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Ultrasensitive detection of non-amplified genomic DNA by nanoparticle-enhanced surface plasmon resonance imaging

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ABSTRACT

Technologies today available for the DNA detection rely on a combination of labeled probes hybridized to target sequences which are amplified by polymerase chain reaction (PCR). Direct detection methods that eliminate the requirement for both PCR and labeling steps could afford faster, cheaper and simpler devices for the analysis of small amounts of unamplified DNA.

In this work we describe the results obtained in the ultrasensitive detection of non-amplified genomic DNA. We analyzed certified reference materials containing different amounts of genetically modified DNA by using a detection method which combines the nanoparticle-enhanced surface plasmon resonance imaging (SPRI) biosensing to the peptide nucleic acids (PNAs) improved selectivity and sensitivity in targeting complementary DNA sequences. The method allowed us to obtain a 41 zM sensitivity in targeting genomic DNA even in the presence of a large excess of non-complementary DNA.

1. Introduction

The development of new devices for the rapid, cheap and sensitive detection of specific sequences of genomic DNA is crucial to future genetic diagnostic applications (Perkel, 2008; Gibson, 2008; Sassolas et al., 2008). Most of the current available methods for genomic DNA detection require the DNA sequence to be identified to be previously amplified by polymerase chain reaction (PCR), as well as labeled with fluorophores. Such procedure require expensive reagents and pitfalls may occur due to contamination or matrix effects (Shi et al., 2008).

High-sensitivity, high-specificity, label-free or non-labeling sensing and multiplexed capability are essential properties required for new devices to be used in genomic DNA detection. Ultrasensitivity is necessary to avoid the sample PCR amplification (Sohni et al., 2008).

A number of different methods have been investigated so far, with the aim to meet the above-mentioned requirements (Sassolas et al., 2008; Rosi and Mirkin, 2005; Totsingan et al., 2008). However, a limited number of label-free or non-labeling applications on non-amplified genomic DNA samples have been so far described (Zhu et al., 2008; Zhang and Appella, 2007; Ho et al., 2005; Minunni et al., 2007; Goodrich et al., 2004; Stoeva et al., 2006; Storhoff et al., 2004). Most of the methods detect genomic DNA with pM–fM sensitivity. The anthrax DNA colorimetric detection was obtained with an aM sensitivity (Zhang and Appella, 2007) while zM sensitivity in targeting a specific human genomic DNA sequence has been achieved by combining a fluorescence-based detection with an optical polymeric transducer (Ho et al., 2005). In the latter case, a target large sample volume was used for the analysis (3 mL) and single detection of the non-amplified genomic DNA was demonstrated.

A multiplexed and label-free or non-labeling detection of non-amplified genomic DNA with zM sensitivity represents a fundamental step toward the identification of innovative genomic applications.

SPR imaging (SPRI) (Rothenhäusler and Knoll, 1988) is an extremely versatile method for detecting the interaction of biomolecules in a microarray format (Scarano et al., 2009; Grasso et al., 2006, 2009a,b; Lee et al., 2006; D'Agata et al., 2006; Arena et al., 2004). Label-free and real-time analyses can be carried out with high-throughput and low sample consumption by cou-

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pling microfluidic devices with the SPRI apparatus (Grasso et al., 2009a,b).

The advantages offered by surface plasmon resonance imaging (SPRI) for the label-free, ultrasensitive and multiplexed detection of DNA and RNA have been recently demonstrated (Wark et al., 2008; Ruemmele et al., 2008; D'Agata et al., 2008). The detection of 7 fM genomic DNA has been obtained by using an enzymatically amplified SPRI-based analytical approach (Goodrich et al., 2004) while the direct detection of 10 ppm solutions of plant, bovine and human genomic DNAs has been obtained by using spectral SPR (Minunni et al., 2007).

Herein, we report on the possibility of using peptide nucleic acids (PNAs) probes for the ultrasensitive nanoparticle-enhanced SPRI detection of non-amplified genomic DNA solutions containing a target sequence as a minor component. PNAs have been shown to be able to improve both selectivity and sensitivity in targeting complementary ssDNA and RNA sequences (D'Agata et al., 2008; Li-Qiang et al., 2007; Rossi et al., 2006).

As a test system, we choose a set of samples containing different amounts of genetically modified (GM) material (Roundup Ready soybean, RR, BF410) for which certified reference materials with different GM mass fractions (from <0.03% to 5.00%) are available. It was possible to detect non-amplified genomic DNA down to 41 zM (4.1×10^{-20} M) in concentration, even in the presence of a large excess of non-complementary DNA, thus affording a direct method for detection of the GMO content which parallels the specificity of PCR-based methods.

2. Experimental

2.1. Materials and reagents

Reagents were obtained from commercial suppliers and used without further purification. Wild-type streptavidin (WT-SA) was purchased from Invitrogen (Italy). Nitrocellulose membrane filters were purchased from Whatman (UK). Trisodium citrate dihydrate, tetrachloroauric(III) acid, ethanol, dimethyl sulfoxide, hexane, sodium hydroxide solutions (10 M in water) and dithiobis(N)succinimidylpropionate (DTSP) were purchased from Sigma–Aldrich (Italy). Phosphate buffered saline (PBS) solutions at pH 7.4 (137 mM NaCl, 2.7 mM KCl, phosphate buffered 10 mM) were obtained from Amresco (Italy). Biotinylated oligonucleotides (Fig. 1) were purchased from Thermo Fisher Scientific, Inc. The certified reference materials ERM-BF410 consisting of <0.03% (GM-free), 0.10%, 0.50%, 2.00% and 5.00% (w/w) of soyabean Roundup Ready™ GMO were purchased from Fluka. SPRI gold chips were purchased from GWC Technologies (USA). Ultra-pure water (Milli-Q Element, Millipore) was used for all the experiments.

2.2. PNA probe synthesis and surface immobilization

The PNA 1 sequence (Fig. 1) was designed, synthesized by using automatic solid phase synthesis, purified and characterized (S1 in Supplementary data) as previously described (Germini et al., 2005).

An optimal surface probe density is required in order to obtain reproducible and consistent SPRI results, since an excessively dense surface decreases the measurement sensitivity in detecting oligonucleotide hybridization (Zanoli et al., 2008).

The PNA 1 probe was immobilized on the DTSP-modified gold chips through the amine-coupling reaction between the N-hydroxysuccinimidyl ester ends of DTSP and the N-terminal group present on the 2-(2-aminoethoxy)ethoxyacetic acid (AEEA) linker (Rossi et al., 2006). The probe immobilization was obtained using 0.1 μM PNA 1 solutions in PBS (flow rate 5 μL min^{-1}) (S2 in Supplementary data) that produced a mean PNA 1 surface coverage of 3×10^{12} molecules cm^{-2}.

2.3. SPRI apparatus and measurements

All the SPRI experiments were carried out by using an SPR imager apparatus (GWC Technologies, USA). SPR images were analyzed by using the V++ software (version 4.0, Digital Optics Limited, New Zealand) and the software package Image J 1.32j (National Institutes of Health, USA). SPRI provides data as pixel intensity units (0–255 scale). Data were converted into percentage of reflectivity (%R), or Δ%R in the case of difference images, by using the formula:

$$\%R = 100 \times \left(\frac{0.85 I_p}{I_s}\right)$$

where I_p and I_s refer to the reflected light intensity detected using p- and s-polarized light, respectively. The experiments were carried out by sequentially acquiring 15 frames averaged SPR images with 5 s time delay between them. Kinetic data were obtained by plotting the difference in percent reflectivity (Δ%R) from selected regions of interest (ROIs) of the SPR images as a function of time. The selected ROIs were chosen in order to include all the SPR chip area involved by the surface interaction experiment. All the SPRI experiments were carried out at room temperature.

A microfluidic device with six parallel microchannels (80 μm depth, 1.4 cm length, 400 μm width) and circular reservoirs (diameter = 400 μm) at the ends of each channel was used for the study. It was fabricated in poly(dimethylsiloxane) (PDMS) polymer through the well established replica molding technique (see also Supplementary data). PEEK tubes (UpChurch Scientific) were inserted in the circular reservoirs in order to connect the PDMS microfluidic cell to an Ismatec IPC (Ismatec SA, Switzerland) peristaltic pump. The microfluidic device was assembled by fixing the PDMS mold on the SPRI gold chip surface. A refractive index matching liquid was used to obtain the optical contact between the flow cell and the prism.

2.4. Preparation of the modified gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized by citrate reduction of $HAuCl_4 \cdot 3H_2O$ according to methods elsewhere described (Grabar et al., 1995) and were characterized by UV–vis spectroscopy (Agilent 8453 spectrometer) and transmission electron microscopy (TEM, Jeol JEM-2000 FX II, operating at 200 kV). The mean diameter of AuNPs was 20 ± 5 nm (S3 in Supplementary data).

AuNPs were conjugated to the biotinylated DNA 13-mer sequence (T_m = 38.0 °C) according to procedures elsewhere described (He et al., 2000). The selection of an appropriate conjugated AuNPs concentration was critical for the success of the nanoparticle-enhanced SPRI experiments. The described experiments were conducted by using 0.3 nM solutions (in PBS). It is here useful to note that similar experiments, carried out by using oligonucleotides instead of genomic DNA, were conducted by using more concentrated AuNPs solutions (1 nM). The actual concentration of the conjugated AuNPs stock solutions (typically, 1–3 nM) was obtained from UV–vis spectroscopy ($\varepsilon_{528} = 2 \times 10^8$ M^{-1} cm^{-1}) (Haiss et al., 2007).

2.5. Genomic DNA sample treatments

Genomic DNA was extracted from each Roundup Ready GMO certified reference material by using the IonForce kit (Generon, Italy) according to the manufacturer's