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A reusable DNA biosensor for the detection of genetically modified organism using magnetic bead-based electrochemiluminescence

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ABSTRACT

A reusable magnetic bead-based electrochemiluminescence (ECL) DNA biosensor for the detection of genetically modified organism (GMO) is proposed. The sensor method consists of immobilization of biotin-probe using streptavidin-coated magnetic beads, hybridization of target DNA with biotin-probe and ruthenium(II) tris-bipyridal (TBR)-probe, detection of target DNA by direct measuring the ECL emission of TBR. The sensor was applied to detect GMO in engineered tobacco samples. Results indicate that the sensitivity of this sensor method was 5 nmol/L of CaMV35S DNA. A stable calibration curve with a wide dynamic range was established. The calibration curve was linear from 5 nmol/L to 5 μmol/L, thus, made quantitative analysis possible. GM tobacco samples and non-GM tobacco samples were clearly discriminated by the sensor method. Results of the study suggest that the reusable DNA biosensor is a feasible tool for daily GMO detection due to its rapidness, simplicity, safety, sensitivity, reliability and low cost.

1. Introduction

Recently, the safety concern of genetically modified organism (GMO) has attracted much attention [1,2]. Many countries require mandatory labeling of GM foods and have adopted a threshold for affirmative GMO labeling (e.g., 0.5% in the European Union) [3]. Hence, demand for testing GM foods has increased dramatically. Polymerase chain reaction (PCR) combined with gel electrophoresis analysis is the most widely used tool for GMO identification [4,5]. However, gel electrophoresis and ethidium bromide (EB) staining are laborious, time-consuming and poisonous. The need for simplicity and speed in these assays has stimulated the development of DNA biosensors for GMO screening, such as quartz crystal microbalance (QCM)-based DNA biosensor [6–8], surface plasmon resonance (SPR) biosensor [9–12], electrochemical genosensor [7,13–15], and nanoparticle-based DNA biosensor [16]. The analytical performances of these biosensors for the detection of GMO are listed in Table 1.

Electrochemiluminescence (ECL), where light-emitting species are produced by reactions between electrogenerated intermediates, has become an important and powerful analytical tool [17–26]. An ECL reaction between ruthenium(II) tris-bipyridal (TBR) and tripropylamine (TPA) has been demonstrated to be a highly sensitive approach to quantify target DNA in our previous study [20]. In the present work, we report a reusable magnetic bead-based ECL DNA biosensor for GMO detection. The cauliflower mosaic virus CaMV35S (CaMV35S) promoter and the nopaline synthase (NOS) terminator commonly used in transgenic products [27] were targeted. As an example, engineered tobacco samples were tested by the sensor.

2. Materials and methods

2.1. Materials

Tobacco samples were gifts from South China Agricultural University, Guangzhou, China. UNIQ-10 column PCR product purification kit was purchased from Shanghai Sangon Biological Engineering Technology & Service Corporation Ltd. (SSBE). Streptavidin-coated magnetic beads (2.8 μm diameter) were products of Dynal Biotech (Lake Success, NY, USA). TPA was purchased from Sigma (Louis, MO, USA). The primers and probes used for GMO detection were synthesized by SSBE. The probes were labeled with biotin by SSBE and labeled with TBR by our laboratory according to Terpetschnig's paper [28]. The sequences of the primers and probes were shown in Table 2.

2.2. Apparatus

The reusable magnetic bead-based ECL DNA biosensor was designed by our laboratory. The heart of it is an electrochemical detection cell, containing a platinum working electrode (disk), a platinum counter electrode (cirque) and an Ag/AgCl reference electrode (thread). A removable magnet is placed under the

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Table 1
Comparison of the analytical performances of current biosensors for the detection of GMO.

Method	Detection limit	Linearity range	Reproducibility	Ref. no.
QCM-based DNA biosensor	0.025 μM	0–0.1 μM	10–15% (CV)	[6]
SPR biosensor	1 nM	1–100 nM for NOS; 1–125 nM for 35S	<3% (CV)	[9]
Enzyme-based electrochemical genosensor	1.2 pM	12 pM to 12 nM	10% (SD)	[14]
Nanoparticle-based DNA biosensor	0.8 fmol (0.16 nM)	0–25 fmol	2.6–12.2% (SD)	[16]

working electrode for immobilization and release of the biotin-probe captured by streptavidin-coated magnetic beads. The ECL emission is received by a single photon counting module (MP-962, PerkinElmer, Germany) and the ECL value is output by a personal computer.

2.3. Sample preparation

The genomic DNAs of the tobacco samples were extracted by the cetyltrimethyl ammonium bromide (CTAB) method. Briefly, the sample was minced with sterile surgical blades and dried to flour, then moistened with threefold amount of water and extracted by CTAB, precipitated, treated with chloroform, and precipitated with isopropanol to obtain the purified genomic DNA. The concentration of the genomic DNA was determined by measuring the absorbance at 260 nm (A_{260}) using a UV Spectrophotometer (BioPhotometer, Eppendorf, Germany). The purity of the DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm (A_{260}/A_{280}).

The genomic DNA with an A_{260}/A_{280} ratio of 1.8–2.0 was used as template to amplify the GMO target DNA (CaMV35S promoter and NOS terminator) following the PCR procedure of Pietsch [29]. The thermocycler (PTC-100 MJ Research Inc., USA) was programmed with an initial step of denaturation at 94 °C for 3 min. Cycling conditions were: denaturation at 94 °C for 20 s, annealing at 54 °C for 40 s and elongation at 72 °C for 1 min. In total 40 cycles of above program were performed. The last round of elongation at 72 °C was for 3 min. Negative controls (PCR without DNA template) were processed in parallel with each batch of samples to monitor the possible contamination. The concentration of PCR products was determined spectrophotometrically at 260 nm. Screening of the PCR products was performed by gel electrophoresis and visualized through a UV transilluminator. The PCR products were then diluted 10 times and used as GMO target DNAs for tobacco samples detection.

2.4. Immobilization of biotin-probe

Twenty microliters of biotin-probe and 10 μL of streptavidin-coated magnetic beads were incubated at 37 °C for 20 min, to form the biotin–streptavidin linkage. Then, the mixture was added to the electrochemical detection cell and the biotin-probe captured by streptavidin-coated magnetic beads was immobilized on the working electrode by activating the magnetic field of the removable magnet.

2.5. Hybridization with GMO target DNA and TBR-probe

Twenty microliters of denatured GMO target DNA and 20 μL of TBR-probe were added to the electrochemical detection cell in turn, and the mixture was hybridized at 50 °C for 10 min, to form a biotin-probe, GMO target DNA and TBR-probe sandwich complex.

2.6. ECL detection

Tris–EDTA (TE) (pH 7.4) buffer containing TPA was added to the electrochemical detection cell, and a voltage of 1.25 V was applied across the electrode to start the reaction between TBR and TPA, which can emit photons for ECL detection. After ECL detection, the sandwich complex with the magnetic beads was washed away by deactivating the magnetic field. To start the next detection cycle, a new biotin-probe bound to streptavidin-coated magnetic beads was immobilized on the working electrode by reactivating the magnetic field.

2.7. Calibration of the sensor method for CaMV35S target DNA

To prepare the calibration standards, PCR product of CaMV35S promoter was purified using UNIQ-10 column PCR product purification kit, and quantified by absorbance measurement at 260 nm. The purified PCR product was then serially diluted into a series of samples containing different concentrations of CaMV35S target DNA, ranging from 5 nmol/L to 5 μmol/L. Three separate samples for each CaMV35S concentration were prepared and evaluated by the sensor method. Each sample was measured 30 times with 1-s data integration. The averages and the standard deviations were calculated using Microsoft Excel spread sheet function. The calibration curve was plotted as the ECL value (counts per second, cps) against the CaMV35S concentration.

A threshold value is calculated based on the average (V_{negative}) and standard deviation ($V_{\text{stdev(neg)}}$) of the ECL value from the negative controls (PCR without DNA template), shown as formula (1), to define if a sample was positive of GMO (containing GM component).

$$V_{\text{threshold}} = V_{\text{negative}} + 3V_{\text{stdev(neg)}} \quad (1)$$

3. Results and discussion

3.1. Principle

Fig. 1 shows the basic principle of the reusable magnetic bead-based ECL DNA biosensor for the detection of GMO.

Table 2
Primers and probes used for GMO detection.

Name	Sequence (5′–3′)	Product size (bp)	GC content (%)
CaMV35S sense primer	gctcctacaaatgccatca	195 (sense primer + antisense primer)	9/19 (47.7%)
CaMV35S antisense primer	gatagtgggattgtgcgtca		10/20 (50%)
NOS sense primer	gaatcctgttgccggtcttg	180 (sense primer + antisense primer)	11/20 (55%)
NOS antisense primer	ttatcctagtttgcgcgcta		9/20 (45%)
CaMV35S biotin-probe	cggcagaggcatcttcaacgatggcc-biotin		16/26 (61.5%)
CaMV35S TBR-probe	TBR-tttccacgatgctcctcgtgggtggg		16/26 (61.5%)
NOS biotin-probe	ccatctaaataacgtcatgcat-biotin		8/22 (36.4%)
NOS TBR-probe	TBR-cgcgtattaaatgtataattgcg		8/23 (48.2%)