNA isolates. (b) The concentration of eluted ssDNA aptamer from each IpaH-SELEX round. Negative selection (NS) was performed after 7th round in order to remove non-specific binders.

Table I. Aptamer sequences for IpaH and summary of kinetic data.

Aptamer	Selected sequence	Size (bp)	K_D (pM)
IpaH-1	GCACCTGATCGAGTGTTCATCT GTTAGCGTCTCACCGACA	40	112.20 ± 7.4138
IpaH-2	CGGCAGTGGCAAACATAAAGC ACATAGTGACAGCACCCGA	40	0.1874 ± 0.0131
IpaH-3	CCCAATAACGAAACGGCGTAT AGACCGCGACCTCAGTTAG	40	0.0047 ± 0.0002
IpaH-4	CGCGAAGTGTGGAATTCGTAGA TATTCCCATCTTGACGTG	40	0.0206 ± 0.0013
IpaH-5	CTATGTACAGTCGCCGGTACGA AGTGGGAAATCACGGCCC	40	0.0368 ± 0.0032
IpaH-6	GCAAGACCTATACGCTTACTTA TTACTGTGTCTTACGCGT	40	0.0700 ± 0.0085
IpaH-7	CGGTGAGTCACGAATGTTCAAC GAACCTACGTGCATTGAG	40	0.0056 ± 0.003
IpaH-8	GTGGAAAGAGGTACAATCATTG CTGGGAGACAGGTAGCTAG	41	0.0032 ± 0.004
IpaH-9	CGGCGCGAGGTTGGGGAGAGGC TGTGTCAACATTGGCGGC	40	0.022 ± 0.0012
IpaH-10	GTCCGGATAGCCCAAAGGGCA CGCAGTCATGCCGTTCCAC	40	0.1640 ± 0.027
IpaH-11	TCGCGGATTCCGATGTGCATG TCACATAAAGTTGAGTCCG	40	0.2180 ± 0.019
IpaH-12	CTACCTGTGGGGGGATCTGTT CCCTCATCTCTCTTCATG	39	0.2420 ± 0.018
IpaH-13	CGACGTTAACAAGTGCACTAG TGCCGTGTTGTGGCCCGGA	40	0.0449 ± 0.035
IpaH-14	CGTAAAGAACGCTGCCATGAA TGTGCTATCCAAGCCCGGG	40	289.0 ± 9.8520
IpaH-15	GGCTGACGGGGAGAACCAATG AAACGGCGTCCAACCACG	39	0.034 ± 0.0014
IpaH-16	GTCCGGTAGCATGAAACACA AAACGCAGGTGGTTCGCTAG	40	0.0078 ± 0.0007
IpaH-17	**CAGTTATAAAGCCTTCCGCC AATTTAGGTCTCTACCGTCG**	**40**	**0.0028 ± 0.0002**
IpaH-18	AGGTGGCTCGGGAATTACCTGA CCAGTGATTTGTGGTGG	39	0.0291 ± 0.0023
IpaH-19	GCGATGCGACCGATGGAATTAT TCTGTAGGGCCCCGCTC	39	0.0284 ± 0.0036

was attached via amine coupling and each aptamer was allowed to interact with immobilized IpaH protein (Fig. 4). The dissociation constant (K_d)/association constant (K_a) for the interaction of IpaH protein-aptamer was determined using BIAevaluation software, version 4.1. As shown in Table I, the automatically calculated K_D values of the selected aptamers range from picomolar to femtomolar scale (289.0 ± 9.9 pM to 2.82 ± 0.2 fM). This result indicates that IpaH-17 aptamer has significant binding affinity for IpaH protein. (2.82 ± 0.2 fM)

2.5. Sensitivity Test and Specificity Test of IpaH17-SPR Sensor

IpaH17 aptamer with the highest affinity to IpaH was immobilized on the sensor chip SA by streptavidin-biotin interaction in order to construct IpaH17-SPR sensor. The response units (RU) values obtained from the different protein concentrations were shown in Figure 5(a).

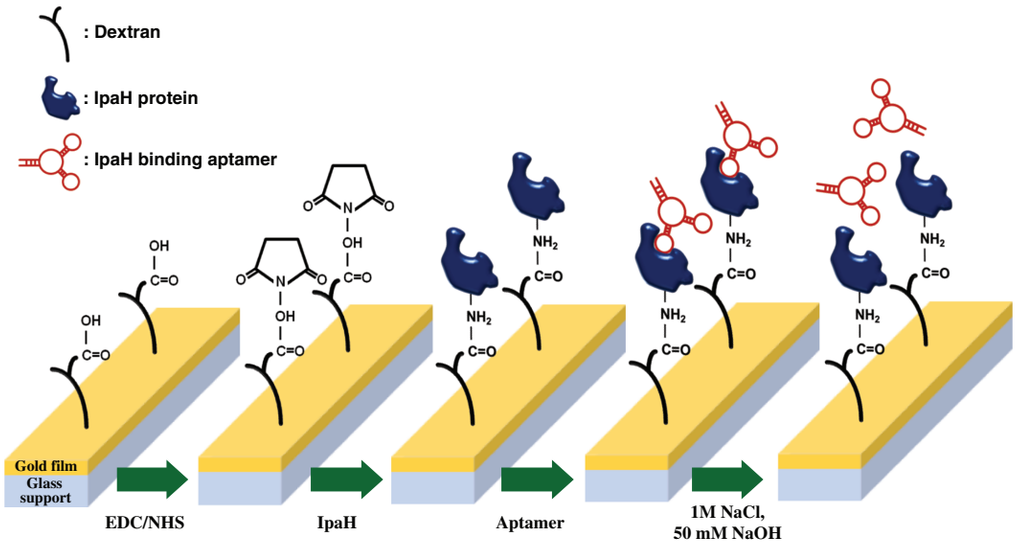

Figure 4. The reaction diagram of IpaH aptamer binding using the surface plasmon resonance (SPR) biosensor platform.

Initially, IpaH17-SPR sensors were used for IpaH detection from PBS buffer solution in a different concentration range of 0∼100 ng/mL. In this experiment, there were significant differences which demonstrated that a higher sensitivity can be achieved. The IpaH concentration dependent IpaH17-SPR sensor response was highly linear with a linear regression constant of 99.4% in the range between 0 and 100 ng/mL (Fig. 5(b)). The reaction time for the sensitive measurement of all samples was around 10 min. The change of RU in quantity was successfully obtained with

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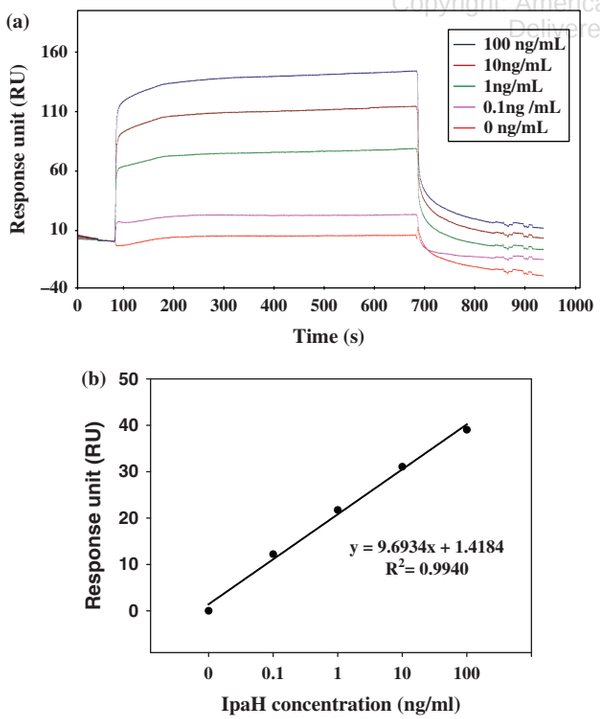

Figure 5. (a) SPR biosensor against IpaH protein at different concentrations. (b) Concentration dependency of a IpaH17-SPR biosensor showing the high linearity of the sensor response in the studied concentration range.

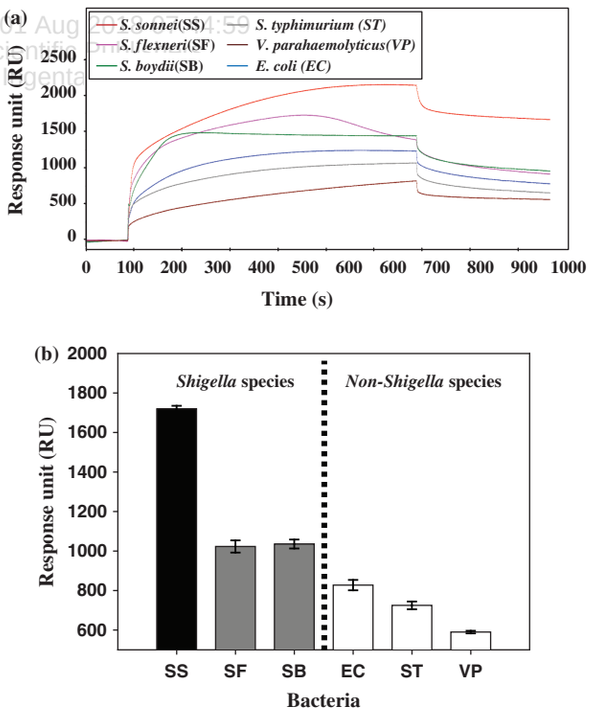

Figure 6. (a) Specific response to six pathogenic food-borne bacteria. Three *Shigella* species (*S. sonnei*, SS; *S. flexneri* SF; *S. boydii*, SB) and three non-*Shigella* food-borne bacteria (*Salmonella typhimurium*, ST; *Escherichia coli*, EC; *Vibrio parahaemolyticus*, VP). (b) Bar graphs (based on triplicate experiments with standard deviation) of IpaH binding preference. All bacterial lysates were applied to IpaH17-SPR sensor.

more than 10 RUs within 2 min. This result indicates that the IpaH17-SPR sensor may be applicable to the quantitative and rapid analysis for discriminating *S. sonnei* within various food-born pathogenic bacteria.

The binding experiment was subsequently performed against a variety of food-born bacterial cell lysate. Three *Shigella* species (*S. sonnei*, SS; *S. flexneri*, SF; *S. boydii*, SB) and non-*Shigella* food-borne bacteria (*Salmonella typhimurium*, ST; *Escherichia coli*, EC; *Vibrio parahaemolyticus*, VP) were used for IpaH17-SPR specificity test. The cell lysate supernatant was injected and the binding performance was monitored by measuring the RUs. Figure 6(a) shows the binding response of the IpaH17-SPR sensor against different cell lysates. The cell lysate of *S. sonnei* (SS) revealed apparent increased RU value compared with other bacterial cell lysate (Fig. 6(b)). This result also shows that the specific response was obtained within 2 min after cell lysates sample injection. Our sensor platform enables the rapid detection of cellular IpaH of *S. sonnei*.

3. CONCLUSIONS

Shigella is a group of facultative intracellular pathogens which were subgrouped into four species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (also designated as serogroups A to D). Especially, *Shigella sonnei* as a major cause of dysentery is largely associated with outbreaks in industrialized nations. Owing to the plasmid-associated virulent determinants that have been common to *Shigella*, development of sensitive and specific detection method strategies is urgently required. The genome of *S. sonnei* includes a virulence plasmid which encodes an invasive intracellular protein, IpaH (Invasion plasmid antigen H), promoting host-invasion and the inflammatory action. In this study, the soluble expression efficiency of recombinant *S. sonnei* specific IpaH was successfully enhanced by co-expressing with molecular chaperons. IpaH was the introduced into the SELEX process in order to isolating the specific aptamer candidates. IpaH17 aptamer with the 2.82 ± 0.2 fM binding affinity was selected through SPR kinetic analysis. IpaH17-SPR biosensor platform showed the IpaH concentration dependent response with a linear regression constant of 99.4% in the range between 0 and 100 ng/mL. The specific response to *S. sonnei* was also critically observed in the cell lysate verification within 2 min. The aptamer-immobilized SPR biosensor enables rapid, sensitive, and selective pathogen detection in food industry and epidemic study.

4. MATERIALS AND METHODS

4.1. Bacterial Strains and Culture Media

Shigella sonnei (KCTC 2518), *Shigella boydii* (KCTC 22528), *Shigella Flexneri* (**KCTC** 2008) *Escherichia coli* (KCTC 1116), *Salmonella typhimurium* (KCTC 2053), and *Vibrio parahaemolyticus* (KCTC 2729) were obtained from Korean collection for type cultures (KCTC). The media for bacterial culture were as follows: nutrient broth (BD Difco, UK) for *S. sonnei*, *S. flexneri* and *S. typhimurium*; Tryptic Soy Broth (BD Difco, UK) for *S. boydii*; Luria-Bertani media (LB; BD Difco, UK) for *Escherichia coli*; nutrient broth, 3% NaCl *for V. parahaemolyticus*. All bacteria were cultured at 37 °C with shaking at 180 rpm.

4.2. Construction of pGEX-4T-1-ipaH Expression Vector

The DNA sequence coding for ipaH, *Shigella sonnei* isolate invasion plasmid antigen protein (accession number ABE28529.1), was amplified using two primers: 5′-ATC GGA TCC CTC ACA TGG AAC AAT CTG-3′ with an endonuclease site of BamHI (underlined) and 5′-ATC CTC GAG ATT CTC TTC ACG GCT TCT with an endonuclease site of XhoI (underlined). Polymerase chain reaction (PCR) of ipaH gene was performed in a total volume of 50 μL with 20 pmol of each primer, 200 μM of dNTP, 1.25 U of Ex Taq polymerase (Takara, Japan). The ipaH gene was amplified through 30 cycles of PCR at 95 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s in a MyCycler™ (BioRad, CA). The PCR product was digested with BamHI and XhoI, then ligated into BamHI and XhoI site on pGEX-4T-1 vector with T4 DNA ligase (Elpis Biotech, South Korea) to generate pGEX-4T-1-ipaH recombinant vector. The pGEX-4T-1-ipaH was transformed *E. coli* BL21(DE3) and recombinant strains were selected for resistance to ampicillin (100 μg/mL). The recombinant construct was confirmed by restriction digestion assay and sequencing (Solgent, South Korea).

4.3. Preparation of Recombinant IpaH Protein

Construction of co-expression system of IpaH protein with pTf16 chaperone was carried out to increase the solubility of recombinant IpaH protein. The final construct, pGEX-4T-1-ipaH was transformed into *E. coli* BL21 (DE3) harboring pTf16 chaperone vector. *E. coli* BL21 (DE3) harboring pGEX-4T-1-ipaH and pTf16 was screened in LB agar plate containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL).

The broth culture of 1 L on LB media supplemented with ampicillin and chloramphenicol was initiated by inoculating 1/100 volume of overnight culture of recombinant strain. When the OD_{600} value reached 0.8, cells were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 18 °C for 20 h. After cultivation, cells were harvested, washed with 1X PBS (Gibco, USA; 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, pH 7.4), and resuspended in 1X PBS. Cells were then lysed by sonication and soluble fraction was separated by centrifugation (4 °C, 13,000 rpm, 10 min). The supernatant was applied to Glutathione Sepharose

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beads (GE healthcare, Sweden) and purified as recommended by the manufacturer. The eluted GST-ipaH fusion protein was followed by dialysis into 1X PBS and further concentrated using Vivaspin centrifugal concentrator (Vivascience, UK). The final purified and concentrated IpaH protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Bradford assay.

4.4. SELEX Process

4.4.1. Initial Random Library

The initial oligonucleotide library consists of 40 nt randomized region (N_{40}) and primer binding sites was chemically synthesized (Bioneer, South Korea): 5′-ATA CCA GCT TAT TCA ATT-N_{40}-AGA TAG TAA GTG CAA TCT-3′. The random library was amplified by PCR using forward primer (5′-ATA CCA GCT TAT TCA ATT) and biotinylated reverse primer (5′-AGA TTG CAC TTA CTA TCT-3′) during the selection process. PCR product was purified using QIAquick PCR purification kit (Qiagen, USA) and single-stranded DNA (ssDNA) was generated by removing the bionitylated ssDNA using streptavidin agarose resin (Pierce, USA). The resulting ssDNA was confirmed by comparison with dsDNA (PCR product) on 10% polyacrylamide gel containing 0.5X TBE buffer (see Fig. 3(a)). Then, the ssDNA library was heat denatured for 5 min at 85 °C and renatured for 2 h at room temperature (RT) to allow stable structure formation of aptamer.

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4.4.2. IpaH-SELEX

The structured DNA aptamer library was incubated with recombinant IpaH protein at 4 °C for 15 h at 10:1 molar ratio in 1X PBS. Aptamer binding and elution steps were performed according to manufacturer's recommendations. In brief, the protein-aptamer mixture was added to pre-activated Glutathione Sepharose 4B (GE Healthcare Life Sciences) and incubated for 1 h at 4 °C for glutathione-GST fused IpaH interaction. After binding step, recombinant IpaH protein-DNA aptamer complexes were purified using Glutathione elution buffer solution (0.154 g of reduced glutathione dissolved in 50 ml of 50 mM Tris-HCl, pH 8.0) and the eluted ssDNA was used as the template DNA for the next round of selection. To remove non-specific binders, negative selection was performed without IpaH protein after the sixth round and a total of 10 rounds of selection were carried out. During the selection process, the eluate concentration of each SELEX round was determined using the nanodrop spectrophotometer (Thermo scientific, USA). The DNA aptamer pool of the optimal round with the highest eluted concentration was cloned into T-blunt vector (Solgent, South Korea) in *E. coli* DH5α using unmodified primers. Purified plasmids from individual clones were analyzed by sequencing and utilizing ClustalX sequence alignment program.

4.5. Affinity Test of Selected Aptamers Using Surface Plasmon Resonance (SPR) Analysis

The binding affinity of selected DNA aptamer candidates for IpaH protein was analyzed by surface plasmon resonance (SPR) using Biacore 3000 instrument (Biacore AB, Sweden) at 25 °C. HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% polysorbate 20 v/v) was used as running buffer at a flow-rate of 5 μL/min and flow cell 1 was used as reference. To immobilize the IpaH protein to the CM5 sensor chip, the Au surface of sensor chip CM5 was pretreated with HBS-EP buffer and activated with a 1:1 mixture of 0.05 M *N*-hydroxysuccinimide (NHS) and 0.2 M *N*-ethyl-*N*′-(dimethylaminopropyl) carbodiimide (EDC) by modifying the carboxymethyl groups of dextran. IpaH protein was diluted in 10 mM sodium acetate (pH 4.5) and then injected over the sensor surface to coat the surface of flow cell 2, and followed by injecting 1 M ethanolamine hydrochloride, pH 8.5 to block remaining active sites. After the baseline stabilization, 19 selected ssDNA aptamers at different concentrations (100 nM, 200 nM, and 300 nM) were injected over the flow cell 1 and 2 for 1 min at a flow-rate of 10 μL/min with a 1 min of dissociation time. Following each experiment, the sensor chip was regenerated with 1 M NaCl, 50 mM NaOH. The SPR analysis and dissociation constants (K_d) of the aptamer-IpaH complexes were determined by BIAevaluation 4.1 software (Biacore, Sweden).

4.6. Sensitivity and Specificity Test of IpaH Binding Aptamer

The sensitivity and specificity test of the selected IpaH-17 aptamer with the lowest K_D value was conducted in BIAcore SPR system. Biotinylated IpaH-17 aptamer (purchased from Bioneer, Korea) was diluted to 10 pmol/μL in 1X PBS, denatured to 95 for 5 min, and cooled at RT for 2 h before use. Biotinylated aptamer was immobilized to flow cell 2 of sensor chip SA by streptavidin-biotin interaction. For sensitivity test of IpaH-17 aptamer, IpaH protein was diluted into 1X PBS to give a series of concentrations of 0.1, 1, 10, 100 ng/mL, then injected over the surface for 10 min at a flow rate of 5 μl/min.

Specificity test was carried out using various foodborne bacteria; *S. sonnei*, *S. boydii*, *S. flexneri*, *E. coli*, *S. typhimurium* and *V. parahaemolyticus*. Each bacterial culture of 10 mL was prepared at 0.9 unit of OD_{600}. The harvested cell pellets were washed with 1X PBS, resuspended in 1 mL 1X PBS, and lysed by sonication for 2 min. The supernatant of each cell lysate was obtained by centrifugation (13,000 rpm, 10 min, 4 °C) and injected over the aptamer-immobilized sensor chip.

After injection of each sample, assay was followed by stabilization for 4 min and regeneration for 1 min with 1 M NaCl, 50 mM NaOH, 50% isoamylalchol. The binding of analyte analyzed through response unit (RU) changes in sensogram.

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