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Original paper

Development of a Simultaneous Detection Method for Foodborne Pathogens Using Surface Plasmon Resonance Biosensors

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Surface plasmon resonance (SPR) biosensors were used to develop a rapid and simultaneous detection method for three important foodborne pathogens, *i.e., Escherichia coli* O157:H7 (O157), *Salmonella* Enteritidis (SE), and *Listeria monocytogenes* (LM). Bacterial homogenates prepared by sonication from bacterial suspensions at various cell concentrations were analyzed using SPR biosensors and sensor chips with polyclonal antibodies specific to each of the target pathogens. The precipitates from the homogenates were demonstrated to be suitable for specific and simultaneous detection of O157, SE, and LM. By using the precipitates and a custom-built multichannel SPR biosensor, the lower detection limit for O157, SE, and LM was determined to be 0.6×10^6, 1.8×10^6, and 0.7×10^7 CFU/mL, respectively, in the presence of non-target pathogens at concentrations of 10^5 to 10^8 CFU/mL.

Keywords: simultaneous detection, foodborne pathogens, SPR, *Escherichia coli* O157:H7, *Salmonella*, *Listeria*

Introduction

Food safety is a global health goal and food poisoning is a major health risk (Velusamy *et al*., 2010). There are approximately 76 million instances of food poisoning, accounting for 324,000 hospitalizations, 5200 deaths and a $23 billion financial loss annually in the United States (Mead *et al*., 2000). Although the safety of food has dramatically improved overall in the past several years, food poisoning still occurs. According to the Statistics of Food Poisoning published by the Food Safety Division, the Ministry of Health, Labor and Welfare of Japan, 1100 incidents of food poisoning occurred in Japan in 2012, causing 26,699 hospitalizations and 11 deaths (i). Foodborne pathogens are a major cause of food poisoning, thus the rapid, sensitive and reliable detection of foodborne pathogens is critical for the effective prevention of outbreaks of food poisoning.

At present, culture-based conventional methods are commonly used in the food industry to detect foodborne pathogens. The conventional methods are considered to be the "gold-standard" and are well known for their cost effectiveness, sensitivity, ability to confirm cell viability, and ease of standardization (Dwivedi and Jaykus, 2011). However, because they have complicated procedures, they are time consuming, taking at least 2 to 3 days for positive presumptive results and 7 to 10 days for confirmation, and are also labor intensive (Bai *et al*., 2010). The rapid growth of the food industry means that these methods can no longer meet the demands of the industry. Thus, the need for rapid and simple detection methods for foodborne pathogens is growing. Several new methods, such as enzyme-linked immunosorbent assay (ELISA) (Park *et al*.,

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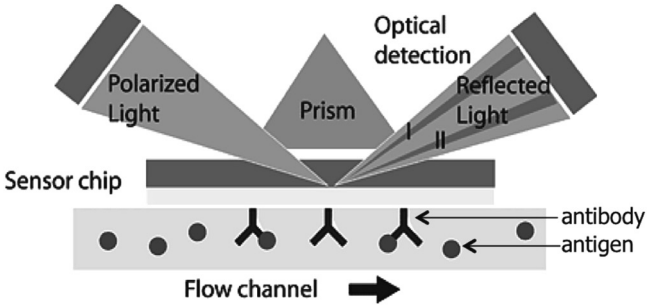

Fig. 1. Schematic representation of an SPR biosensor. The ligand (shown here as an antibody) is immobilized on the sensor chip surface. The analyte (shown here as an antigen) passes through the microfluidic flow channels.

2012; Zhong *et al*., 2012) and polymerase chain reaction (PCR) (Liu *et al*., 2012; Wang and Mustapha, 2010), have been developed for the rapid detection of foodborne pathogens. While PCR and ELISA have improved detection time, biosensors can perform faster detection, maintaining high sensitivity and specificity (Taylor *et al*., 2006).

In recent years, there has been much research activity in the area of developing biosensors for detecting foodborne pathogens (Velusamy *et al*., 2010). Surface plasmon resonance (SPR)-based optical biosensors, which allow for real-time and label-free detection, are of great utility. SPR biosensor can detect minute changes in the refractive index, which occur when an analyte binds to the ligand immobilized on the transducer surface. The sensor measures the change in the angle of the reflected light due to the change in the density of the medium with time (Fig. 1) (Rich and Myszka, 2001). SPR biosensors have been used by many research groups for the detection of foodborne pathogens (Meeusen *et al*., 2005; Oh *et al*., 2004; Subramanian *et al*., 2006; Tawil *et al*., 2012; Waswa *et al*., 2007; Wei *et al*., 2007). However, all these studies detected only one species of target pathogen in one detection period. In this study, we used a multichannel SPR biosensor to develop a method for simultaneously detecting three important foodborne pathogens, *i.e*., *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*.

Materials and Methods

Bacteria and cultivation *Escherichia coli* O157:H7 (O157) and *Listeria monocytogenes* LIS 21 (LM) were obtained from the Fukuoka City Institute for Hygiene and the Environment, Fukuoka, Japan. *Salmonella* Enteritidis (SE) IFO 3313 was purchased from the Institute for Fermentation, Osaka, Japan. Each of the three bacterial strains was cultured in 5 mL tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) at 37℃ for 18 h with shaking at 130 rpm. Cultures were used for the following sample preparations.

Sample preparation Cultures of O157, SE, and LM were centrifuged at 5,800 × *g* for 5 min. The pellets were resuspended in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) surfactant P20), centrifuged at 5,800 × *g* for 5 min, and the pellets were resuspended again in HBS-EP buffer. The bacterial suspensions in an ice bath were sonicated until the bacteria were disrupted thoroughly by using a TOMY Ultrasonic Disruptor UD-201 (TOMY SEIKO CO., LTD, Tokyo, Japan) at an output of 50 W. The whole homogenates were directly used as whole sonicated samples. After centrifuging 1 mL of the homogenates at 15,000 × *g* and 4℃ for 10 min, 850 μL of the supernatant was withdrawn and mixed with 150 μL of HBS-EP buffer. The mixtures were used as the supernatants of the sonicated samples. HBS-EP buffer (850 μL) was added to and mixed with the remaining precipitate and supernatant (150 μL). These mixtures were used as the precipitates of the sonicated samples. These samples were serially diluted and mixed to produce samples for SPR analysis and stored at −20℃ until use.

Previous studies showed that the lower detection limit for bacteria using an SPR biosensor is not only dependent on the sensitivity of the instrument and the specificity and affinity of the surface chemistry, but also on the sample preparation method (Taylor *et al*., 2005; Zhang *et al*., 2013). Compared with whole bacterial cells, small pieces of cells are easier to detect using an SPR biosensor (Bhunia *et al*., 2004). Each bacterium contains many antigens. Breaking a bacterium into small pieces will not only increase the number of analytes available for detection, but also improve the diffusion of the analytes. Both factors will affect the ability of the analytes to bind to the ligand immobilized on the sensing surface (Taylor *et al*., 2005). Sonication, which appears to weaken microbial membranes through cavitation induced by ultrasonic shock waves (Wong *et al*., 2012), is often used to break cells into small pieces and release cellular contents. Sonication has been proven to be an effective way to improve the lower detection limit for bacteria using an SPR biosensor (Zhang *et al*., 2013). Thus, the whole sonicated samples were used here for bacterial detection. However, for the specific detection of bacteria, the cross-reactivity of antibody against non-target bacteria should be minimized. The whole sonicated sample was thus separated into supernatant and precipitate by centrifugation and used for SPR detection to minimize the cross-reactivity of antibodies against non-target bacteria.

Viable counts Viable bacterial counts were determined by plating 100 μL of 10-fold serial dilutions of the bacterial suspensions in phosphate buffered saline (PBS; 0.1 M phosphate buffer containing 0.15 M NaCl, pH 7.2) on tryptic soy agar (TSA; Becton, Dickinson and Company) plates. After cultivation at 37℃ for 24 h, formed colonies were counted.

SPR instrumentation Two SPR biosensors were used for the detection of foodborne pathogens. One was a Biacore J (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and the other one was a custom-built multichannel SPR biosensor (Kyushu Keisokki Co., Ltd., Fukuoka, Japan). Biacore J was equipped with two flow channels: flow channel 1 for detection and flow channel 2 for

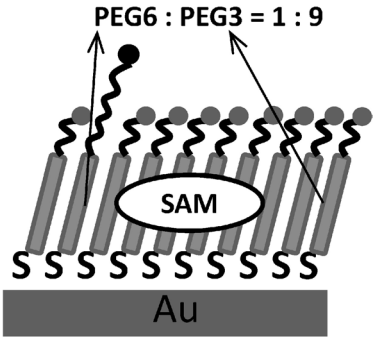

Fig. 2. Schematic illustration of a self-assembled monolayer formed on Sensor Chip Au.

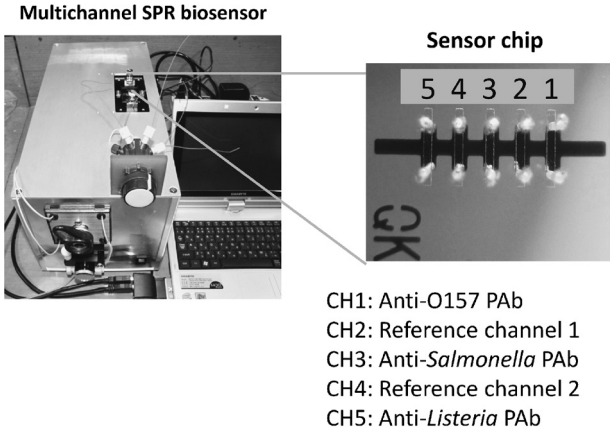

Fig. 3. Multichannel SPR biosensor and sensor chip with five flow channels.

reference. The multichannel SPR biosensor was equipped with five flow channels. Two flow channels served as reference channels and the other three flow channels were used for detection.

Surface modification of sensor chips The gold surfaces of Sensor Chips Au (GE Healthcare Bio-Sciences AB and Kyushu Keisokki Co., Ltd.) were cleaned by washing in acetone for 10 min, ethanol for 2 min, and 2-propanol for 2 min with sonication, washing with deionized water, immersing in a mixed solution (deionized water: 28% ammonia solution: 30% H_2O_2 = 5:1:1) for 30 min at 90℃, and washing again with deionized water. The surfaces were subsequently modified with the formation of a mixed self-assembled monolayer (SAM) by immersing in ethanol with 0.1 mM carboxy-EG_6-undecanethiol (PEG6; DOJINDO Laboratories, Kamimashiki, Kumamoto, Japan) and 0.9 mM hydroxy-EG_3-undecanethiol (PEG3; DOJINDO Laboratories) for 24 h at room temperature (Fig. 2). The carboxyl group of PEG6 was used for subsequent immobilization of antibody, while PEG3 was used to create a non-fouling background. The 1:9 ratio of PEG6:PEG3 maximized antibody binding while minimizing non-specific binding, based on our preliminary study. After the formation of the mixed SAM, the chips were washed in ethanol for 5 min with sonication and then washed with deionized water.

Antibody immobilization on sensor chips Polyclonal goat antibody (PAb) for O157, *Salmonella* and *Listeria* were purchased from Kirkegaard & Perry Laboratories, Inc. (KPL, Inc., Gaithersburg, Maryland, USA). Antibodies were immobilized onto the surface of sensor chips using the amine-coupling method (Löfås and Johnsson, 1990). The surfaces of the flow channels were activated for 10 min with a 1:1 mixture of 0.1 M *N*-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) at medium flow rate (approximately 30 μL/min). In the case of Biacore J, antibody was immobilized on flow channel 1 at a concentration of 100 μg/mL in 10 mM acetate buffer with the corresponding pH (pH 4.0 for anti-*Salmonella* PAb, pH 5.0 for anti-O157 PAb and anti-*Listeria* PAb) at medium flow rate (approximately 30 μL/min) for 12 min. Flow channel 2 was left blank to serve as a reference channel. In the case of the multichannel SPR biosensor, anti-O157 PAb, anti-*Salmonella* PAb and anti-*Listeria* PAb were immobilized on flow channel 1 (CH1), flow channel 3 (CH3) and flow channel 5 (CH5), respectively. Flow channel 2 (CH2) and flow channel 4 (CH4) were left blank to serve as the reference channels (Fig. 3). After antibody immobilization, the surfaces of all flow channels were blocked with 1.0 M ethanolamine-HCl (pH 8.5) for 10 min and 1% bovine serum albumin in HBS-EP buffer for 6 min at medium flow rate (approximately 30 μL/min) to minimize non-specific adsorption (Rich and Myszka, 2001).

SPR detection of samples Sensor chips with antibodies were docked into Biacore J and the multichannel SPR biosensor. HBS-EP buffer was used as a running buffer and run at medium flow rate (approximately 30 μL/min) and 25℃. HBS-EP buffer was first injected for 5 min to establish a baseline. Then, the corresponding sample was injected for 5 min for antigen-antibody binding. The signal change for the sample was obtained by subtracting the signal recorded at 30 s before the start of the sample injection from the signal recorded at 60 s after the end of the sample injection. After measurement of each sample, regeneration solution (10 mM glycine-HCl, pH 1.7) was injected for 1 min to dissociate antigen-antibody binding. After regeneration, sensor chip surfaces were equilibrated with running buffer (HBS-EP buffer).

The unit of signal change is the resonance unit (RU). For instance, 1,000 RU represents a mass change of 1 ng per mm^2 on the surface of the sensor chip and also represents a resonance angle change of 0.1 degree. To compensate the detection channel for variations in sample composition and non-specific adsorption, the signal of the reference channel was subtracted from that of the detection channel. This is important for determining the accurate lower limit of detection (Taylor *et al*., 2005). Moreover, the accuracy of detection results could be improved by setting the baseline. For this purpose, the signal of HBS-EP buffer was subtracted from the signals of samples. This compensation removed the sensor responses of baseline drift and measurement noise of the instrument. This is particularly important when the value of the signal change derived from the binding of target antigen in the sample is small or the capture method is used for SPR analysis.

Statistical analysis Triplicate experiments were run for every Biacore J experiment and standard deviation (SD) was calculated for the results. Student's *t*-test (one-tailed distribution, paired type) was performed to determine significant ($p < 0.05$ and $p < 0.01$) differences between means. All statistical analyses were performed using Microsoft Excel for Mac 2011 (V 14.3.1, Microsoft Corporation, Redmond, WA, USA).

Results and Discussion

Detection of pathogens using whole sonicated samples and Biacore J The whole sonicated samples were applied to Biacore J equipped with sensor chip functionalized with the corresponding PAb. Fig. 4 shows the signal changes determined on the whole sonicated samples. Two kinds of samples, samples containing the target pathogen only and samples containing both target and non-target pathogens, were used for detection. Compared with the signal changes of samples containing target pathogen only, samples containing the same level of target pathogen in the presence of non-target pathogens produced higher positive signal changes. In the case of the detection of O157 using anti-O157 PAb (Fig. 4a), the signal change (84 RU) of the sample containing only O157 at 1.8×10^6 CFU/mL was not significantly different from the signal change (100 RU) of the sample containing O157 at the same concentration, and same level of SE and LM, at 1.2×10^6 and 1.8×10^6 CFU/mL, respectively. However, in the presence of SE and LM at 1.2×10^8 and 1.8×10^8 CFU/mL, respectively, the signal change (148 RU) was significantly different from that (84 RU) of O157 alone at 1.8×10^6 CFU/mL. The same results were obtained on the whole sonicated samples of O157 at 1.8×10^5 CFU/mL. For detection of SE by anti-*Salmonella* PAb, the presence of O157 and LM at a cell concentration of about 100-fold of SE significantly affected the detection of SE (Fig. 4b). For the detection of LM, the presence of O157 and SE at a cell concentration of about 10-fold of LM significantly affected the detection of LM (Fig. 4c). The significant differences between the signal changes of samples containing target pathogen in the presence and absence of non-

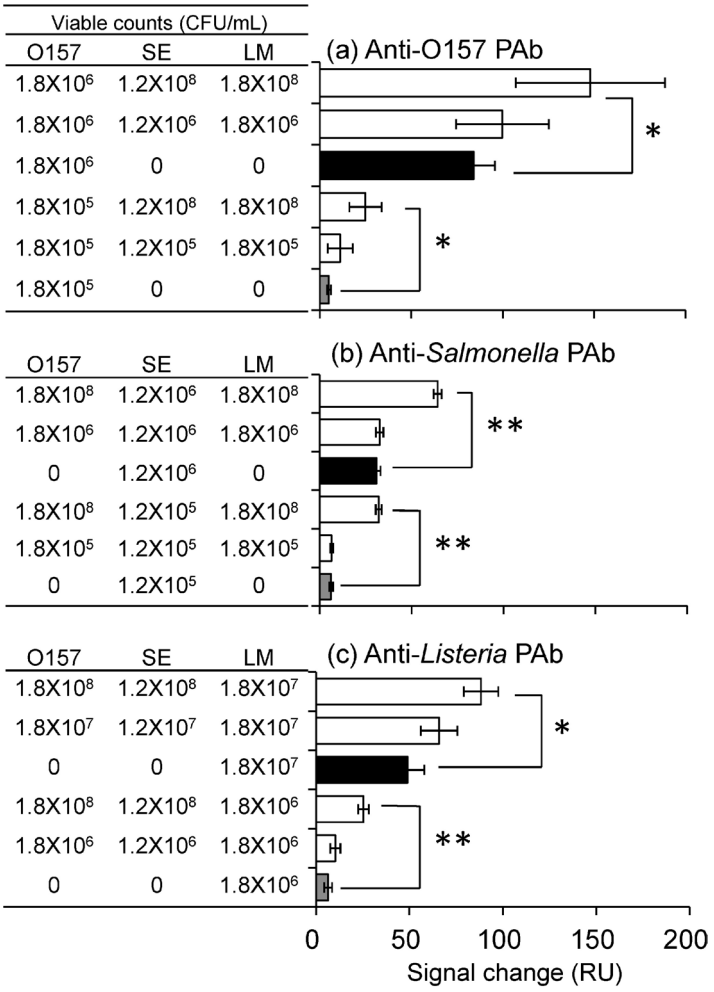

Fig. 4. Signal changes determined on the whole sonicated samples prepared from the target pathogens at various cell concentrations in the presence and absence of non-target pathogens using Biacore J. Suspensions of *E. coli* O157:H7 (O157), *S.* Enteritidis (SE), and *L. monocytogenes* (LM) at about 10^9 CFU/mL were sonicated and diluted with HBS-EP buffer to prepare the whole sonicated samples for Biacore J analysis. The viable counts in the samples are shown in the figure. Signal changes of the samples were determined with sensor chips functionalized with (a) anti-O157 PAb, (b) anti-*Salmonella* PAb, and (c) anti-*Listeria* PAb. Results are shown as mean ± SD (n = 3). Symbols: *, $p < 0.05$; **, $p < 0.01$.

target pathogens seem to be related to cross-reactivity of antibody against non-target pathogens.

Cross-reactivity test on supernatants and precipitates of sonicated samples using Biacore J Fig. 5 shows the signal changes obtained when the supernatants and precipitates of sonicated samples containing non-target pathogens at various cell concentrations were run across the sensor chip surfaces functionalized with the corresponding PAbs. No significant positive signal changes were obtained with both the supernatants and precipitates of sonicated samples containing non-target pathogens at low cell concentrations (below 10^6 CFU/mL) (Fig. 5a, b, and c). In contrast, at a high cell concentration of 10^8 CFU/mL, both the supernatants and precipitates of sonicated samples containing non-target pathogens produced positive signal changes of less than 25 RU for anti-O157 PAb and anti-*Salmonella* PAb (Fig. 5a and b). For anti-*Listeria* PAb, the supernatant of the sonicated sample containing non-target pathogens produced a positive signal change of 7 RU while the precipitate of the sonicated sample produced no signal change (Fig. 5c). Compared to the signal changes of the supernatants of sonicated samples, the signal changes of the precipitates of sonicated samples were much lower in the case of anti-O157 PAb and anti-*Listeria* PAb (Fig. 5a and c). For anti-*Salmonella* PAb, the signal change of the precipitate of the sonicated sample was slightly higher than that of the supernatant of the sonicated sample (Fig. 5b). Hence, for specific and simultaneous detection of the three pathogens using an SPR biosensor, the precipitate of the sonicated sample seems more suitable than the supernatant of the sonicated sample as it minimized the overall cross-reactivity of PAbs against non-target pathogens.

Cross-reactivity arises because the non-target pathogen shares an epitope in common with the target pathogen or because it has an epitope that is structurally similar to that of the target pathogen (ii). For anti-O157 PAb and anti-*Listeria* PAb, the concentration of shared epitope or similar epitope of the non-target pathogens in the supernatant of the sonicated sample appears to be higher than that in the precipitate of the sonicated sample. On the other hand, the situation is opposite for anti-*Salmonella* PAb.

Specific detection of each pathogen using the precipitates of sonicated samples and SPR biosensors (Biacore J and multichannel SPR biosensor) Fig. 6 shows the signal changes for target pathogens at various cell concentrations (10^5, 10^6, and

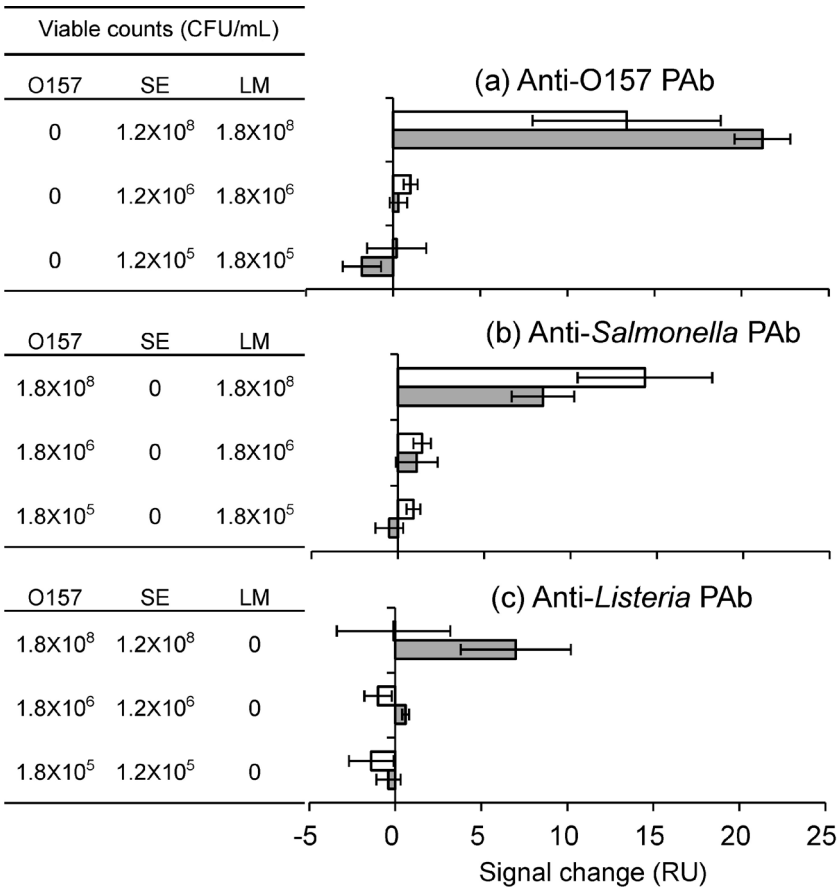

Fig. 5. Cross-reactivity of PAbs against supernatants and precipitates of the sonicated non-target pathogens. Suspensions of *E. coli* O157:H7 (O157), *S.* Enteritidis (SE), and *L. monocytogenes* (LM) at about 10^9 CFU/mL were sonicated and diluted with HBS-EP buffer to prepare the whole sonicated samples. The supernatants (■) and precipitates (□) were prepared from the whole sonicated samples as described in the text. The viable counts in samples are shown in the figure. Signal changes of the samples were determined using Biacore J equipped with sensor chip functionalized with (a) anti-O157 PAb, (b) anti-*Salmonella* PAb, and (c) anti-*Listeria* PAb. Results are shown as mean ± SD (n = 3).

10^7 CFU/mL) and control samples containing the other two non-target pathogens at 10^8 CFU/mL. These results were obtained by using the precipitates of sonicated samples and Biacore J. O157 at 1.8×10^6 CFU/mL, SE at 0.9×10^7 CFU/mL and LM at 1.8×10^7 CFU/mL produced positive signal changes of 39, 65, and 22 RU, respectively, and were significantly higher than those of the control samples. It has been reported that the cell concentration of O157, SE, and LM was less than 10^8 CFU/mL after the simultaneous enrichment of six foodborne pathogens, including these three pathogens (Kobayashi *et al*., 2009). Hence, the lower limit of detection using an SPR biosensor with specific PAbs for O157, SE, and LM was determined to be 1.8×10^6, 0.9×10^7, and 1.8×10^7 CFU/mL, respectively.

The above results demonstrate that the precipitate of the sonicated sample is more suitable for the specific and simultaneous detection of pathogens than the supernatant of the sonicated sample in cross-reactivity test using Biacore J and specific PAbs. Similar results were obtained in cross-reactivity tests using the multichannel SPR biosensor and specific PAbs (data not shown). Hence, the precipitates of the sonicated samples were applied to the multichannel SPR biosensor for pathogens detection.

Fig. 7 shows the signal changes obtained by using the multichannel SPR biosensor for the precipitates of sonicated samples prepared from each pathogen at about 10^5 to 10^8 CFU/mL. For CH1 (anti-O157 PAb), non-target pathogens of SE and LM produced positive signal changes ranging from 4 to 25 RU and 4 to 28 RU, respectively, which indicated that the cut-off value for the detection of O157 with CH1 should be 25 + 28 = 53 RU. Similarly, the cut-off value for the detection of SE with CH3 is 42 RU, and for the detection of LM with CH5 is 13 RU. The cut-off value was used as a reference when the lower detection limit for each species of pathogen was calculated. The lower detection limit was calculated from the data shown in Fig. 7 by regression analysis based on three times the standard deviation of the baseline noise (Taylor *et al*., 2006; Thomsen *et al*., 2003), which is 4 RU of signal change for the multichannel SPR biosensor. The calculated lower detection limit for O157, SE and LM is 1.1×10^6, 1.9×10^6, and 1.3×10^7 CFU/mL, respectively. Signal changes obtained for target pathogens at various cell concentrations using the multichannel SPR biosensor were similar to those obtained by using Biacore J. This indicated that the custom-built multichannel SPR biosensor used in this study has the same detection sensitivity as that of Biacore J.

Many researchers have applied SPR biosensors for the detection of O157 (Meeusen *et al*., 2005; Subramanian *et al*., 2006; Taylor *et al*., 2005), SE (Bhunia *et al*., 2004; Bokken *et al*., 2003), and LM (Hearty *et al*., 2006; Leonard *et al*., 2004). The lower detection limit was reported to be 10^5 to 10^7 CFU/mL for O157, 10^6 to 10^7 CFU/mL for SE, and 10^5 to 10^7 CFU/mL for LM, respectively. The different lower detection limits from these studies may be attributed to the use of different SPR biosensors, surface chemistries, antibodies, assay types (direct, sandwich, and subtractive inhibition assay), sample preparation methods, antibody immobilization methods, and linker molecules.

Simultaneous and specific detection of pathogens using the precipitates of sonicated samples of a mixture of three pathogens

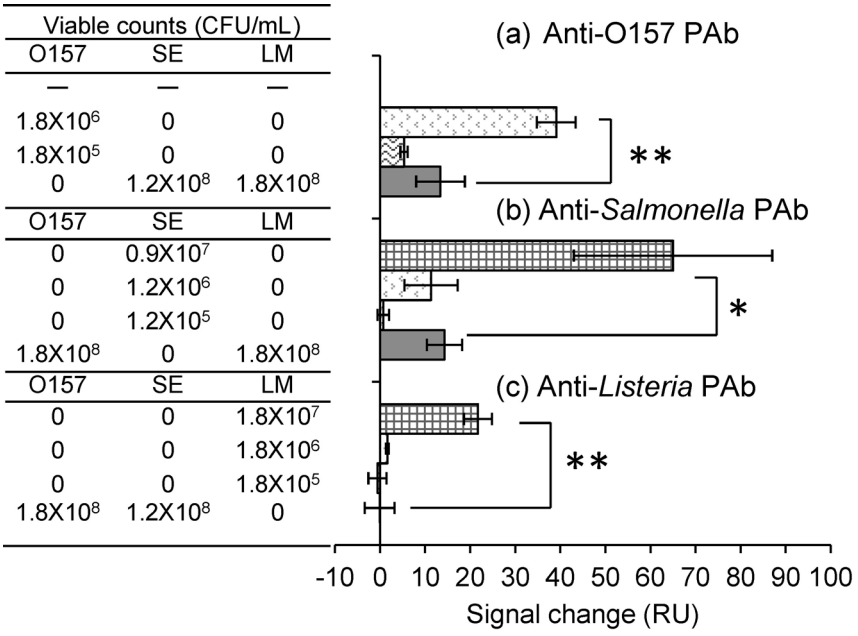

Fig. 6. Signal changes determined on the precipitates of sonicated samples prepared from the target pathogens at various cell concentrations and non-target pathogens using Biacore J. Suspensions of *E. coli* O157:H7 (O157), *S.* Enteritidis (SE), and *L. monocytogenes* (LM) at about 10^9 CFU/mL were sonicated and the precipitates obtained were resuspended and diluted with HBS-EP buffer to prepare the precipitates of sonicated samples for Biacore J analysis. The viable counts in samples are shown in the figure. Signal changes of samples were determined with sensor chips functionalized with (a) anti-O157 PAb, (b) anti-*Salmonella* PAb, and (c) anti-*Listeria* PAb. Results are shown as mean ± SD (n = 3). Symbols: *, $p < 0.05$; **, $p < 0.01$; −, not done.

and the multichannel SPR biosensor Successful biosensor detection must discriminate a target analyte from coexisting complex constituents in the sample (Taylor *et al*., 2006). The multichannel SPR biosensor used in this work could discriminate multiple pathogens simultaneously from a complex bacterial mixture in the sample. Samples containing the three pathogens at cell concentrations of 10^5, 10^6, and 10^7 CFU/mL were applied to a multichannel SPR biosensor equipped with sensor chip functionalized with PAbs on corresponding flow channels. Fig 8 shows the corresponding signal changes of samples for CH1 (anti-O157 PAb), CH3 (anti-*Salmonella* PAb) and CH5 (anti-*Listeria* PAb). Cell concentration-dependent increases in signal were observed for all three channels. The lower detection limit for O157, SE and LM calculated from the data shown in Fig. 8 by regression analysis was 0.6×10^6, 1.8×10^6, and 0.7×10^7 CFU/mL, respectively.

It has been reported that an eight-channel SPR biosensor based on wavelength division multiplexing achieved a lower detection limit for O157 and LM of 1.4×10^4 and 3.5×10^3 CFU/mL, respectively (Taylor *et al*., 2006). The study adopted the sandwich assay method for bacterial detection, in which secondary antibody was used to amplify the signal change of detection. However, the use of secondary antibody increased the cost of detection significantly because of the high price of antibody. Moreover, the use of secondary antibody also increased the detection time up to 30 min for one sample. In contrast, the detection time for one sample was only 5 min in our study since we used the direct assay method without using secondary antibody.

Conclusion

In this work, SPR biosensors were used to develop a rapid and simultaneous detection method for three important foodborne pathogens, O157, SE, and LM. It was demonstrated that the precipitates of sonicated samples are more suitable for the

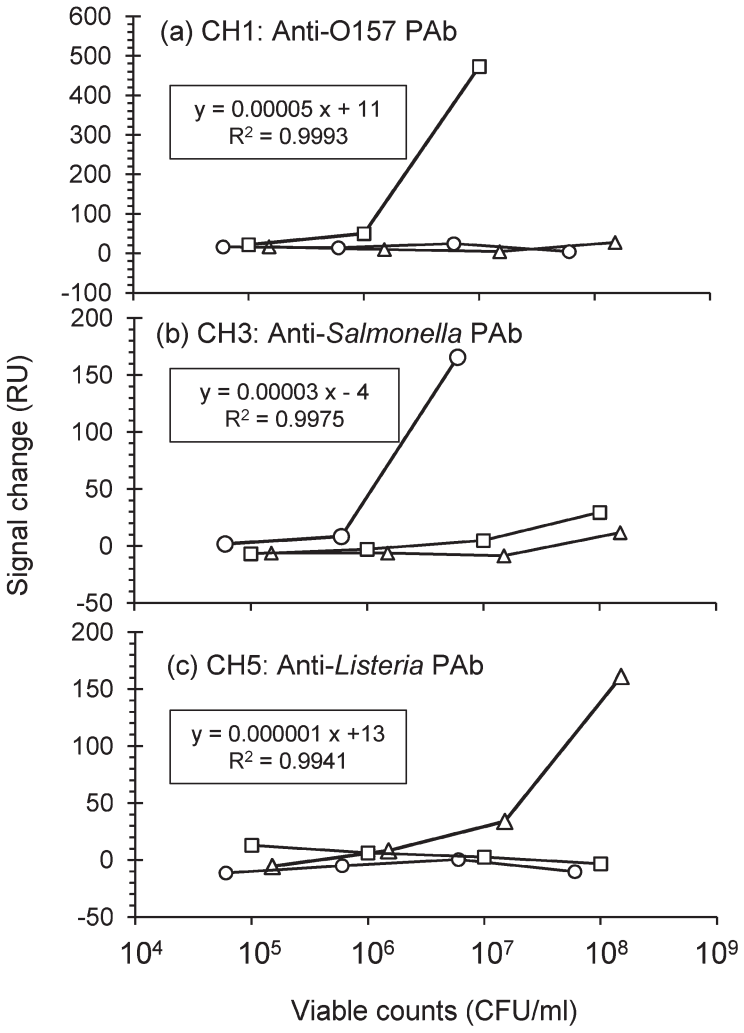

Fig. 7. Signal changes of each of the channels of the multichannel SPR biosensor functionalized with the specific PAbs against precipitates of the sonicated samples of target and non-target pathogens at various cell concentrations. *E. coli* O157:H7(O157, □), *S.* Enteritidis (SE, ○), and *L. monocytogenes* (LM, △) at 1.0×10^9, 0.6×10^9, and 1.5×10^9 CFU/mL, respectively, were sonicated to prepare the precipitates of sonicated samples. The precipitates of the sonicated samples were 10-fold serially diluted with HBS-EP buffer to attain cell concentrations from 10^5 to 10^8 for multichannel SPR analysis. The equation in each figure, Fig. 7a, b, and c, is the regression equation used to calculate the lower detection limit for the corresponding target pathogen.

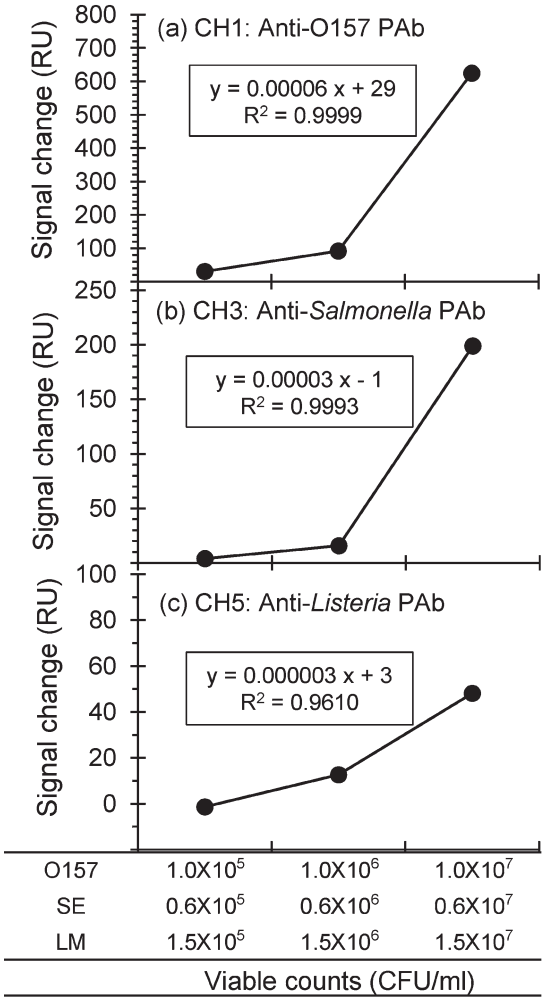

Fig. 8. Simultaneous detection of the three foodborne pathogens from mixtures of precipitates of sonicated samples prepared from *E. coli* O157:H7 (O157), *S.* Enteritidis (SE), and *L. monocytogenes* (LM) at various cell concentrations using the multichannel SPR biosensor. O157, SE, and LM at 1.0×10^9, 0.6×10^9, and 1.5×10^9 CFU/mL, respectively, were sonicated to prepare the precipitates of the sonicated samples. The mixtures of the precipitates were analyzed using the multichannel SPR biosensor. The equation in each figure, Fig. 8a, b, and c, is the regression equation used to calculate the lower detection limit for the corresponding target pathogen.

simultaneous and specific detection of the three pathogens than the supernatants of sonicated samples. The three pathogens were detected simultaneously by using the precipitates of sonicated samples and a custom-built multichannel SPR biosensor with sensor chip functionalized with polyclonal antibodies specific to the target pathogens. The presence of non-target pathogens interfered with the detection of target pathogens due to cross-reactivity of the immobilized PAbs against non-target pathogens. This interference was eliminated by setting an appropriate cut-off value for each detection channel.

The three important foodborne pathogens were detected simultaneously and specifically from a sample containing pathogens in buffer. Detection limits and/or signal changes for pathogens in buffer may be different from that for pathogens in food samples, as the components of food may affect the detection of pathogens. Further work is required to develop a simultaneous detection method for multiple foodborne pathogens in food samples.

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