JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Surface Plasmon Resonance Detection of Transgenic *Cry1Ac* Cotton (*Gossypium* spp.)

Zhuoya Zhao,[†,‡] Yanshan Chen,[†,‡] Wenzhong Xu,*[,†] and Mi Ma[†]

[†]Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People's Republic of China

[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

ABSTRACT: The detection and identification of genetically modified (GM) plants are challenging issues that have arisen from the potential negative impacts of extensive cultivation of transgenic plants. The screening process is a long-term focus and needs specific detection strategies. Surface plasmon resonance (SPR) has been used to detect a variety of biomolecules including proteins and nucleic acids due to its ability to monitor specific intermolecular interactions. In the present study, two high-throughput, label-free, and specific methods based on SPR technology were developed to detect transgenic *Cry1Ac* cotton (*Gossypium* spp.) by separately targeting protein and DNA. In the protein-based detection system, monoclonal anti-Cry1Ac antibodies were immobilized on the surface of a CM5 sensor chip. Conventional cotton samples were used to define the detection threshold. Transgenic cotton was easily identified within 5 min per sample. For the DNA-based model, a 25-mer biotinylated oligonucleotide probe was immobilized on an SA sensor chip. PCR products of *Cry1Ac* (230 bp) were used to investigate the reaction conditions. The sensitivity of the constructed sensor chip was identified at concentrations as low as 0.1 nM based on its complementary base pairing.

KEYWORDS: *surface plasmon resonance, Cry1Ac, cotton, protein-based detection, DNA-based detection*

INTRODUCTION

Genetic modification (GM) technology, which involves the integration of an exogenous gene into the genome of an organism with consequent gene expression and access to new traits or functions such as insect resistance or herbicide tolerance, has been widely applied in crops. Insecticidal crystal proteins (ICPs) encoded by the *Cry1Ac* gene have been introduced into several crops for their high toxicity to lepidopteran pests.[1−3] However, the gradually increasing cultivation of transgenic crops has raised concerns about the environment. These concerns include gene flow, the enhancement of insect resistance, and the decrease of natural enemies.[4−6] Therefore, the identification of genetically modified organisms (GMO) has become of considerable interest, and various methods for the detection of GMOs have accordingly been developed.[7−12]

Strategies employed in testing for GMOs can be divided into two categories. The first is based on detecting the exogenous gene itself using techniques such as polymerase chain reaction (PCR), Southern blotting, and DNA microarrays.[13,14] Some innovative methods based on PCR have been developed recently.[10,15,16] As a routine analytical method for GMO detection, PCR is not only highly sensitive but also relatively accurate and reliable. However, PCR methods have difficulties in distinguishing similarly sized DNA fragments.

The second group of methods is based on detecting the proteins expressed by the exogenous gene. These include Western blotting, enzyme-linked immunosorbent assays (ELISAs), and lateral flow strips.[17] Western blotting is the most accurate of these detection methods, and it can identify the size of the protein expressed in the transgenic materials.[18] ELISAs and lateral flow strips are the most widely used techniques in GMO identification.[18,19] The sandwich ELISA format has been employed to detect novel proteins in GMOs for its features of high sensitivity and specificity, which are induced by enzymatic substrate reaction and antibody−antigen interaction, separately. The lateral flow format, which is a variation on ELISA, using strips rather than microtiter wells, is rapid and simple and does not require the use of specialized equipment. These characteristics make lateral flow strips suitable as an initial screening method early in the food chain.[20] Another approach to GMO detection is surface plasmon resonance (SPR), which is used extensively for monitoring interactions between biomolecules.

SPR is accurate and fast, allowing high-throughput, real-time results. In addition, no molecular labels are required, and SPR can be used to detect both protein−protein and DNA−DNA interactions.[21,22] The feasibility of using SPR technology to monitor DNA-based interactions has attracted considerable attention in the GMO detection field.[20,23−25] For example, SPR technology coupled with asymmetric and multiplex PCR has been developed to detect genetically modified Roundup Ready soybean gene sequences and Bt-176 maize genomic sequences.[23,24] Public reports of protein-based SPR technology being used for GMO identification are, however, rare. This paper describes a single technology, employing both protein- and DNA-based strategies, to identify GMOs.

The specific recognition of antigen−antibody binding, using a CM5 sensor chip coated with Cry1Ac monoclonal antibody, was the basis of the protein-based component of the SPR device. The DNA-based component made use of a biotinylated

Received: November 26, 2012
Revised: February 27, 2013
Accepted: March 7, 2013
Published: March 7, 2013

Table 1. PCR Primers and SPR Probe Used in This Study

primer/probe	use	sequence (5′−3′)
Bt-FW	primer	AAC*CTCGAG*ATGGACAACAACCCAAACATCAAC
Bt-RV	primer	ACC*AAGCTT*CGCTGAAATTCCTAACACCCACGAT
Biot-Bt	probe	AATCCTGGTCCTGAAATGACAGAAC
Short-BtFW	primer	AGGATTCTCCCACAGGTTGAGCCAC
Short-BtRV	primer	GTCTAACGAGGTCTCCACCAGTGAA
Com-Bt	sample	GTTCTGTCATTTCAGGACCAGGATT
Non-Bt	sample	GTTTCTGCTCAGCGAGTTCGTGCCA

25-mer oligonucleotide probe, containing the conserved sequence of the *Cry1Ac* gene, immobilized on the surface of an SA sensor chip.

MATERIALS AND METHODS

Cotton Samples and DNA Isolation. Conventional cotton DP5415 and transgenic Bt cotton variety Nucotn33B, which expresses the Cry1Ac protein, were obtained from the Chinese Academy of Agricultural Sciences. Genomic DNA was isolated from leaves of the cotton using the Quick Plant Genomic DNA Kit (Nova Science Ltd., Los Angeles, CA, USA).

Synthetic Oligonucleotides. The nucleotide sequences of the biotinylated oligonucleotides probe (Biot-Bt), complementary target (Com-Bt), noncomplementary target (Non-Bt), and the PCR primers (Invitrogen, Carlsbad, CA, USA) used in this research are provided in Table 1.

***Cry1Ac* Clone and Vector Construction.** To sequence the *Cry1Ac* gene, genomic DNA of DP5415 and Nucotn33B were amplified by high-fidelity PrimeSTAR HS DNA polymerase (TaKaRa, Tokyo, Japan) using Bt-FW and Bt-RV primers (Table 1). The PCR cycles were as follows: 13 cycles consisting of denaturation at 94 °C for 30 s, first annealing at 60 °C for 30 s, final annealing at 48 °C for 30 s with a 1 °C decrease per cycle, and elongation at 72 °C for 90 s. These were followed by 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and elongation at 72 °C for 90 s. A 1780 bp specific DNA fragment, *Cry1Ac*, appeared only in the PCR products of Nucotn33B. The *Cry1Ac* fragment was amplified by PCR with the primers of Bt-FW and Bt-RV (Table 1) to introduce two restriction endonuclease sites of *Xho*I at the 5′ end and *Hin*dIII at the 3′ end, respectively. This PCR fragment was cloned into the prokaryotic expression vector pGEX-KG (Amersham, New York, NY, USA) between *Xho*I and *Hin*dIII to generate pGEX-KG-*Cry1Ac*.

Protein Sample. The soluble and activated Cry1Ac proteins were purified from the noninclusion bodies of the pGEX-KG-*Cry1Ac* prokaryotic expression system, which was used to optimize the conditions for protein-based SPR detection. The pGEX-KG-*Cry1Ac* plasmid was transformed into the *E. coli* Transetta (DE3) host strain, induced with 400 μM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 12 h at 16 °C. The negative control, which consisted of the same conditions without IPTG, proceeded in parallel, was used to explore whether the Cry1Ac protein could be induced in noninclusion bodies. The Cry1Ac protein was expressed with a GST-tag and could therefore be purified by affinity chromatography. The sample was purified further by gel filtration in accordance with the instructions for the GSTrap FF and Superdex 200 columns (GE Healthcare, New York, NY, USA).

Crude protein extracts of cotton leaves for SPR analyses were prepared by grinding the leaves with liquid nitrogen using a mortar and pestle and extracting with PBST buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.05% (v/v) Tween 20, pH 7.4). Four times the volume of sample buffer was added, and the extract mixture was shocked for 2 h at 4 °C. Only soluble samples were analyzed.

DNA Sample. The synthetic oligonucleotide of complementary target Com-Bt and noncomplementary target Non-Bt were used to test the specificity of the constructed sensor chip, and the concentration gradient of Com-Bt was used to define the sensitivity of the sensor chip. DNA fragments for SPR detection were amplified by *Taq* DNA polymerase (TaKaRa) using Short-BtFW and Short-BtRV primers (Table 1). The 35 PCR cycles were as follows: denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and elongation at 72 °C for 45 s. The PCR products were 230 bp and degenerated for 5 min at 98 °C followed by 1 min of cooling on ice prior to detection. The PCR products were purified using EasyPure PCR Purification Kit (TRANS, Beijing, China) and quantified at 260 nm on the UV−vis spectrophotometer NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA).

Surface Plasmon Resonance. The detection of Cry1Ac cotton by SPR was performed on a Biacore3000 (GE Healthcare). For protein-based detection, the Cry1Ac monoclonal antibody (Fitzgerald, Acton, MA, USA) was immobilized as a ligand on a CM5 sensor chip (GE Healthcare) to a level of nearly 8000 resonance units (RU; 1 RU corresponds to a change in surface concentration of about 1 pg/mm^2). The analyte solution was flowed over the sensor chip surface. The response increased in the presence of the Cry1Ac protein. No interaction signal was generated in the absence of Cry1Ac protein. Results were obtained from a continuous flow of 30 μL/min with a total sample volume of 60 μL. Phosphate-buffered saline (PBS; pH 7.4), containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 0.0005% (v/v) Tween 20, was used as the running buffer. Ten microliters of 50 mM NaOH was required to regenerate the sensor chip.

For DNA-based detection, 25-mer biotinylated *Cry1Ac* probe Biot-Bt was immobilized as a ligand onto an SA sensor chip (GE Healthcare). Responses were generated in the presence of oligonucleotide or denatured DNA sequences complementary to that of the *Cry1Ac* probe. Otherwise, no response was generated. The results were obtained with a continuous flow of 5 μL/min over the sensor chip with a total sample volume of 20 μL. PBM buffer (pH 7.4), containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.0005% (v/v) Tween 20, and 15 mM $MgCl_2$, was used as the running buffer. The regeneration buffer was the same as those described above. Only 5 μL of regeneration buffer was required to regenerate the chip.

Control blanks for each experiment were performed using the same protocols on an empty flow cell. All experiments were conducted at 25 °C, and control blanks were subtracted from the corresponding experimental data.

Western Blotting. Western blotting was used to identify the Cry1Ac-GST fusion protein using a Cry1Ac monoclonal antibody (Fitzgerald). For Western blot analysis, 5 μL aliquots of the protein samples were boiled for 5 min, separated by 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The blots were subsequently treated with 5% (w/v) skimmed milk in TBST buffer (0.1% (v/v) Tween 20 in 50 mM Tris-HCl, pH 7.5, and 150 mM NaCl) overnight at 4 °C. Blots were washed three times with TBST for 10 min, incubated with anti-Cry1Ac antibody (1:500 dilution) for 1 h, and washed following the same procedure described above. The membrane was incubated with goat anti-mouse IgG (1:4000 dilution) for 45 min and washed three times with TBST.

Lateral Flow Strip Detection. Cotton samples were ground and diluted in SEB4 sample extraction buffer (Agdia, Elkhart, IN, USA) at room temperature for lateral flow strip analyses. For best results, 20 times the volume of sample buffer was added. Soluble samples were

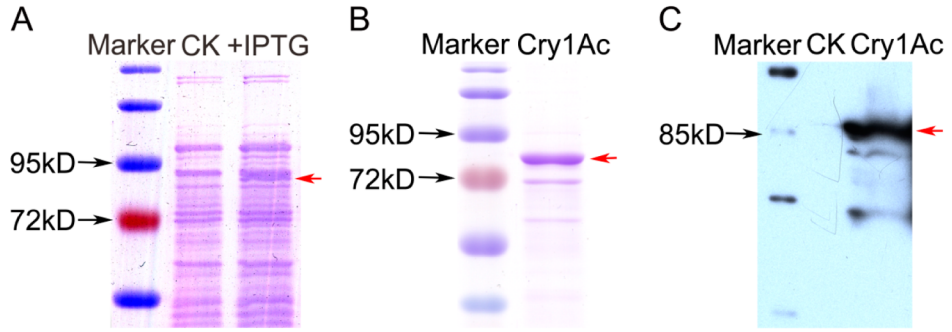

Figure 1. Expression, purification, and identification of Cry1Ac-GST protein: (A) 10% SDS-PAGE of Cry1Ac-GST expressed in noninclusion body (lane CK, induced without IPTG; lane +IPTG, induced with 400 μM IPTG); (B) 12% SDS-PAGE of Cry1Ac-GST purification (lane Cry1Ac, purified Cry1Ac-GST; red arrow indicates Cry1Ac-GST); (C) Western blotting for identification of the Cry1Ac-GST protein (lane CK, induced without IPTG; lane Cry1Ac, purified Cry1Ac-GST; red arrow indicates Cry1Ac-GST).

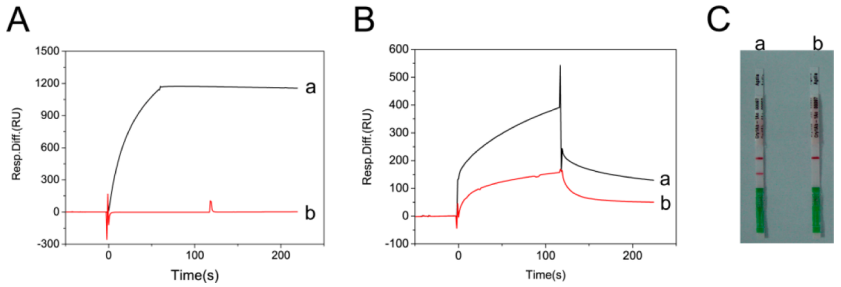

Figure 2. Protein-based detection: (A) positive and negative controls for evaluating the specificity of the constructed sensor chip (curve a, purified Cry1Ac-GST protein, positive control; curve b, BSA protein, negative control); (B) SPR detection of two cotton varieties (curve a, Nucotn33B cotton protein; curve b, DP5415 cotton protein); (C) lateral flow strip detection of cotton proteins (strip a, Nucotn33B cotton protein; strip b, DP5415 cotton protein). The red line at the top of the assay is the control line used to ensure that the test functioned properly. The red line below is the test line, appearing only in response to a positive result.

analyzed in accordance with the instructions for the Bt-Cry1Ab/1Ac ImmunoStrip Test (Agdia).

RESULTS AND DISCUSSION

Cry1Ac Protein-Specific Expression, Purification, and Identification. The pGEX-KG-*Cry1Ac* plasmid in *Escherichia coli* Transetta (DE3) was induced with 400 μM IPTG for 12 h at 16 °C. The negative control was the proteins extracted from the *E. coli* Transetta (DE3) transformed with pGEX-KG-*Cry1Ac* without IPTG induction. SDS-PAGE analyses showed that the Cry1Ac protein was soluble in the noninclusion body (Figure 1A). The Cry1Ac-GST protein was further purified by affinity chromatography and gel filtration (Figure 1B). The Western blot in Figure 1C shows the Cry1Ac-GST protein target line.

Cry1Ac Protein-Based Detection by SPR. To test the specificity of the constructed sensor chip and to optimize regeneration conditions, purified Cry1Ac-GST protein was used as a positive control; bovine serum albumin (BSA) was used as a negative control. Figure 2A shows that the SPR response increased nearly 1200 RU when the Cry1Ac-GST protein was injected. In contrast, no signal was generated when a BSA solution was flowed over the chip. This suggests that the specificity of the sensor chip would be sufficient to detect Cry1Ac protein in the test samples.

Crude proteins from Nucotn33B and DP5415 cotton leaves were extracted and injected over the sensor chip surface. With Nucotn33B protein, the sensor average response approached 397 ± 22.76 RU (n = 3) (Figure 2B, curve a). With wild-type DP5415 protein, the average response approached 131 ± 18.98 RU (n = 3) (Figure 2B, curve b). The latter responses are due either to sample contamination, nonspecific adsorption of antibody, or a combination of both. A lateral flow strip was used to detect whether the samples were contaminated. The test line appeared with cotton Nucotn33B but not with cotton DP5415, indicating that the samples were not cross-contaminated (Figure 2C). Therefore, the SPR response measured with conventional cotton was most likely due to nonspecific adsorption, which is often a factor in immunoreactions involving complex samples.[26] The SPR sensor response to solutions containing Cry1Ac was significantly greater than to solutions without Cry1Ac. This indicates that the current, protein-based SPR method could be used for detection of *Cry1Ac* cotton when wild-type samples are used as a negative control. Sample analysis was complete within 5 min per sample, saving considerable time over traditional Western blotting. Western blotting is the authoritative identification method for proteins and is widely used in many biological fields. The CP4 EPSPS protein in soybeans and the Cry1Ab protein in maize were detected using this method, although its time-consuming nature limits its use for high-throughput GMO detection.[18,26] Western blotting is therefore considered to be better suited for research applications than for routine testing. ELISAs coupled with lateral flow strips have been widely used to detect GMOs. Separately, the two techniques offer high specificity and simple operation.[18,19] To detect the targeting protein in GMOs, the most preferred immunoassay is a sandwich ELISA, which needs two specific antibodies with high affinity per one analyte.[12,27] It is laborious and time-consuming to develop a successful ELISA method for the detection of novel protein expressed in

dx.doi.org/10.1021/jf3050439 | J. Agric. Food Chem. 2013, 61, 2964−2969

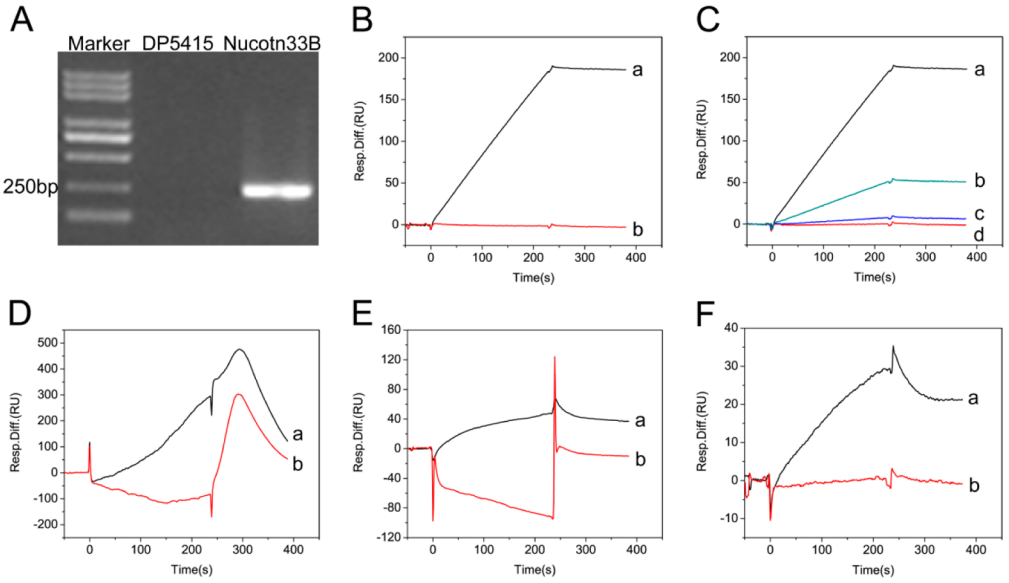

Figure 3. DNA-based detection: (A) 0.8% (w/v) agarose gel electrophoresis of PCR products (left lane, genome of conventional cotton DP5415; right lane, genome of transgenic cotton Nucotn33B; Short-BtFW and Short-BtRV were used as primers); (B) positive and negative controls for defining the specificity of the constructed SA sensor chip (curve a, complementary target Com-Bt used as positive control; curve b, noncomplementary target Non-Bt as negative control); (C) determination of sensor chip detection limit (analyte concentrations were curve a, 10 nM; curve b, 1 nM; curve c, 0.1 nM; and curve d, 0.01 nM); (D) unpurified PCR products used for SPR detection (curves a and b, unpurified PCR products containing a 230 bp fragment of *Cry1Ac*); (E) PCR products purified in different buffers (curve a, purified PCR products containing a 230 bp fragment of *Cry1Ac* dissolved in PBM buffer; curve b, purified PCR products containing a 230 bp fragment of *Cry1Ac* dissolved in deionized water); (F) SPR detection of two purified cotton genomes dissolved in PBM buffer (curve a, Nucotn33B cotton; curve b, DP5415 cotton).

transgenic crops. However, the SPR assay allows for the sensitive detection of target analyte using only one antibody with low or high affinity in a label-free environment.[28−30] The lateral flow strips method was adopted to detect Cry1Ac protein in rice, which can provide a yes/no determination within 5−10 min.[19] However, commercially available lateral flow strips are currently limited to a few biotechnology-derived GM proteins. Compared with these two methods, the protein-based SPR detection scheme described herein was more time-saving in experimental operation and exhibited a wider range of applications.

***Cry1Ac* DNA-Based Detection by SPR.** Probe sequences (Biot-Bt) and PCR primers (Short-BtFW and Short-BtRV) were designed to contain a conserved *Cry1Ac* sequence. DNA agarose gel electrophoresis showed that the conserved sequences of PCR products appeared only in transgenic cotton (Figure 3A). Therefore, the probe and primers were appropriate for detection of the *Cry1Ac* gene.

On the basis of the strong affinity of the biotin−streptavidin interaction, Biot-Bt probes were immobilized onto the surface of the SA sensor chip. An interaction occurred upon injection of 20 μL of 10 nM complementary target Com-Bt, eliciting a response that approached 180 RU. The interaction was stable with very little dissociation following injection of PBM buffer (Figure 3B, curve a). No response was observed upon injection of 20 μL of 10 nM noncomplementary target Non-Bt (Figure 3B, curve b). These results indicated that the constructed sensor chip was specific for the target analyte. A concentration gradient of complementary target Com-Bt (10, 1, 0.1, 0.01 nM) was used to define the sensitivity of the sensor chip. The response decreased with decreasing analyte concentration. At 0.1 nM, the response approached 8 RU (Figure 3C, curve c) with no response at 0.01 nM (Figure 3C, curve d). Thus, the sensitivity of the current DNA-based SPR sensor was 0.1 nM.

Plasmid pGEX-KG-*Cry1Ac* was used as the template, and Short-BtFW and Short-BtRV were used as the primers to amplify a 230 bp DNA fragment containing the Biot-Bt sequence to optimize testing conditions for double-stranded analytes. The PCR products were dissolved in PBM buffer prior to injection. A PCR response was detected as the solution flowed over the sensor chip (Figure 3D, curve a). However, the response was unstable. At times, no response was observed (Figure 3D, curve b), and in the dissociation stage there was an abnormal increase followed by a decrease. This instability was most likely due to the complex mixture of PCR products. When purified PCR products were dissolved in deionized water, no interaction was detected (Figure 3E, curve b). However, a significant response was observed when the PCR products were dissolved in PBM buffer (Figure 3E, curve a). Thus, the condition of the reactions influenced the accuracy of DNA-based SPR detection. Purified analyte dissolved in PBM buffer was suitable for SPR detection. Unpurified samples could be used as a screen prior to specific identification because most of the results obtained with unpurified samples were credible.

Degenerated and purified PCR products of cotton DP5415 and Nucotn33B were detected separately using the optimized testing conditions. The average response following injection of 20 μL of 5 ng/μL Nucotn33B PCR products approached 27.3 ± 1.92 RU (n = 3) (Figure 3F, curve a). This indicated that the constructed SA sensor chip and the process used for detection were capable of identifying transgenic *Cry1Ac* cotton. DNA-based SPR detection was based on PCR amplification and hybridization of the target DNA strand to the probe strand on the chip. In most PCR-based systems used for GMO detection, target fragments are amplified until detected by a nonspecific indicator.[8,31,32] The specificity of the current SPR technology is therefore greater than that of most PCR-based detectors. Recently, other DNA-based sensors have been designed for

dx.doi.org/10.1021/jf3050439 | J. Agric. Food Chem. 2013, 61, 2964−2969

GMO detection. For example, a surface-enhanced Raman scattering (SERS)−barcoded nanosensor was used to detect genetically modified rice with a detection limit of 0.1 pg/mL.[16] However, the detection time was 1 h, whereas the time to detect in the current SPR system was only 4 min. The SA sensor chip with the immobilized *Cry1Ac* probe was stable during detection.

In this study, an SPR device was fabricated and used to identify *Cry1Ac* cotton on the basis of separate protein- and DNA-based interactions. The results support the possibility of SPR-based sensor technology to trace the novel protein and transgenic DNA in complex materials. Although the current cost of SPR assay is expensive and relatively inhibitive in GMO detection, this technology can be rapidly established to detect novel proteins and genes in new trangenic crops. While producing data quickly with accuracy and sensitivity in real time, SPR technology enables rapid and routine analysis of antigen antibody interactions for antibody engineering, which also helps to construct advanced ELISA for GMO detection. Moreover, the development of SPR technology is ongoing at a rapid pace. For example, a highly sensitive and rapid procedure on an SPR sensor chip was optimized, which was based on the covalent-orientated immobilization of antibody and demonstrated to be more cost-effective than the conventional procedure on a commercial CM5 sensor chip.[33] Multiple channels in each chip and in array-based formats are also available, for example, the Biacore TM4000 using 4 × 5 array fromats that enables high-throughput analysis.[34] Besides, DNA array combined with SPR imaging measurements was developed to simultaneously detect transgenic DNA,[35] which enhanced accuracy for a single target or could expand the detection range with the simultaneous detection of multiple targets, thereby allowing high-throughput applications and reducing the per-sample cost.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China. Phone: +86 10 62836690. Fax: +86 10 62836690. E-mail: xuwzh@ibcas.ac.cn.

Funding

This work was financially supported by the National Transgenic Plant Special Fund (2008ZX08012-04) and the National Natural Science Foundation of China (Grant 91117010).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Dr. Yanhui Lu, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, for providing the seeds of Cotton DP5415 and Nucotn33B. We also thank Prof. Shanjin Huang, Institute of Botany, Chinese Academy of Sciences, for providing prokaryotic expression vector pGEX-KG.

ABBREVIATIONS USED

SPR, surface plasmon resonance; GM, genetically modified; GMOs, genetically modified organisms; RU, response unit; ICPs, insecticidal crystal proteins; PCR, polymerase chain reaction; ELISAs, enzyme-linked immunosorbent assays; GST, glutathione *S*-transferase; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; IPTG, isopropyl β-D-1-thiogalactopyranoside

REFERENCES

(1) Tabashnik, B. E.; Gassmann, A. J.; Crowder, D. W.; Carriere, Y. Insect resistance to Bt crops: evidence versus theory. *Nat. Biotechnol.* **2008**, *26*, 199−202.

(2) Snow, A. A.; Pilson, D.; Rieseberg, L. H.; Paulsen, M. J.; Pleskac, N.; Reagon, M. R.; Wolf, D. E.; Selbo, S. M. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecol. Appl.* **2003**, *13*, 279−286.

(3) Wu, K. M.; Guo, Y. Y.; Lv, N.; Greenplate, J. T.; Deaton, R. Efficacy of transgenic cotton containing a cry1Ac gene from *Bacillus thuringiensis* against *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. *J. Econ. Entomol.* **2003**, *96*, 1322−1328.

(4) Snow, A. A.; Andow, D. A.; Gepts, P.; Hallerman, E. M.; Power, A.; Tiedje, J. M.; Wolfenbarger, L. L. Genetically engineered organisms and the environment: current status and recommendations. *Ecol. Appl.* **2005**, *15*, 377−404.

(5) Lu, Y.; Wu, K.; Jiang, Y.; Xia, B.; Li, P.; Feng, H.; Wyckhuys, K. A.; Guo, Y. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* **2010**, *328*, 1151−1154.

(6) Duke, S. O.; Powles, S. B. Glyphosate: a once-in-a-century herbicide. *Pest Manag. Sci.* **2008**, *64*, 319−325.

(7) Meng, Y.; Liu, X.; Wang, S.; Zhang, D.; Yang, L. Applicability of plasmid calibrant pTC1507 in quantification of TC1507 maize: an interlaboratory study. *J. Agric. Food Chem.* **2012**, *60*, 23−28.

(8) Li, X.; Pan, L.; Li, J.; Zhang, Q.; Zhang, S.; Lv, R.; Yang, L. Establishment and application of event-specific polymerase chain reaction methods for two genetically modified soybean events, A2704-12 and A5547-127. *J. Agric. Food Chem.* **2011**, *59*, 13188−13194.

(9) Mano, J.; Yanaka, Y.; Ikezu, Y.; Onishi, M.; Futo, S.; Minegishi, Y.; Ninomiya, K.; Yotsuyanagi, Y.; Spiegelhalter, F.; Akiyama, H.; Teshima, R.; Hino, A.; Naito, S.; Koiwa, T.; Takabatake, R.; Furui, S.; Kitta, K. Practicable group testing method to evaluate weight/weight GMO content in maize grains. *J. Agric. Food Chem.* **2011**, *59*, 6856−6863.

(10) Bai, S.; Zhang, J.; Li, S.; Chen, H.; Terzaghi, W.; Zhang, X.; Chi, X.; Tian, J.; Luo, H.; Huang, W.; Chen, Y.; Zhang, Y. Detection of six genetically modified maize lines using optical thin-film biosensor chips. *J. Agric. Food Chem.* **2010**, *58*, 8490−8494.

(11) Randhawa, G. J.; Chhabra, R.; Singh, M. Decaplex and real-time PCR based detection of MON531 and MON15985 Bt cotton events. *J. Agric. Food Chem.* **2010**, *58*, 9875−9881.

(12) Miraglia, M.; Berdal, K. G.; Brera, C.; Corbisier, P.; Holst-Jensen, A.; Kok, E. J.; Marvin, H. J. P.; Schimmel, H.; Rentsch, J.; van Rie, J.; Zagon, J. Detection and traceability of genetically modified organisms in the food production chain. *Food Chem. Toxicol.* **2004**, *42*, 1157−1180.

(13) Guo, J.; Chen, L.; Liu, X.; Gao, Y.; Zhang, D.; Yang, L. A multiplex degenerate PCR analytical approach targeting to eight genes for screening GMOs. *Food Chem.* **2012**, *132*, 1566−1573.

(14) Morisset, D.; Dobnik, D.; Hamels, S.; Zel, J.; Gruden, K. NAIMA: target amplification strategy allowing quantitative on-chip detection of GMOs. *Nucleic Acids Res.* **2008**, DOI: 10.1093/nar/gkn524.

(15) Shrestha, H. K.; Hwu, K.-K.; Chang, M.-C. Advances in detection of genetically engineered crops by multiplex polymerase chain reaction methods. *Trends Food Sci. Technol.* **2010**, *21*, 442−454.

(16) Chen, K.; Han, H.; Luo, Z.; Wang, Y.; Wang, X. A practicable detection system for genetically modified rice by SERS-barcoded nanosensors. *Biosen. Bioelectron.* **2012**, *34*, 118−124.

(17) Holst-Jensen, A. Testing for genetically modified organisms (GMOs): past, present and future perspectives. *Biotechnol. Adv.* **2009**, *27*, 1071−1082.

(18) Margarit, E.; Reggiardo, M. I.; Vallejos, R. H.; Permingeat, H. R. Detection of BT transgenic maize in foodstuffs. *Food Res. Int.* **2006**, *39*, 250−255.

(19) Akiyama, H.; Watanabe, T.; Kikuchi, H.; Sakata, K.; Tokishita, S.; Hayashi, Y.; Hino, A.; Teshima, R.; Sawada, J.; Maitani, T. A detection method of CryIAc protein for identifying genetically modified rice using the lateral flow strip assay. *J. Food Hyg. Soc. Jpn.* **2006**, *47*, 111−114.
(20) Ahmed, F. E. Detection of genetically modified organisms in foods. *Trends Biotechnol.* **2002**, *20*, 215−223.
(21) Nelson, B. P.; Grimsrud, T. E.; Liles, M. R.; Goodman, R. M.; Corn, R. M. Surface plasmon resonance imaging measurements of DNA and RNA hybridization adsorption onto DNA microarrays. *Anal. Chem.* **2001**, *73*, 1−7.
(22) Homola, J. Surface plasmon resonance sensors for detection of chemical and biological species. *Chem. Rev.* **2008**, *108*, 462−493.
(23) Feriotto, G.; Borgatti, M.; Mischiati, C.; Bianchi, N.; Gambari, R. Biosensor technology and surface plasmon resonance for real-time detection of genetically modified Roundup Ready soybean gene sequences. *J. Agric. Food Chem.* **2002**, *50*, 955−962.
(24) Feriotto, G.; Gardenghi, S.; Bianchi, N.; Gambari, R. Quantitation of Bt-176 maize genomic sequences by surface plasmon resonance-based biospecific interaction analysis of multiplex polymerase chain reaction (PCR). *J. Agric. Food Chem.* **2003**, *51*, 4640−4646.
(25) Gambari, R.; Feriotto, G. Surface plasmon resonance for detection of genetically modified organisms in the food supply. *J. AOAC Int.* **2006**, *89*, 893−897.
(26) Rogan, G. J.; Dudin, Y. A.; Lee, T. C.; Magin, K. M.; Astwood, J. D.; Bhakta, N. S.; Leach, J. N.; Sanders, P. R.; Fuchs, R. L. Immunodiagnostic methods for detection of 5-enolpyruvylshikimate-3-phosphate synthase in Roundup Ready® soybeans. *Food Control* **1999**, *10*, 407−414.
(27) Kamle, S.; Ojha, A.; Kumar, A. Development of enzyme-linked immunosorbent assay for the detection of Bt protein in transgenic cotton. *Methods Mol. Biol.* **2013**, *958*, 131−138.
(28) Avramis, V. J.; Avramis, E. V.; Hunter, W.; Long, M. C. Immunogenicity of native or pegylated *E. coli* and *Erwinia* asparaginases assessed by ELISA and surface plasmon resonance (SPR-Biacore) assays of IgG antibodies (Ab) in sera from patients with acute lymphoblastic leukemia (ALL). *Anticancer Res.* **2009**, *29*, 299−302.
(29) Heinrich, L.; Tissot, N.; Hartmann, D. J.; Cohen, R. Comparison of the results obtained by ELISA and surface plasmon resonance for the determination of antibody affinity. *J. Immunol. Methods* **2010**, *352*, 13−22.
(30) Nakamura-Tsuruta, S.; Yasuda, M.; Nakamura, T.; Shinoda, E.; Furuyashiki, T.; Kakutani, R.; Takata, H.; Kato, Y.; Ashida, H. Comparative analysis of carbohydrate-binding specificities of two anti-glycogen monoclonal antibodies using ELISA and surface plasmon resonance. *Carbohydr. Res.* **2012**, *350*, 49−54.
(31) Alexander, T. W.; Reuter, T.; McAllister, T. A. Qualitative and quantitative polymerase chain reaction assays for an alfalfa (*Medicago sativa*)-specific reference gene to use in monitoring transgenic cultivars. *J. Agric. Food Chem.* **2007**, *55*, 2918−2922.
(32) Grohmann, L.; Bruenen-Nieweler, C.; Nemeth, A.; Waiblinger, H.-U. Collaborative trial validation studies of real-time PCR-based GMO screening methods for detection of the bar gene and the ctp2-cp4epsps construct. *J. Agric. Food Chem.* **2009**, *57*, 8913−8920.
(33) Vashist, S. K. A highly-sensitive and rapid surface plasmon resonance immunoassay procedure based on the covalent-orientated immobilization of antibodies. *Protocol Exchange* **2011**, DOI: 10.1038/protex.2011.259.
(34) Helmerhorst, E.; Chandler, D. J.; Nussio, M.; Mamotte, C. D. Real-time and label-free bio-sensing of molecular interactions by surface plasmon resonance: a laboratory medicine perspective. *Clin. Biochem. Rev.* **2012**, *33*, 161−173.
(35) Scarano, S.; Ermini, M. L.; Spirit, M. M.; Mascini, M.; Bogani, P.; Minunni, M. Simultaneous detection of transgenic DNA by surface plasmon resonance imaging with potential application to gene doping detection. *Anal. Chem.* **2011**, *83*, 6245−6253.