International Food Research Journal 17: 477-483 (2010)

Surface plasmon resonance biosensor for real-time detection of genetically modified organisms

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Abstract: Application of surface plasmon resonance (SPR) biosensor in detection of genetically modified organism (GMO) is demonstrated. A total of four biotinylated probes namely Tnosb, P35Sb, LECb and TSQb were successfully immobilized onto the SA chip. Results analysis indicated that the SPR system with the sensor chip immobilized with the Tnosb, P35Sb, LECb and TSQb biotinylated probes potentially detect complementary standard fragments as low as 1 nM. Biospecific interaction analysis (BIA), employing surface plasmon resonance (SPR) and biosensor technologies provide easy, rapid and automatable approach in detection of GMOs. Short assay times, label free DNA hybridization reaction and no toxic compounds are required, i.e. ethidium bromide, and the reusability of the sensor surface are some of the factors that contribute to the general advantages of the surface plasmon resonance (SPR) biosensor system in detection of GMOs.

Keywords: SPR biosensor, GMO

Introduction

Genetically modified organisms are of great interest due to its broad geographic distribution and tremendous diversity and currently, great advances have been achieved in the detection of genetically modified organisms. In Malaysia, it is now established that GMO related products are available in the market (Tung *et al*., 2008; Tung *et al*., 2009; Jasbeer *et al*., 2009), and this may aroused ideological and ethical concerns among the public in relation to the issue of safety and labelling, and raising the need for the accuracy of GMOs quantification and makes GMO's labelling possible. For example, biospecific interaction analysis (BIA) was performed using surface plasmon resonance (SPR) and biosensor technologies to detect genetically modified Roundup Ready soybean, lectin, 35S promoter and NOS terminator gene sequences. Moreover, the SPR based biosensors enables real time monitoring variety of molecule reactions via BIA. The adsorption of biomolecules to an immobilized ligand on a sensor chip is measured in the same time and place as it occurs. The analytical system, Biacore, is based on a biosensor that utilizes SPR to monitor the adsorption of biomolecules on a sensor chip. This optical technique measures changes in refractive index in the vicinity of the sensor chip surface. Such changes are directly proportional to the change in adsorbed mass, which makes it suitable for the detection of biomolecules. Since the ligand in this study is a biotinylated single-stranded DNA, SPR technology could easily monitor DNA-DNA hybridization in the same time as it occurs (Wood, 1993; Nilsson *et al.,* 1995).

As molecules are immobilized on a sensor surface, the refractive index at the interface between the surface and a solution flowing over the surface changes, altering the angle at which reduced-intensity polarized light is reflected from a supporting glass plane. The change in angle, caused by binding or dissociation of molecules from the sensor surface, is proportional to the mass of bound material and is recorded in a sensorgram. When sample is passed over the sensor surface, the sensorgram shows an increasing response as molecules interact. The response remains constant if the interaction reaches equilibrium. When sample is replaced by buffer, the response decreases as the interaction partners dissociate. Complete profiles of recognition, binding and dissociation

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are generated in real time. From these profiles, data such as specificity, affinity, kinetic behavior and sample concentration can be determined. For most applications, a dextran matrix covering the gold layer enables molecules to be immobilized to a sensor surface and provides a hydrophilic environment for interactions. Surface specificity is determined by the nature of the immobilized molecule. Since light does not penetrate the sample, interactions can be followed in colored, turbid or opaque samples. No labels are required and detection is instantaneous.

In Biacore systems, SPR phenomenon occurs when polarized light, under conditions of total internal reflection, strikes an electrically conducting gold layer at the interface between media of different refractive index: the glass of a sensor surface (high refractive index) and a buffer (low refractive index). A wedge of polarized light, covering a range of incident angles, is directed toward the glass face of the sensor surface. Reflected light is detected within a Biacore system. Electric field intensity, known as an evanescent wave, is generated when the light strikes the glass. This evanescent wave interacts with, and is absorbed by, free electron clouds in the gold layer, generating electron charge density waves called plasmons and causing a reduction in the intensity of the reflected light. The resonance angle at which this intensity minimum occurs is a function of the refractive index of the solution close to the gold layer on the opposing face of the sensor surface.

Probe molecules used in this technology can be varying from small metabolites or drugs to large transcription complexes, and their interactions with the target range from the highly specific to the nonspecific. In interaction processes that are complicated, there can be multiple binding sites, cooperative interactions, and so forth.

No labeling of molecules is required in the SPR detection method, and the binding of probes with molecular weights greater than 200 daltons can usually be detected quite accurately. With this BIACORE technology, the SPR angle change is reported as resonance units (RU), where 1000 RU correspond to an angle change of approximate 0.1°. The exact relation between RU and nanograms of material bound will vary with the refractive index (Davis and Wilson, 2000). If the added molecule does not bind to a target or receptor, the SPR angle change in the sample and reference flow cells will be the same, and, after subtraction, will give a zero net RU response that indicates no binding occurred. Only bound molecules generate a positive SPR signal. That signal, recorded over time, produces a sensorgram. In a typical sensorgram, a baseline signal with no change in RU over time is followed by sample injection, which produces the association phase where RU increases with time. If the reaction rates are fast enough, it is possible to reach a steady state region, where the rates of association and dissociation are equal. Resuming buffer flow causes the complex to dissociate, and the kinetics of the dissociation can be recorded. At a desired time, a regeneration solution can be injected to remove remaining bounded molecules from the surface, and the original RU value is re-established. Thus, both kinetics and the equilibrium constants can be determined from a single experiment (Myszka, 1999; Myszka, 2000).

In this study, purified DNA was chosen as target of investigations. The objective of this study is to develop a method for the GMO detection using SPR biosensor technology.

Materials and Methods

DNA extraction

Testing samples and Roundup Ready soybean powders (IRMM, Geel, Belgium) were subjected to DNA isolation using DNeasy Plant Mini Kit (QIAGEN, Germany). The extraction procedure was according to the manufacturer's instructions. The DNA concentration of solutions was determined by measuring the UV absorption at 260 nm. The purity of the extracted DNA was evaluated by agarose gel electrophoresis using UV absorption ratios of 260/280 nm and 260/230 nm.

Synthetic oligonucleotides

The target oligonucleotides, the biotinylated oligonucleotide probes, and the PCR primers used in this study are reported as in Table 1.

Asymmetry polymerase chain reaction

The PCR was performed in a final volume of 100 µl volume containing 1X PCR buffer (10 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100) (Finnzymes, Espoo, Finland), 100 µM dNTPs (Finnzymes, Espoo, Finland), 0.5 µM of forward primer, 0.01 µM of reverse primer, 2U of DyNAzyme™ II DNA polymerase (Finnzymes, Espoo, Finland), sterile ultrapure deionized water and 30 ng of genomic DNA template. Amplification was performed in the personal Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94°C for 4 minutes followed by 50 cycles of denaturation at 94°C for 1 minute, annealing for 45 seconds at 58°C and polymerization at 72°C for 90 minutes. Final elongation was at 72°C for 5 minutes.

Table 1. Nucleotide sequences used in Biosensor (Surface Plasmon Resonance) analysis

Oligonucleotide	Use	Sequence (5' – 3')	References
35S-2	PCR primer (forward)	GATAGTGGGATTGTGCGTCA	
35S-1	PCR primer (reverse)	GCTCCTACAAATGCCATCA	
P35b	Biotinylated probe	biotin-GGCCATCGTTGAAGATGCCTCTGC	
Target P35b	Synthetic Target	GGCAGAGGCATCTTCAACGATGGCC	Mannelli *et al.,*
Tnos-1	PCR primer (forward)	GAATCCTGTTGCCGGTCTTG	2003
Tnos-2	PCR primer (reverse)	TTATCCTAGTTTGCGCGCTA	
Tnosb	Biotinylated probe	biotin-AATGATTAATTGCGGGACTCTAATC	
Target Tnosb	Synthetic Target	GATTAGAGTCCCGCAATTAATCATT	
TSQf	PCR primer (forward)	GTCTTCCCGTTACCTTGCGC	
TSQr	PCR primer (reverse)	CTCGATGACCGTCGTGATGC	
TSQb	Biotinylated probe	biotin-AGGTGATCGGCGTCGGCGTCTTCG	
Target TSQb	Synthetic Target	CGAAGACGCCGACGCCGATCACCT	This work
LQf	PCR primer (forward)	CTCTTCCCGAGTGGGTGAGG	
LQr	PCR primer (reverse)	AAGCACGTCATGCGATTCCC	
LECb	Biotinylated probe	biotin-GAGTCCCGTGGCAGCAGAGAACCCT	
Target LECb	Synthetic Target	AGGGTTCTCTGCTGCCACGGGACTC	

Surface plasmon resonance

BIAcore 3000 analytical system (BIAcore AB, Uppsala, Sweden) was used in these experiments. Sensor chips SA (research grade), recoated with streptavidin were from BIAcore AB (Uppsala, Sweden). Running buffer was HEPES buffered saline –EP (HBS-EP), which contains 10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.005% (v/v) surfactant P20 (BIAcore AB, Uppsala, Sweden). The experiments were conducted at 25°C. The flow rate was 5µl/min. Sensorgrams were analyzed with BIAevaluation 2.1 software. The flow cells were regenerated by performing a 5 µl pulse of regeneration buffer that contains 50 mM NaOH and 1 M NaCl for 1 minute.

Immobilization of biotinylated probes

Biotinylated probes (P35b, Tnosb, TSQb, LECb) were immobilized onto different flow cell of SA sensor chip (Biacore AB, Uppsala, Sweden). The immobilization of the biotinylated oligonucleotide (probe 80 pmol) on to the gold sensor chip was performed at 25°C; the liquid flow was set at 5 µl/ min. The total volume of biotinylated probe used in the immobilization was 20 µl.

Hybridisation with synthetic oligoncleotides

The synthetic oligonucleotides (Target P35b, Target Tnosb, Target TSQb, and Target LECb) fully complementary to the immobilised probe were used for the characterization of the biosensor. The hybridization with the target oligonucleotides was performed at 25°C injecting the oligonucleotide solution in hybridization buffer in the SPR flow cell; the flow rate was set at 5 µl/min. The oligonucleotides were diluted in the HBS-EP in the presence of 0.5 M NaCl. NaCl stops electrostatic repulsion of the oligonucleotide. The reaction was monitored for few min and the sensor chip was then washed with the hybridization buffer to remove the unbound oligonucleotide. The analytical signal, reported as resonance units (RU), is the difference between the

value after the hybridization value and the value recorded before the hybridization (baseline). Both values are taken when the sensor chip is in contact with the same buffer solution (hybridization buffer) so that the shift is related only to compounds fixed on the sensor chip during the reaction. In all the experiments, the single stranded probe was regenerated by a 1 min treatment with regeneration buffer. After each regeneration cycle a successive hybridization reaction can to be monitor. Such treatment could be performed up to 100-200 times without affecting the hybridization efficiency of the immobilized probe (Mariotti *et al.,* 2002).

Results and Discussion

In surface conditioning test, all of the flow cells were successfully well conditioned and the surface performance test indicated that the regeneration solution is not affecting the baseline or ligand (personal communication with Rick Filonzi, BIACORE, Australia). The sample binds reproducibly over a series of injections indicate reproducibility of the system.

The surface performance test was successfully performed separately on each of the immobilized flow cell with the injection of respective single stranded synthetic oligonucleotide (Target P35b, Target Tnosb, Target TSQb and Target LECb) at the flow rate of 5 µl/min for 2min. Besides that, the response level indicates that the value obtained is between 10% difference and therefore can be tolerate for every surface performance test (personal communication with Henry, GE Healthcare, U.S.A).

In the SPR measurement of the immobilized biotinylated probes, the resonance units after injection of the Tnosb, P35b, LECb and TSQb were 1286.7, 1115.6, 816.4 and 1106.4 respectively. These quantities of immobilized biotinylated probe were enough to detected minute amount of GMO material (verbal communication with Rick Filonzi, BIACORE, Australia). Results shown in Table 2 indicated that the SPR system with the immobilized biotinylated probes onto the SA sensor chip capable detecting complementary standard fragments as low as 1 nM. On the other hand, Wolcott (1992) concluded that the SPR system is sensitive enough to detect 320 fg (3.2 X 10^{-13} g) of a 97-bp molecule or 24 fg of a 7,200-bp DNA molecule (compared with 100 fg of DNA on a Southern blot).

The results of the SPR analysis shown in Table 3 indicate that sample labeled as POP gave the lowest average response values among all the samples tested with Tnos, P35S, LEC and TSQ gene fragment detection with the resonance unit of 10.70, 21.58, 420.36 and 675.68 respectively. However, the highest average response values recorded from the SPR analysis of Tnos, P35S, LEC and TSQ gene fragment detection derived from the 5% GMO standard, with the resonance unit of 18.78, 26.54, 449.40 and 692.40 respectively.

Sensorgrams generated from the SPR analysis as shown in Figure 1, Figure 2, Figure 3 and Figure 4 that corresponds with the resonance unit listed in Table 3 suggested that 24-25-mer oligonucleotides were appropriate probes for the detection of genetically modified Roundup Ready soybean in foods samples. On the other hand, results obtained from the studies conducted by Feriotto *et al*. (2002) indicated that 15-mer oligonucleotides were suitable probes for the detection of genetically modified Roundup Ready soybean in foods under standard BIA experimental conditions. By contrast, when 11-mer DNA probes were employed, no efficient hybridization was obtained because of the low stability of the hybridization complexes generated (Feriotto *et al*., 2002).

According to Malmqvist (1993), the stable binding of the specific ligands on the sensor chip allows regeneration of the sensor surface and 50-100 analytical cycles can be performed on one and the same surface. Furthermore, the SPR is an easy to use programming environment for automating analytical procedures allows the system to run overnight and at weekends, leaving the daytime free for developing new analyses and evaluation of results (Malmqvist, 1993). Besides that, the system can also be used for standardized concentration analysis.

Conclusion

In this study, the Tnosb, P35Sb, LECb and TSQb biotinylated probes were successfully immobilized onto the SA sensor chip with the resonance unit of 1286.7, 1115.6, 816.4 and 1106.4 respectively. Results analysis indicated that the SPR system with the sensor chip immobilized with the Tnosb, P35Sb, LECb and TSQb biotinylated probes potentially detected complementary standard fragments as low as 1 nM. This study strongly suggests that biospecific interaction analysis (BIA), utilizing surface plasmon resonance (SPR) is appropriate for GMO detection. In consequence with the study conducted by Mannelli *et al.* (2003), the biosensor clearly demonstrated the applicability to GMO detection both in environmental and food analysis. Moreover, the advantages of the system versus the electrophorectical post PCR detection is the label free DNA hybridisation reaction

Table 2. Resonance unit of SPR after injection of standards into the respective flow cell containing immobilized biotinylated probe on a sensor chip SA

Cycle	Flow Cell**	RelResp1 RU*	RelResp2 RU*	Average RU*	Concentration nM
Tnos					
1,2	1	911.2	891.17	901.18	100
3,4	1	885.04	875.05	880.04	10
5,6	1	836.11	831.20	833.66	1
7,8	1	381.31	400.59	390.95	0.1
9,10	1	102.93	105.78	104.36	0.01
11,12	1	45.62	44.87	45.24	0.001
35S					
1,2	2	811.46	795.55	803.50	100
3,4	2	790.24	790.55	790.40	10
5,6	2	734.58	732.63	733.60	1
7,6	2	398.86	406.02	402.44	0.1
8,9	2	121.45	124.95	123.20	0.01
LEC					
1,2	3	498.89	498.80	498.84	100
3,4	3	494.45	467.23	480.84	10
5,6	3	206.54	206.87	206.70	1
7,8	3	88.94	78.23	83.58	0.1
9,10	3	37.88	37.72	37.80	0.01
11,12	3	31.52	31.33	31.42	0.001
TSQ					
1,2	4	756.82	756.21	756.52	100
3,4	4	763.71	758.09	760.90	10
5,6	4	731.62	723.19	727.40	1
7,8	4	509.69	477.78	493.74	0.1
9,10	4	89.67	84.51	87.09	0.01
11,12	4	38.49	37.79	38.14	0.001

* RelResp – Real Response
RU – Resonance Unit
**FC1: Tnosb, FC2: P35Sb, FC3: LECb, FC4: TSQb

Table 3. Resonance unit of SPR after injection of samples into the respective flow cell containing immobilized biotinylated probe on a sensor chip SA

Sample	Flow Cell**	RelResp 1 RU*	RelResp 2 RU*	Average RU*
Tnos				
5% (standard)	1	19.77	17.78	18.78
SBH (soy bean hull pellet)	1	16.54	15.78	16.16
POP (chicken feed)	1	11.56	9.83	10.70
AFM (animal feed)	1	11.49	11.84	11.66
35S				
5% (Standard)	2	25.3	27.78	26.54
SBH (Soy bean hull pellet)	2	25.82	25.92	25.87
POP (chicken feed)	2	21.42	21.75	21.58
AFM (animal feed)	2	28.21	24.3	26.26
LEC				
5% (Standard)	3	451.17	447.10	449.14
SBH (Soy bean hull pellet)	3	431.54	428.77	430.16
POP (chicken feed)	3	421.41	419.30	420.36
AFM (animal feed)	3	436.84	433.16	435.00
TSQ				
5% (Standard)	4	692.89	691.91	692.40
SBH (Soy bean hull pellet)	4	686.26	683.05	684.66
POP (chicken feed)	4	677.33	674.02	675.68
AFM (animal feed)	4	688.27	684.13	686.20

* RelResp – Real Response
RU – Resonance Units
**FC1: Tnosb, FC2: P35Sb, FC3: LECb, FC4: TSQb

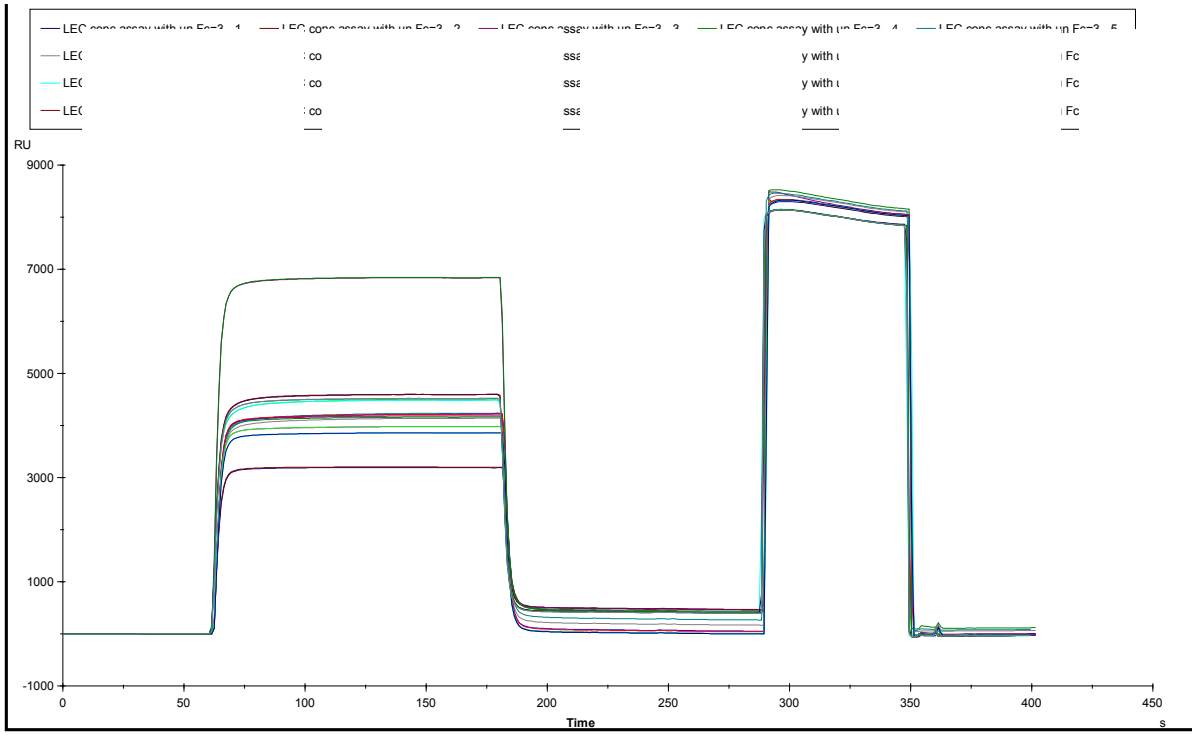

Figure 1. Sensorgram obtained after injection of LEC standards and asymmetry PCR products into the flow cell containing immobilized biotinylated probe (LECb) on a SA sensor

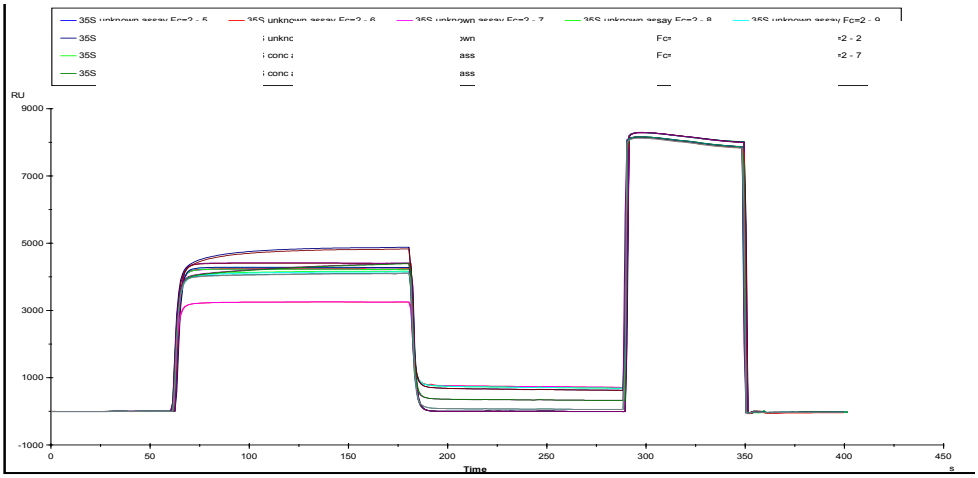

Figure 2. Sensorgram obtained after injection of P35S standards and asymmetry PCR products into the flow cell containing immobilized biotinylated probe (P35Sb) on a SA sensor

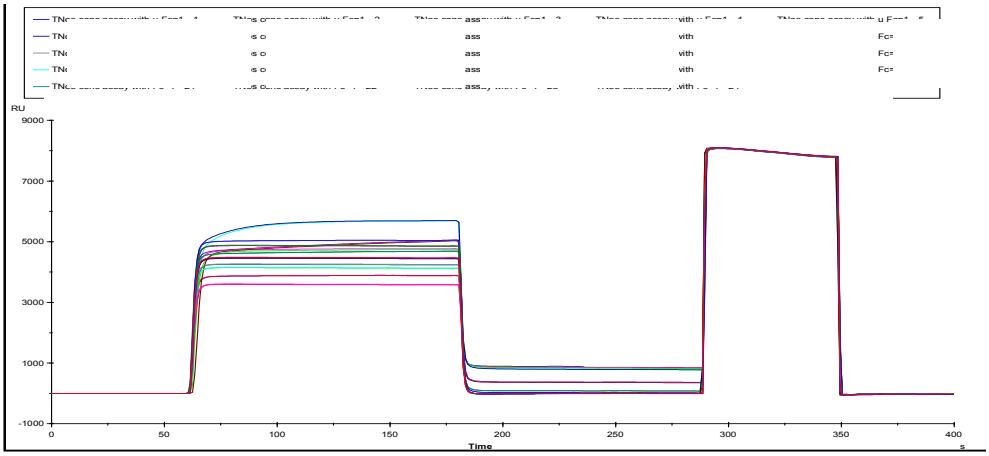

Figure 3. Sensorgram obtained after injection of Tnos standards and asymmetry PCR products into the flow cell containing immobilized biotinylated probe (Tnosb) on a SA sensor

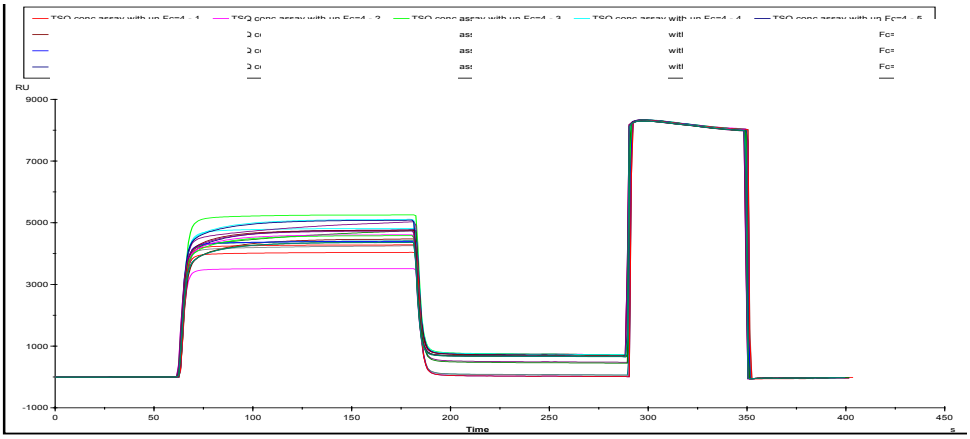

Figure 4. Sensorgram obtained after injection of TSQ standards and asymmetry PCR products into the flow cell containing immobilized biotinylated probe (TSQb) on a SA sensor

and no toxic compounds are required, i.e. ethidium bromide, and the reusability of the sensor surface for more than 20 measurements cycles. Since light does not penetrate the sample, interactions can be followed in colored, turbid or opaque samples. No labels are required and detection is instantaneous. According to Malmqvist (1993), biospecific interaction analysis (BIA), employing surface plasmon resonance (SPR) and biosensor technologies are an easy, rapid and automatable techniques and this study revealed the application of this approach to detect GMO. Therefore, all the SPR-based formats introduced in this study were found to be useful for the detection of Roundup Ready, lectin, 35S promoter and NOS terminator gene sequences.

Acknowledgements

The authors thank Dr. Henry K.L. Tan, Mr. T.M. Chang (G.E. Healthcare, Biosciences, Malaysia) and Mr. Rick Filonzi (BIACORE, Australia) for the valuable technical advices, and Institute of Bioscience, Universiti Putra Malaysia for the facilities. This work was supported by Research University Funding (04/01/07/0062RU).

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