JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biosensor Technology and Surface Plasmon Resonance for Real-Time Detection of Genetically Modified Roundup Ready Soybean Gene Sequences

GIORDANA FERIOTTO,† MONICA BORGATTI,‡ CARLO MISCHIATI,‡ NICOLETTA BIANCHI,‡ AND ROBERTO GAMBARI*,†,‡

Biotechnology Center, Ferrara University, 44100 Ferrara, Italy, and Department of Biochemistry and Molecular Biology, Ferrara University, Via L. Borsari n. 46, 44100 Ferrara, Italy

Biospecific interaction analysis (BIA) was performed using surface plasmon resonance (SPR) and biosensor technologies to detect genetically modified Roundup Ready soybean gene sequences. We first immobilized, on SA sensor chips, single-stranded biotinylated oligonucleotides containing soybean lectin and Roundup Ready gene sequences, and the efficiency of hybridization to oligonucleotide probes differing in length was determined. Second, we immobilized biotinylated PCR products from nontransgenic soybeans (genomes carrying only the lectin gene), as well as from genetically modified Roundup Ready soybean, and we injected the oligonucleotide probes. Furthermore, we used the sensor chips carrying either lectin and Roundup Ready soybean PCR products or 21-mer oligonucleotide as probes, and we injected both nonpurified and purified asymmetric PCR products. The results obtained show that 13 and 15 mer oligonucleotides are suitable probes to detect genetically modified Roundup Ready soybean gene sequences (either target oligonucleotides or PCR products) under standard BIA experimental conditions. By contrast, when 11 mer DNA probes were employed, no efficient hybridization was obtained. All the SPR-based formats were found to be useful for detection of Roundup Ready gene sequences, suggesting that these procedures are useful for the real-time monitoring of hybridization between target single-stranded PCR products, obtained by using as substrates DNA isolated from normal or transgenic soybeans, and oligonucleotide or PCR-generated probes, therefore enabling a one-step, nonradioactive protocol to perform detection.

KEYWORDS: Polymerase chain reaction; GMO; biosensors; real-time assays; surface plasmon resonance

INTRODUCTION

Identification of genetically modified organisms (GMO) in foods is becoming an issue of great interest, because of the increasing number of GMO-derived products launched into the food market (*1*, *2*) and the increased customer demand for strict regulations and labeling of such foods (*3*, *4*). Accordingly, a variety of methods for the detection of GMO were developed that can be used for screening purposes. For instance, the fast and simple polymerase-chain reaction (PCR) procedure was recently applied for detection of FLAVR SAVR tomato (*5*), Roundup Ready soybean (*6*, *7*), Bt-maize (*8*), and gbss-antisense transgene potatoes (*9*). As specific examples, Vollenhofer et al. (*1*) described the specific detection of glyphosate-tolerant soybean and insect-resistant maize in food. Primers were designed to amplify parts of the 35S promoter derived from Cauliflower Mosaic Virus, the NOS terminator derived from *Agrobacterium tumefaciens*, and the antibiotic marker gene NPTII (neomycin-phosphotransferase II), to allow for general screening of foods. It should be noted that in this technology, confirmation of the results using restriction enzyme analysis or hybridization is strongly recommended. Accordingly, different methods were recently reported to confirm specificity of PCR products. For instance, Hoef et al. (*10*) applied a method based on a nested PCR procedure to samples of soybean, soy-meal pellets, and soybean flour, as well as a number of processed complex products. On the other hand, Vaitilingom et al. (*11*) reported a fast and quantitative method to detect transgenic "Maximizer" maize, "event 176" (Novartis), and "Roundup Ready" soybean (Monsanto) in food by real-time quantitative PCR by the determination of the amplified product accumulation through a fluorogenic probe. Fluorescent dyes were chosen in such a way as to co-amplify wild type and transgenic DNA in the same tube (*10*).

The interest of the scientific community for the development of efficient methodologies for detection of GMO is certainly related to the fact that there is a general agreement for the need

* To whom correspondence should be addressed. Telephone: 39-0532-291448. Telefax: 39-0532-202723. E-mail: gam@dns.unife.it.
† Biotechnology Center.
‡ Department of Biochemistry and Molecular Biology.

10.1021/jf0109773 CCC: $22.00 © 2002 American Chemical Society
Published on Web 01/30/2002

Table 1.

oligonucleotide	use	sequence
RupR1 (forward)	PCR primer	5′-TGTATCCCTTGAGCCATGTTG-3′
RupR2 (reverse)	PCR primer	5′-CGCACAATCCCACTATCCTTC-3′
biot-RupR2 (reverse)	biotinylated PCR primer	5′biot-CGCACAATCCCACTATCCTTC-3′
Lec1 (forward)	PCR primer	5′-ATGGGCTTGCCTTCTTTCTC-3′
Lec2 (reverse)	PCR primer	5′-CCGATGTGTGGATTTGGTG-3′
biot-Lec2 (reverse)	biotinylated PCR primer	5′biot-CCGATGTGTGGATTTGGTG-3′
RupR-11 (forward)	probe	5′-AGAGTCAGCTT-3′
RupR-13 (forward)	probe	5′-TAGAGTCAGCTTG-3′
RupR-15 (forward)	probe	5′-CTAGAGTCAGCTTGT-3′
biot-RupR-21 (reverse)	biotinylated target DNA	5′biot-CTG*ACAAGCTGACTCTAG*CAG-3′
Lec-11 (forward)	probe	5′-AAGTCGTCGCT-3′
Lec-13 (forward)	probe	5′-CAAGTCGTCGCTG-3′
Lec-15 (forward)	probe	5′-TCAAGTCGTCGCTGT-3′
biot-Lec-21 (reverse)	biotinylated target DNA	5′biot-TC*AACAGCGACGACTTGA*TCA-3′

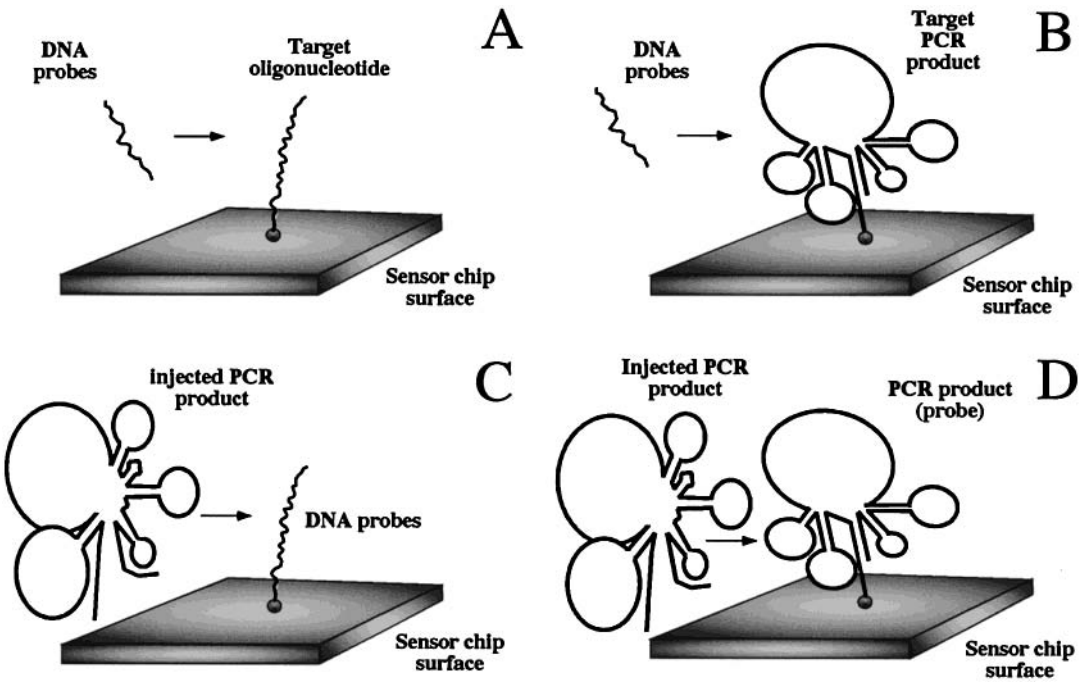

Figure 1. Experimental strategies and formats for GMO detection using SPR-based BIA and sensor chips carrying lectin or Roundup Ready oligonucleotides or PCR products.

of highly automated detection systems for the identification of GMO-free foods as well as quantitative assays to verify the levels of GMO within foods.

The recent development of surface plasmon resonance (SPR) based biosensors (*12*−*14*) enables one to perform biospecific interaction analysis (BIA) for monitoring a variety of molecular reactions in real time. This optical technique detects and quantifies changes in refractive index in the vicinity of the sensor chips surfaces to which ligands have been immobilized (*14*), allowing detection of biomolecules (analytes) interacting with the ligand (*13*, *14*). If the ligand is a biotinylated single-stranded DNA, SPR technology could easily monitor DNA−DNA hybridization in the same time as it occurs (*15*, *16*) and has been applied to detect HIV-1 infection (*17*) and genetic mutations (*18*−*20*).

Despite the fact that this approach could be of great interest in genetic analyses, no data are in the available literature on the possible use of SPR-based biosensors for the identification of GMO.

In the present paper we report how we first immobilized, on two different flow cells of a SA sensor chip, single-stranded biotinylated oligonucleotides containing the sequence of the soybean lectin and the Roundup Ready genes, and the efficiency of hybridization of oligonucleotide probes differing in length was determined. Second, we immobilized on different SA sensor chips biotinylated PCR products from nontransgenic soybeans (genomes carrying only the lectin gene), as well as from genetically modified Roundup Ready soybean. Then, we injected the oligonucleotide probes. Furthermore, we used the sensor chips carrying either lectin and Roundup Ready soybean PCR products or 21-mer oligonucleotide as probes, and we injected both nonpurified and purified asymmetric PCR products.

MATERIALS AND METHODS

Soybean Samples and DNA Isolation. Roundup Ready and conventional soybean powders were obtained from Fluka (Buchs, Switzerland). Genomic DNA was isolated using the Wizard Magnetic DNA Purification System for Food (Promega Corporation, Madison, WI).

Synthetic Oligonucleotides. The target oligonucleotides, the oligonucleotide probes, and the PCR primers were purchased from Sigma-Genosys (Cambridge, UK) and purified by HPLC. The nucleotide sequences of the oligonucleotides used are reported in **Table 1**.

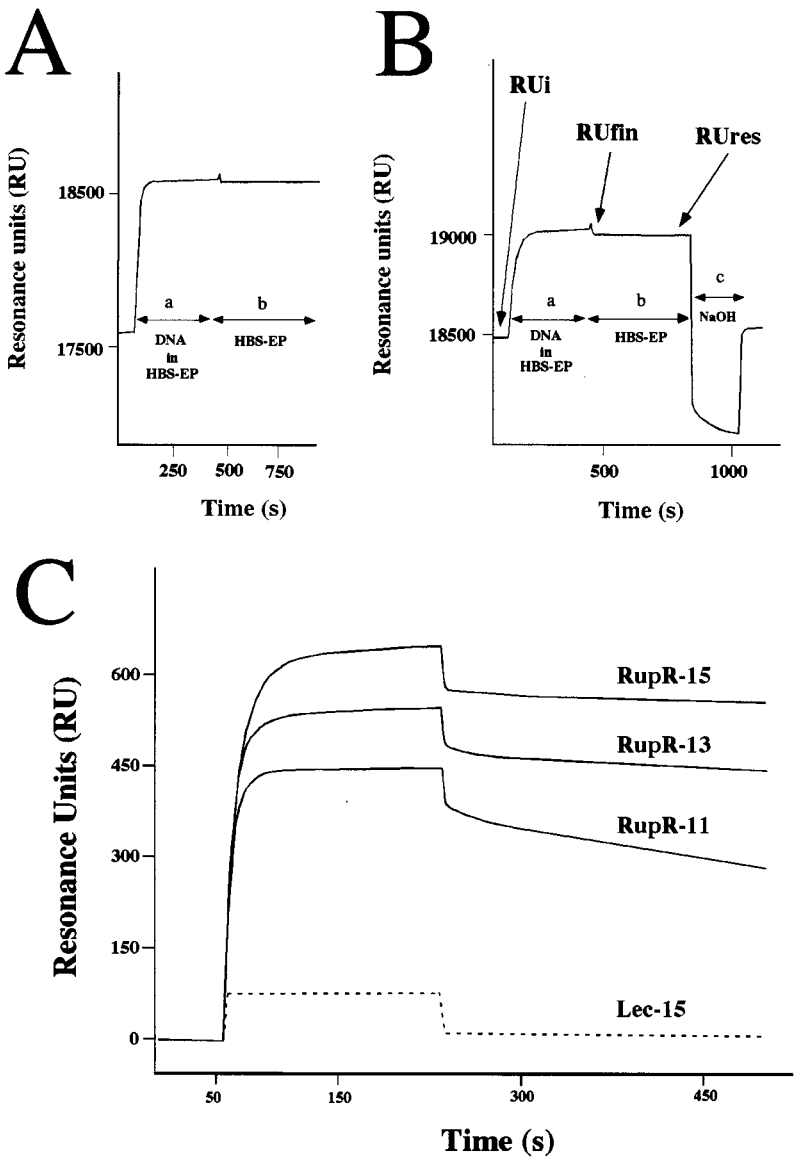

Figure 2. (A) Sensorgram obtained after injection of 25 μL containing 80 pmol of biotinylated Roundup Ready 21-mer target DNA on a SA sensor chip. The Roundup Ready 21-mer was dissolved in HBS-EP. a = injection of the oligonucleotide; b = injection of HBS-EP. (B) Sensorgram obtained after injection of 25 μL containing 40 pmol of Roundup Ready 15-mer probe to the SA sensor chip containing the target Roundup Ready 21-mer DNA. a = injection of the oligonucleotide; b = injection of 20 μL of HBS-EP; c = injection of 10 μL of 50 mM NaOH. Positions for identification of the values of RUi (initial RU), RUfin (RU at the end of the injection), and RUres (residual RU) are indicated. (C) Sensorgrams obtained after injection of 25 μL containing 40 pmol of Roundup Ready 11-mer, 13-mer, and 15-mer probes (solid lines) or lectin 15-mer probe (dotted line) to the SA sensor chip containing the target Roundup Ready 21-mer DNA. The sensorgrams shown in this panel have been obtained by subtracting from the experimental sensorgrams the sensorgrams obtained by injecting HBS-EP.

Table 2.

immobilized DNA sample	Injected DNA[a]					
			purified PCR products		nonpurified PCR	
	RupR 15-mer	lectin 15-mer	RupR	lectin	RupR	lectin
RupR 21-mer	554	17	39	1	N. D.	N. D.
lectin 21-mer	56	396	2	31	N. D.	N. D.
RupR PCR products	38	2	282	1	184	29
lectin PCR products	5	32	8	367	2	302

[a] Numbers represent RUfin–RUi values.

Polymerase-Chain Reaction. In each PCR reaction (*21*), 10 ng of genomic DNA from Roundup Ready soybeans and conventional soybeans were amplified by Taq DNA polymerase using the Lec1, Lec2, RupR1, and RupR2 primers, amplifying soybean lectin and Roundup Ready gene sequences, respectively. The nucleotide sequences of these PCR primers are shown in **Table 1**. PCR was performed in a final volume of 100 μL containing 50 mM KCl, 10 mM TRIS−HCl pH 8.8, 2.5 mM $MgCl_2$ by using 2U/reaction of Taq DNA polymerase, 100 μM dNTPs, 0.5 μM PCR primers, 200 ng BSA, and 50 ng of genomic DNA. The 40 PCR cycles used were as follows: denaturation,

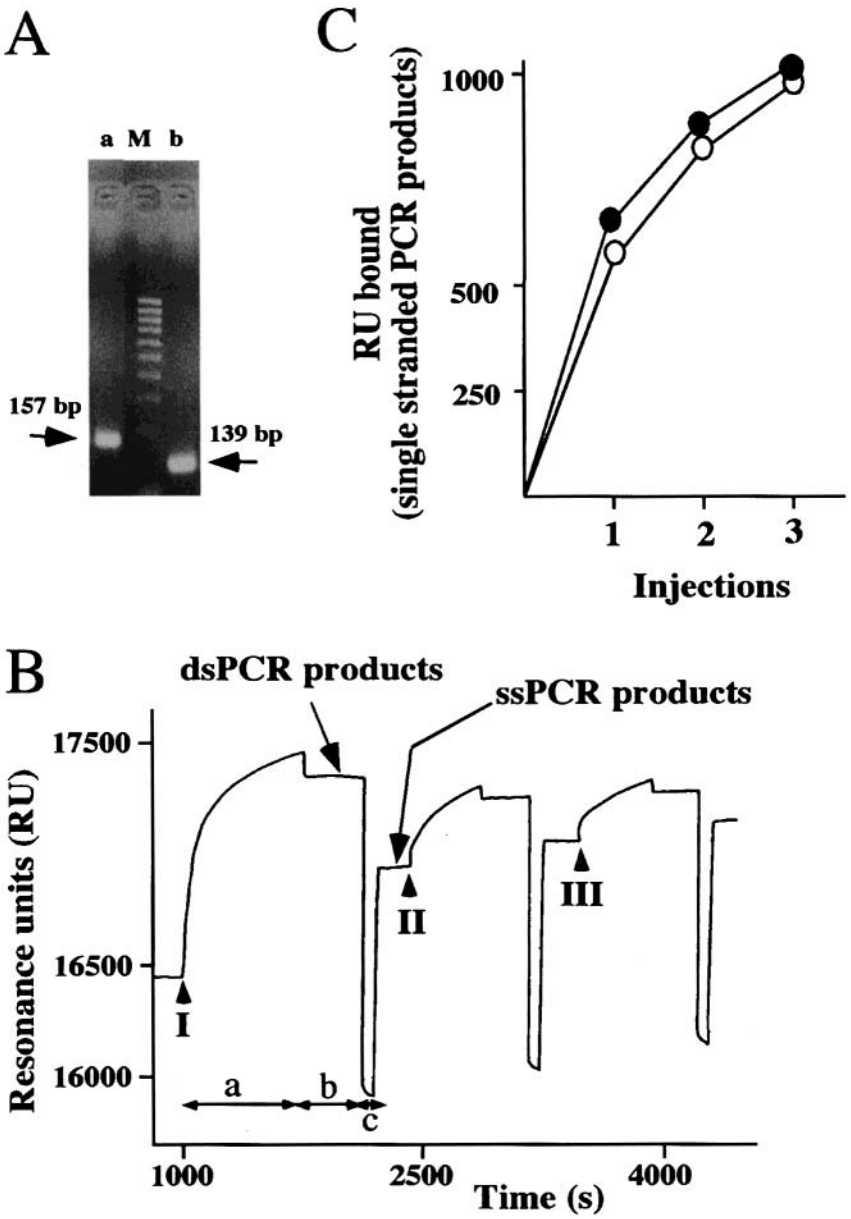

Figure 3. (A) Agarose-gel electrophoretic analysis of the injected lectin (a) and Roundup Ready (b) biotinylated PCR products. M = 100 bp ladder. (B) Representative example of the increase of resonance units following injection on biotinylated Roundup Ready PCR products. Three injections were consecutively performed (I–III). PCR products were injected in HBS-EP; after each injection (segments "a" of the panel), injections of HBS-EP (segments "b" of the panel), and 50 mM NaOH (segments "c" of the panel) were performed. (C) Comparison of the increase of resonance units following three consecutive injections of Roundup Ready (closed symbols) and lectin (open symbols) biotinylated PCR products on SA sensor chips.

30 s, 96 °C; annealing, 30 s, 62 °C; elongation, 10 s, 72 °C. The lengths of the lectin and Roundup Ready PCR product were 157 and 139 bp, respectively.

For immobilization onto SA5 sensor chips, PCR products were obtained by using, in addition to RupR1 and Lec1, the biotinylated biot-RupR2 and biot-Lec2 primers. PCR products were purified using Microcon-30 (Millipore Corporation, Bedford, MA).

Asymmetric PCR products were obtained by 50 amplification cycles using 0.5 μM RupR1 or Lec1 primer, 0.01 μM RupR2 or Lec2 primer, and 25 ng of genomic DNA or 5 ng of the relative PCR products.

Surface Plasmon Resonance. BIAcore 1000 analytical system (BIAcore AB, Uppsala, Sweden) was used in all experiments. Sensor chips SA (research grade), precoated 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 5 μL/min. Sensorgrams were analyzed with the BIAevaluation 2.1 software (*17*). Blank subtractions were performed in all the experiments by subtracting from the experimental sensorgrams the sensorgrams obtained by injections of buffers. To obtain an efficient capture of lectin and Roundup Ready 21 target DNA onto the sensor chip, the well-documented streptavidin−biotin interaction was employed (*22*). After a pretreatment with 10 μL-pulse with 50 mM NaOH, 80 pmoles in HBS-EP of the biotinylated biot-Lec-21 or biot-RupR-21 oligonucleotides were injected over the sensor chip SA5. Hybridization was carried out by injecting 25 μL of lectin and Roundup Ready probes of different lengths (5−10 μM in HBS-EP) at 25 °C, 5 μL/min flow rate. After hybridization and washing with 20 μL of HBS-EP, the flow cells were regenerated by performing a 5-μL pulse of 50 mM NaOH. The nucleotide sequences of targets and probes are shown in **Table 1**. In our hands, the regenerated flow cells can be reused for at least 70 times (data not shown and ref. *19*).

Sequencing of PCR Products. The lectin and Roundup Ready PCR products were purified with Microcon-30 (Millipore Corporation, Bedford, MA) and sequenced using the BigDye terminator cycle

sequencing kit and the ABI Prism 377 DNA sequencer (PE Applied Biosystems, Foster City, CA).

RESULTS

Surface Plasmon Resonance (SPR)-Based Biospecific Interaction Analysis (BIA) Formats for GMO Detection. In **Table 1** the nucleotide sequences of soybean lectin and Roundup Ready biotinylated target oligonucleotides and DNA probes are reported, together with the PCR primers used. Biotinylated lectin and Roundup Ready PCR products or 21 mer target DNAs were stably immobilized onto a SA5 sensor chip by taking advantage from the streptavidin−biotin interaction (*22*) as elsewhere described (*19*, *20*).

In **Figure 1** the employed SPR-based BIA approaches are described. The first approach (**Figure 1A**) is based on immobilization of target single-stranded synthetic oligonucleotides on the sensor chip and injection of specific DNA probes differing in length. This format is of great importance for studying relationships between length of the probes, efficiency of hybridization to target DNA, and stability of the generated molecular hybrids. The second, third, and fourth formats are relevant for diagnostic purposes and are based on the PCR-mediated amplification of gene sequences to be identified. In the SPR-based format described in **Figure 1B**, the PCR is performed using a biotinylated PCR primer immobilized on a sensor chip and analyzed by injection of suitable oligonucleotide probes. In the format outlined in **Figure 1C**, the probes are immobilized on the sensor chip and the asymmetric PCR product to be analyzed is injected. In the last proposed format (**Figure 1D**), PCR-generated probes are immobilized on the sensor chip and the asymmetric PCR products to be analyzed are injected. In our experiments the DNA material to be analyzed has been defined as the "target" and the biomolecules used to detect it (either synthetic oligonucleotides or PCR products) have been referred to as "probes".

Hybridization between Lectin and Roundup Ready Oligonucleotide Probes Differing in Length and 21mer Target DNAs Immobilized on a SA Sensor Chip. Concerning the production of flow cells carrying target DNA oligonucleotides, the data obtained demonstrate that rapid capture of 400−800 RU (resonance units) of biotinylated 21-mer target oligonucleotides was obtained within 4−6 min in different experiments by injecting 25 μL containing 80 pmol of the lectin and Roundup Ready mers in HBS-EP (an example is shown in **Figure 2A**). Preliminary experiments demonstrated that more than 90% of saturation of the lectin and Roundup Ready 21-mer flow cell surfaces is obtained by injecting 40 pmol/25 μL of the specific complementary 15-mer probes, and that satisfactory regeneration of the flow cell with a pulse of 50 mM NaOH is obtained (**Figure 2B** and data not shown). The data of the % of saturation were obtained by using the BIAevaluation software and the algorithm

$$\text{expected } \underset{\text{(analyte)}}{\text{RUfin} - \text{RUi}} = \frac{\underset{\text{(ligand)}}{\text{RUfin} - \text{RUi}} \times \underset{\text{(analyte)}}{\text{M.W.}}}{\text{M.W. (ligand)}}$$

where RUi (initial RU) and RUfin (final RU) are RU at the start and the end of the DNA injection respectively, and RUres (residual RU) is RU at the end of the washing with HBS-EP.

As expected, the generated hybrids were stable (RUres − RUi = 95% ± 3.5% of RUfin − RUi in eight different experiments).

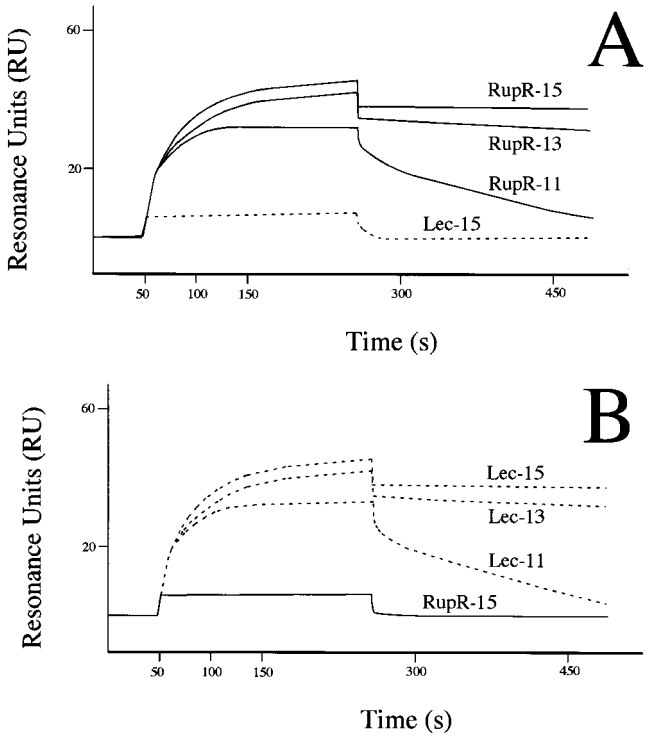

Figure 4. Sensorgrams obtained after injection of 25 μL containing 40 pmol of Roundup Ready (solid lines) and lectin (dotted lines) 11-mer, 13-mer, and 15-mer probes, as indicated, on sensor chips carrying Roundup Ready (A) or lectin (B) PCR products. The sensorgrams shown in this panel have been obtained by subtracting from the experimental sensorgrams the sensorgrams obtained by injecting HBS-EP.

In **Figure 2C** (solid lines) we report the sensorgrams obtained by injecting Roundup Ready DNA probes of different lengths on the sensor chips carrying the Roundup Ready 21-mer target DNA sequences (see scheme depicted in **Figure 1A**). As clearly evident, both Roundup 13- and 15-mers efficiently hybridize to the target 21-mers leading to stable hybrids. On the contrary, the 11-mer Roundup Ready probe hybridized with lower efficiency, giving rise to hybrids exhibiting lower stability when compared to the hybrids generated by RupR-15 and RupR-13. Similar results were obtained using the 11-, 13-, and 15-mer lectin probes and a flow cell carrying the 21-mer target lectin DNA (data not shown). Furthermore, we found that the lectin 15-mer does not hybridize to the Roundup Ready 21-mer target (**Figure 2C**, dotted lines) and the Roundup Ready 15-mer does not hybridize to the lectin 21-mer targets (data not shown).

Some of the relevant data obtained, analyzed with the BIAevaluation software, are reported in **Table 2**. Taken together, these results conclusively demonstrate that, in the BIA experimental conditions, the employed 13-mer and 15-mer DNA probes are suitable for identification of lectin and Roundup Ready soybean DNA sequences.

Immobilization on a SA Sensor Chip of Target Lectin and Roundup Ready PCR Products. Table 1 shows the nucleotide sequences of the PCR primers used to amplify lectin and Roundup Ready gene regions. To produce double-stranded target gene sequences, PCR was performed in two steps by first using, in addition to RupR1 and Lec1, the biotinylated biot-RupR2 and biot-Lec2 primers in a 1:1 ratio. The second PCR step was performed by using 1% of the PCR products as template in the excess of the RupR1 and Lec1 primers and in the presence, respectively, of biotinylated biot-RupR2 and biot-Lec2 primers. This was done in order to minimize the presence

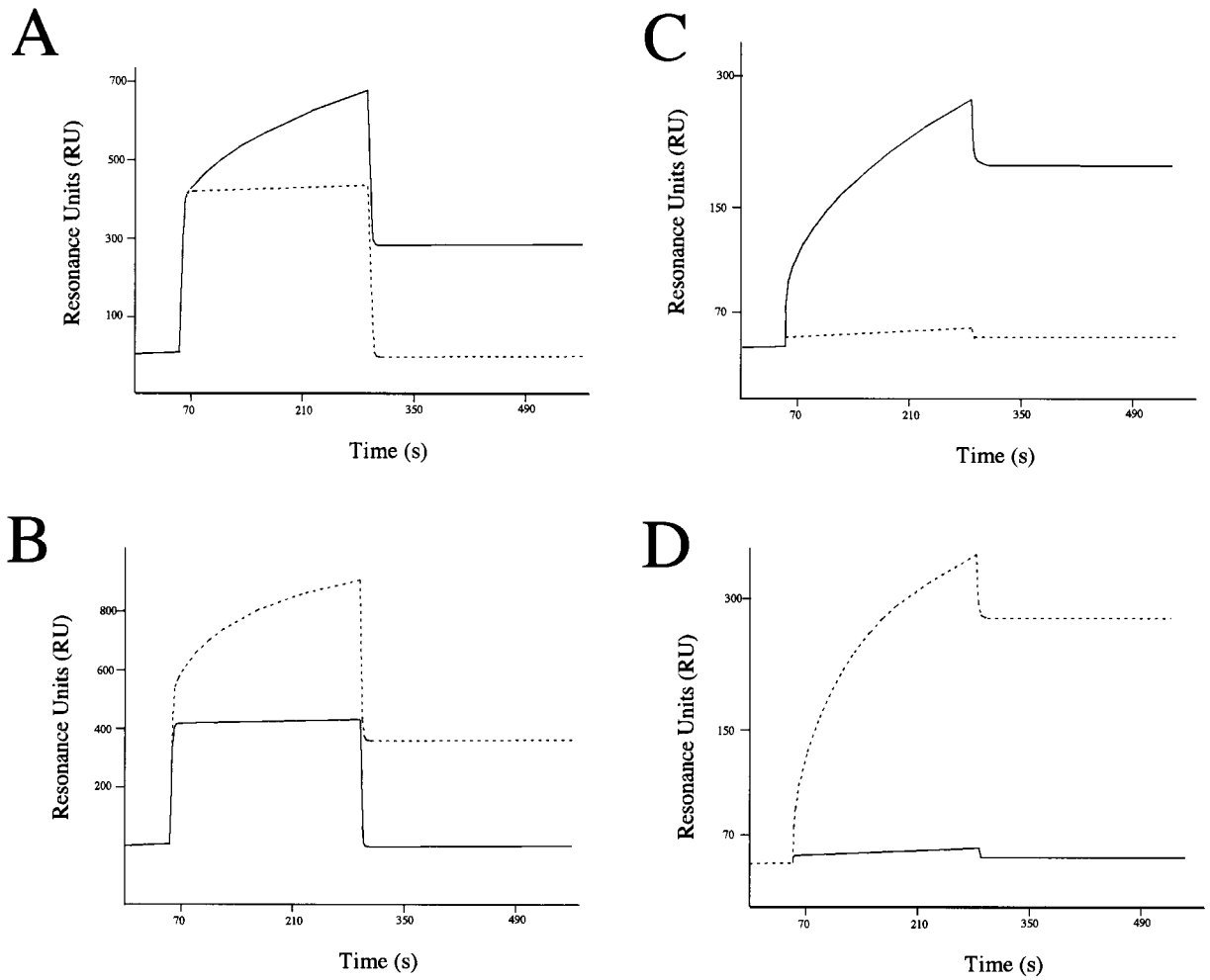

Figure 5. Sensorgrams obtained after injection of 25 μL containing Roundup Ready (solid lines) and lectin (dotted lines) asymmetric PCR products on sensor chips carrying Roundup Ready (A) or lectin (B) PCR generated probes. In panels C and D, the sensorgrams shown in panels A and B have been subtracted using a blank sensorgram obtained after injection of the PCR probes onto an unrelated target DNA.

of biotinylated, unincorporated, biot-RupR2 and biot-Lec2 PCR primers in the mixture after the polymerase chain reaction. The final lectin and Roundup Ready PCR products were, in any case, further purified with Microcon-30. Agarose gel electrophoretic analysis (**Figure 3A**) and direct sequencing (data not shown) of the PCR products confirmed the specificity of the PCR reaction. To reach high levels of binding of the PCR products, three consecutive injections were performed using 60 μL of 3.5 μM PCR products in HBS-EP (**Figure 3B**). Pulses with 50 mM NaOH were performed after each injection for the production of single-stranded PCR products able to hybridize with the lectin and Roundup Ready probes. We obtained about 1000 RU of immobilized PCR products from lectin and Roundup Ready gene sequences (**Figure 3C**).

Hybridization of Roundup Ready and Lectin DNA Probes Following Injection to Immobilized Target Roundup Ready and Lectin PCR Products. A representative example of the binding of Roundup Ready and lectin 11-15 mer DNA probes to Roundup Ready and lectin immobilized PCR products is shown in **Figure 4**. As expected from the data shown in **Figure 2**, RupR-15, RupR-13, Lec-15, and Lec-13 DNA probes are able to specifically hybridize to the Roundup Ready (**Figure 4A**) and lectin (**Figure 4B**) PCR products, respectively. On the contrary, the 11-mer probes hybridize generating unstable probes/PCR products complexes. The Roundup Ready 15-mer probe does not hybridize to the lectin PCR target DNA (**Figure 4B**, solid line); conversely, the lectin 15-mer probe does not hybridize to the Roundup Ready PCR target DNA (**Figure 4A**, dotted line).

Injection of Asymmetric Roundup Ready and Lectin PCR Products to Sensor Chips Carrying 21-mer or PCR-Generated Probes. Figure 5 shows the results concerning a representative example of injection of asymmetric Roundup Ready and lectin PCR products onto sensor chips carrying Roundup Ready (**Figure 5**, **A** and **C**) and lectin (**Figure 5**, **B** and **D**) PCR-generated probes. The results obtained demonstrate that high-level gene-specific binding is obtained. It should be noted that in the experiment shown in **Figure 5** purified PCR products were used. However, from the data shown in **Table 2**, it is clear that both purified and nonpurified asymmetric PCR can be injected onto sensor chips carrying Roundup Ready and lectin PCR-generated probes.

In addition, **Table 2** shows that, with respect to injection of asymmetric PCR products flow cells carrying 21-mer DNA probes, discrimination is also readily obtained, although the level of hybridization (RUfin − RUi) is much lower than that obtained using PCR-generated probes. In our experimental conditions, this format exhibits, among the tested formats (see **Figure 1**, **C**−**D**), the lowest efficiency in terms of obtained/expected RUfin − RUi values.

DISCUSSION

In the present paper we demonstrate that biospecific interaction analysis (BIA), employing surface plasmon resonance (SPR) and biosensor technologies (*14*) is an easy, speedy, and automatable approach to detect GMO.

We first immobilized on two different flow cells of a SA sensor chip single-stranded biotinylated oligonucleotide containing the sequence of the lectin and the Roundup Ready genes, and the efficiency of hybridization of oligonucleotide probes differing in length was determined. Second, we immobilized on different SA sensor chips biotinylated PCR products from nontransgenic soybeans (genomes carrying only the lectin gene), as well as from genetically modified Roundup Ready soybean. Then, we injected the oligonucleotide probes. Furthermore, we used the sensor chips carrying lectin and Roundup Ready soybean PCR products as probes, and we injected both nonpurified and purified asymmetric PCR products.

The results obtained show that 15-mer oligonucleotides are suitable probes for 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 hybidization was obtained because of the low stability of the hybridization complexes generated.

All the SPR-based formats were found to be useful for detection of RoundUp Ready gene sequences (see **Table 2**), demonstrating that SPR-based BIA is a flexible, easy, speedy, and automatable approach to detect genetically modified Roundup Ready soybean in foods. By this procedure, the real-time monitoring of hybridization between target single-stranded PCR products, obtained by using as substrates DNA isolated from normal or transgenic soybeans, and oligonucleotide or PCR-generated probes is possible, therefore enabling a one-step, nonradioactive protocol to perform detection.

The described method is, to our knowledge, the first application of BIA and SPR to detection of GMO.

We like to point out that the procedure described in the present paper is rapid, and informative results are obtained within about 40 min. We also like to underline that new-generation biosensors (for instance BIAcore-2000 and BIAcore-3000) exhibit technical innovations (among which are multi-sample analysis, increased sensitivity, and smaller flow cells) allowing multiple testing and shorter injection periods.

Advantages of the methodology described in the present paper are (a) that it is a nonradioactive methodology and (b) that gel electrophoresis and/or dot-spot analysis are not required.

In addition, we underline that our approach could be proposed for all the SPR-based biosensors that are commercially available, including IBIS-I and IBIS-II (*23*), IAsys and IAsys Plus (*24*), SPR670 and SPR-CELLIA biosensors (*25*). Some of these optical SPR-based biosensors use, in addition to flow-based sensor chips, stirred micro-cuvettes, reducing the costs of the experimentation (*26*).

On the other hand, we like to point out that both sensitivity and limit of detection of this methodology have still to be determined. These are, of course, very important parameters, because information on low percentage of GMO is of great value. Determination of the ability of SPR-based BIA in detecting very low amounts of GMO should be considered a major research field for future studies.

Finally, we like to emphasize that this method is expected to be suitable in multiplex determinations and arrayed detections. With respect to DNA microarrays (*27*), it should be pointed out that this novel high-throughput methodology allows the simultaneous analysis of high numbers of food samples and is expected to be the major field of applied research in GMO detection (*28*−*30*). In this respect, a recent paper by O'Brien et al. (*31*) describes a surface plasmon resonance array biosensor based on spectroscopic imaging. Briefly, these authors developed a multi-element transduction system which combines conventional SPR spectroscopy with one-dimensional SPR microscopy to create an effective platform for monitoring binding events on macro- or micro-patterned arrays created on disposable sensor chips (*32*), allowing the analysis of several independent biospecific binding events simultaneously. Studies aimed at developing SPR-based arrayed biosensors have been undertaken by other research groups with very interesting preliminary results (*32*, *33*).

The data presented in our paper strongly suggest the use of Roundup Ready and lectin target DNAs in multiple arrayed samples, thereby introducing the possibility of high-throughput SPR-based screening of GMO in food.

ABBREVIATIONS USED

GMO, genetically modified organisms; SPR, surface plasmon resonance; BIA, biospecific interaction analysis; RU, resonance units; RupR, Roundup Ready.

LITERATURE CITED

(1) Vollenhofer, S.; Burg, K.; Schmidt, J.; Kroath, H. Genetically modified organisms in food-screening and specific detection by polymerase chain reaction. *J. Agric. Food Chem.* **1999**, *47*, 5038−5043.

(2) Lipp, M.; Anklam, E.; Stave, J. W. Validation of an immunoassay for detection and quantitation of a genetically modified soybean in food and food fractions using reference materials: interlaboratory study. *J. AOAC Int.* **2000**, *83*, 919−927.

(3) Vollenhofer, S.; Burg, K.; Schmidt, J.; Kroath, H. Short communication: Detection of Genetically Modified Organisms in Food. *Dtsch. Lebensm. Rundsch.* **1999**, *95*, 275−278.

(4) Studer, E.; Rhyner, C.; Lüthy, J.; Hübner, P. Quantitative competitive PCR for the detection of genetically modified soybean and maize. *Z. Lebensm.-Unters. Forsch.* **1998**, *207*, 207−213.

(5) Meyer, R. Detection of genetically engineered plants by polymerase chain reaction (PCR) using the FLAVR SAVR tomato as an example. *Z. Lebensm.-Unters. Forsch.* **1995**, *201*, 583−586 (German).

(6) Meyer, R.; Chardonnens, F.; Hubner, P.; Luthy, J. Polymerase chain reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products. *Z. Lebensm.-Unters. Forsch.* **1996**, *203*, 339−344.

(7) Wurz, A.; Willmund, R. Identification of Transgenic Glyphosate-resistant soybeans. In *Foods Produced by Means of Genetic Engineering, 2nd Status Report*; Schreiber, G. A., Bögl, K. W., Eds.; Federal Institute for Health Protection of Consumers and Veterinary Medicine: Berlin, 1997; BgVV Hefte 1, pp 115−117.

(8) Hupfer, C.; Hotzel, H.; Sachse, K.; Engel, K. H. Detection of the genetic modification in heat-treated products of Bt maize by polymerase chain reaction. *Z. Lebensm.-Unters. Forsch.* **1998**, *206*, 203−207.

(9) Hassan-Hauser, C.; Mayer, W.; Hortner, H. Detection of the starch modifying gbss-antisense construct in transgenic potatoes. *Z. Lebensm.-Unters. Forsch.* **1998**, *206*, 83−87.

(10) Hoef, A. M.; Kok, E. J.; Bouw, E.; Kuiper, H. A.; Keijer, J. Development and application of a selective detection method for genetically modified soy and soy-derived products. *Food Addit. Contam.* **1998**, *15*, 767−774.

(11) Vaitilingom, M.; Pijnenburg, H.; Gendre, F.; Brignon, P. Real-time quantitative PCR detection of genetically modified Maximizer maize and Roundup Ready soybean in some representative foods. *J. Agric. Food Chem.* **1999**, *47*, 7(12), 5261−5266.

(12) Jonsson, U.; Fagerstam, L.; Ivarsson, B.; Johnsson, B.; Karlsson, R. Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. *BioTechniques* **1991**, *11*, 620−627.
(13) Vadgama, P.; Crump, P. W. Biosensors: recent trends, a review. *Analyst* **1992**, *117*, 1657−1670.
(14) Malmqvist, M. Biospecific interaction analysis using biosensor technology. *Nature* **1993**, *361*, 186−187.
(15) Wood, S. J. DNA-DNA hybridization in real time using BIAcore. *Microchem. J.* **1993**, *47*, 330−337.
(16) Nilsson, P.; Persson, B.; Uhlén, M.; Nygren, P. A. Real-time monitoring of DNA manipulations using biosensor technology. *Anal. Biochem.* **1995**, *224*, 400−408.
(17) Bianchi, N.; Rutigliano, C.; Tomassetti, M.; Feriotto, G.; Zorzato, F.; Gambari, R. Biosensor technology and surface plasmon resonance for real-time detection of HIV-1 genomic sequences amplified by polymerase chain reaction. *Clin. Diagn. Virol.* **1997**, *8*, 199−208.
(18) Nilsson, P.; Persson, B.; Larsson, A.; Uhlen, M.; Nygren, P.-A. Detection of mutations in PCR products from clinical samples by surface plasmon resonance. *J. Mol. Recognit.* **1997**, *10*, 7−17.
(19) Feriotto, G.; Lucci, M.; Bianchi, N.; Mischiati, C.; Gambari, R. Detection of the ΔF508 (F508del) Mutation of the Cystic Fibrosis Gene by Surface Plasmon Resonance and Biosensor Technology. *Hum. Mutat.* **1999**, *13*, 390−400.
(20) Feriotto, G.; Ferlini, A.; Ravani, A.; Calzolari, E.; Mischiati, C.; Bianchi, N.; Gambari, R. Biosensor Technology for Real-Time Detection of the Cystic Fibrosis W1282X Mutation in CFTR. *Hum. Mutat.* **2001**, *18*, 70−81.
(21) Saiki, R. K.; Scharf, S.; Faloona, F.; Mullis, K. B.; Horn, G. T.; Erlich, H. A.; Arnheim, N. Enzymatic amplification of *β*-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **1985**, *230*, 1350−1354.
(22) Leblond-Francillard, M.; Dreyfus, M.; Rougeon, F. Isolation of DNA-protein complexes based on streptavidin and biotin interaction. *Eur. J. Biochem.* **1987**, *166*, 351−355.
(23) de Mol, N. J.; Plomp, E.; Fischer, M. J.; Ruijtenbeek, R. Kinetic analysis of the mass transport limited interaction between the tyrosine kinase lck SH2 domain and a phosphorylated peptide studied by a new cuvette-based surface plasmon resonance instrument. *Anal. Biochem.* **2000**, *279*, 61−70.
(24) Edwards, P. R.; Maule, C. H.; Leatherbarrow, R. J.; Winzor, D. J. Second-order kinetic analysis of IAsys Biosensor data: its use and applicability. *Anal. Biochem.* **1998**, *263*, 1−12.
(25) Senzaki, K.; Ogawa, M.; Yagi, T. Proteins of the CNR family are multiple receptors for Reelin. *Cell* **1999**, *99*, 635−647.
(26) Myszka, D. G.; Rich, R. L. Implementing surface plasmon resonance biosensors in drug discovery. *Pharm. Sci. Technol. Today* **2000**, *9*, 3310−3317.
(27) Hacia, J. G.; Fan, J. B.; Ryder, O.; Jin, L.; Edgemon, K.; Ghandour, G.; Mayer, R. A.; Sun, B.; Hsie, L.; Robbins, C. M.; Brody, L. C.; Wang, D.; Lander, E. S.; Lipshutz, R.; Fodor, S. P.; Collins, F. S. Determination of ancestral alleles for human single-nucleotide polymorphisms using high-density oligonucleotide arrays. *Nat. Genet.* **1999**, *22*, 164−167.
(28) Eggers, M. Hig-throughput microarray technology. *Innov. Pharm. Technol.* **2000**, *6*, 36−44.
(29) Shi, M. M.; Bleavins, M. R.; de la Iglesia, F. A. Technologies for detecting genetic polymorphisms in pharmacogenomics. *Mol. Diagn.* **1999**, *4*, 343−351.
(30) Shi, M. M. Enabling large-scale pharmacogenetic studies by high-throughput mutation detection and genotyping technologies. *Clin. Chem.* **2001**, *47*, 164−72.
(31) O'Brien, M. J., II; Perez-Luna, V. H.; Brueck, S. R.; Lopez, G. P. A surface plasmon resonance array biosensor based on spectroscopic imaging. *Biosens. Bioelectron.* **2001**, *16*, 97−108.
(32) 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.
(33) Brockman, J. M.; Nelson, B. P.; Corn, R. M. Surface plasmon resonance imaging measurements of ultrathin organic films. *Annu. Rev. Phys. Chem.* **2000**, *51*, 41−63.

Received for review July 25, 2001. Revised manuscript received November 26, 2001. Accepted November 26, 2001. This research was supported by Ministero della Sanità, Italy, by P.F. Biotecnologie, and by MURST Cofin-2000 (Tracciabilità di frammenti di DNA e proteine lungo la filiera alimentare e mangimistica con metodi applicati alla etichettatura volontaria − EC1139/98). The BIAcore-1000 was obtained with a grant from the "Grandi attrezzature ad uso comune" fund of Ferrara University.

JF0109773