Electrochemical genosensor for the rapid detection of GMO using loop-mediated isothermal amplification†

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In this study, we are reporting for the first time an efficient, accurate and inexpensive rapid detection system which employs the integration of isothermal amplification and subsequent analysis of unpurified amplicons by an electrochemical system. In our experiments, loop-mediated isothermal amplification (LAMP) with its higher efficiency than PCR was performed at a constant temperature (65 °C). Amplification products were combined with a redox active molecule Hoechst 33258 [H33258, 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi(1H-benzimidazole)] and analyzed by a DNA stick (DS) which is integrated with a disposable electrochemical printed (DEP) chip using linear sweep voltammetry (LSV). The DNA minor groove binding of the H33258 molecule causes a significant drop in the peak current intensity of the H33258 oxidation. The phenomenon of DNA binding induced by H33258, in addition to changes in the anodic current peak, was used to detect maize CBH 351 variety (StarLink™). Since laborious probe immobilization was not required, and amplification and detection were performed on a single device, our biosensor eliminates potential cross-contamination. We believe that this type of sensor will have an unprecedented impact for environmental protection.

Introduction

The genetically modified organism (GMO) can be defined as plants or animals (or products thereof) whose genetic make-up has been determined or altered by genetic engineering. Numerous analytical methods, both qualitative and quantitative, have been developed to determine reliably the presence and/or the amount of GMO in agricultural commodities, in raw agricultural materials, and in processed and refined ingredients.

Although much progress has been achieved in the development of genetic analysis methods, such as those based on the use of polymerase chain reaction (PCR) and real-time PCR, several other analytical technologies that can provide solutions to current technical issues in GMO analysis are emerging. Those include, mass spectrometry,[1] chromatography,[2] near-infrared spectroscopy,[3] micro-fabricated devices,[4–6] DNA chip technology[7] and nanoscale GMO analysis.[8]

The development and application of reliable detection and quantitative analytical methods are essential for the implementation of labelling rules. Appropriate sampling strategies and methods for sample preparation, as well as methods of development and application to detect GMOs and their derivatives, must then be followed by the establishment of validation criteria and performance assessment.

We know that the GMOs are diverse, and include transgenic experimental animals such as mice, several fish species, transgenic plants, and various microscopic organisms altered for the purposes of genetic research or for the production of pharmaceuticals. By far the most common genetically modified (GM) organisms are crop plants. But the technology has now been applied to almost all forms of life, from pigs bearing spinach genes to goats that produce spider silk.[9]

Maize line CBH 351 (trade name StarLink™) is a corn line that contains a modified *cry9c* gene from *Bacillus thuringiensis* subsp. *Tolworthi* and the *bar* gene from *Streptomyces hygroscopicus*. For both introduced genes, expression is driven by the 35s promoter, and termination of transcription for the *cry9c* and the *bar* gene are regulated by the nos adenylation signal and the 35s terminator, respectively.[10] The *cry9c* gene product confers resistance to feeding damage of lepidopteran insects, such as the European corn borer (ECB). The *bar* gene is a source of resistance against the herbicide phosphinotricin.

Although the use of StarLink™ corn was not approved for human consumption, recently, traces of *cry9c* were detected in taco shells[11,12] and in maize seeds of a non-StarLink™ variety.[13] These indicate that the GM corn had mingled with the human food chain. So, it has become a great concern and it is highly important to develop a novel sensor which can detect this GMO within a very short time with high specificity. Recently, an impressive number of inventive designs for electrochemical DNA sensing became available as reported in numerous publications,[14] and the strategies include: metal nanoparticle-based detection,[15–17] enzyme-amplified electronic transduction,[18,19] electrocatalysis,[20,21] polymer- or film-based detection,[22,23]

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surfactant bilayers,[24] surface-attached molecular beacons,[25] ferrocene-labelled signalling probes[26] and electroactive proteins, *etc*.[27]

Fan *et al.* investigated the conformational changes of DNA in the reagentless method for the sequence-specific detection.[25] Yeung *et al.* have reported an electrochemical real-time PCR system using ferrocene-labelled dUTP.[28] Hou *et al.* have developed a field-effect transistor system for microelectronic PCR quantification without using any labels.[29] Sun *et al.* utilized the interaction of DNA with phenosafranine for the detection of PCR amplicons related to GMOs.[30] Most devices and indicators are still at the investigation stage, and applications are yet to be developed.

Label-based electrochemical methods using H33258 have been well-described in numerous publications.[31–33] However, these label-based methods require the immobilization of the sensing layer, an oligonucleotide that is called a 'probe' on the electrode surface. In contrast, our method does not require any probe immobilization.[34,35] We detected the aggregation of DNA in the presence of H33258 in solution.[36] H33258 is a well-defined and widely-used molecule in biochemical applications. H33258 binds to the minor grooves of AT-rich regions of DNA;[37,38] in addition, the anodic current peaks of the DNA–H33258 complex decreases in proportion to double-stranded DNA (dsDNA) titration. This decrease in the current response is due to the slow diffusion of the H33258–DNA complex to the chip surface.[39]

Several isothermal amplification methods have been proposed, including nucleic acid sequence-based amplification,[40] self-sustained sequence replication,[41] and strand displacement amplification,[42,43] but these still have drawbacks to overcome.[44,45] They require either a precision instrument for amplification, or an elaborate method for detecting the amplified products due to poor specificity of target sequence selection. Loop-mediated isothermal amplification (LAMP) is another newly-invented method that requires only 4 different primers designed to recognize 6 distinct regions.[46–49] The LAMP method for GMO detection required a set of two specially-designed inner and two outer primers, and relied on auto-cycling strand displacement DNA synthesis that is performed by a DNA polymerase with high strand displacement activity.[46] For each LAMP reaction, all four primers are used, but later during cycling reaction, only the inner primers are used for strand displacement DNA synthesis. The LAMP reaction should also be preceded by non-denatured template DNA because the primers can penetrate into partially single-stranded template DNA at 65 °C. The final products are stem-loop DNAs with several inverted repeats of target, and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the same target strand. However, without any target DNA the amplification cannot be performed. As LAMP recognizes the target by six distinct sequences initially, and then by four distinct sequences, it is expected to amplify the target sequence with high selectivity. With the 2% agarose gel electrophoresis of the LAMP product, a characteristic ladder of multiple bands can be seen, unlike PCR products. This ladder pattern is characteristic of the LAMP amplification, and indicated that stem-loop DNA with inverted repeats of the target sequence was produced. The LAMP product is also a mixture of products of several different sizes, with an average molecular size of 2 kbp.[46]

Therefore, LAMP might be quite suitable for target GMO amplification in an integrated electrochemical device like a DNA stick (DS). In this paper, we are reporting for the first time, the rapid and effective detection of the CBH 351 maize variety of GMO using the DS which contains a disposable electrochemical printed (DEP) chip. Post-LAMP products were analyzed directly on-chip without the purification and immobilization process using linear sweep voltammetry (LSV). Therefore, we would like to propose that this sensor system can be an effective, cheap and rapid method of environmental protection.

Materials and methods

Hoechst 33258 [2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi(1H-benzimidazole), H33258] was purchased from Sigma-Aldrich Co. (MO, USA). H33258 stock solution was prepared by directly dissolving it in high purity water (ρ = 18.3 MΩ cm, Millipore, Bedford, MA, USA), and was kept in the dark at 0–4 °C and used up within seven days. Phosphate buffer solution (PBS, 20 mM, 0.04 M K_2HPO_4, 0.01 M KH_2PO_4, 20 mM NaCl, pH 7.4) was used throughout the experiments. DEP chips contained a three-electrode system including a carbon-based working electrode, Ag/AgCl reference electrode and a carbon-based counter electrode. The area of the working electrode was 1.96 mm^2. DEP chips were obtained from Bio-device Technology, Co. (Ishikawa, Japan) and used once for each experiment, and discarded after each measurement.

DNA extraction from *Zea mays*

Genuine seeds of this particular GM variety were imported directly from the United States. Then, grinding of a single maize seed was performed by using a Multi-Beads Shocker (YASUI KIKAI Co., Osaka, Japan) with a 12 mL tube holder (Type SH-123) at 1800 rpm for 30 s. Before grinding, the seed was washed with 1% sodium dodecyl sulfate (SDS), rinsed with distilled water, and dried to remove powders and broken pieces of other seeds. DNA from the ground single maize seed (*ca.* 300 mg/seed) was extracted by using a DNeasy Plant Mini Kit (QIAGEN GmbH), according to the manufacturer's manual, except for elution by distilled water. For maize test samples, six mixing levels containing 0.1% and 0.05% of each GM line were prepared to evaluate our method.[50] Different copy numbers of maize genomic DNA were also prepared to compare between PCR and LAMP amplicons and to identify the limit of detection of the DS based on target DNA.

LAMP reaction for CBH 351

The LAMP method requires a set of four specially-designed primers (F3, B3, the forward inner primer [FIP], and backward inner primer [BIP]) that recognize a total of six distinct regions (F1, F2, F3, B1, B2 and B3) in the target DNA (Fig. 1). The two inner primers, FIP and BIP, contain two distinct sequences corresponding to the sense and antisense sequences of the DNA, one for priming in the first stage and the other for self-priming in later stages. FIP consists of complementary sequence F1 (F1c) and direct sequence F2 (F2). BIP consists of complementary sequence B1 (B1c) and direct sequence B2 (B2). The two outer primers, F3 and B3c (the sequence complementary to B3) are

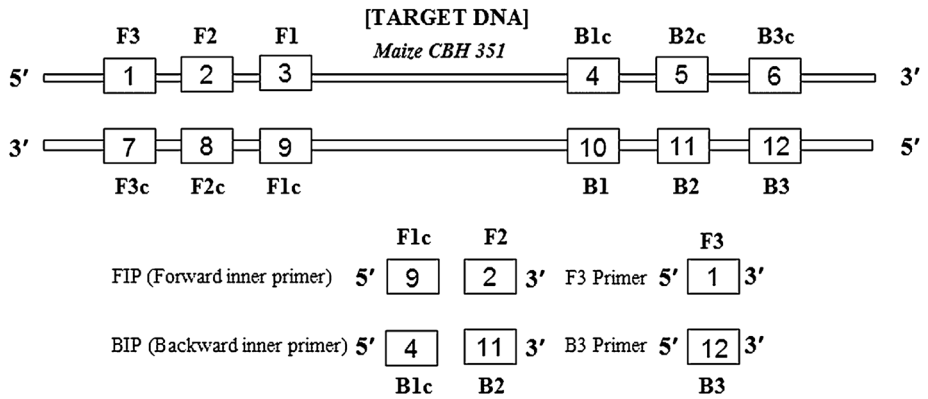

Fig. 1 Double-stranded target DNA and the design of the LAMP primers. The LAMP inner (FIP and BIP), and outer (F3 and B3) primer pairs are shown. All of these primers recognize a total of six distinct regions (F1, F2, F3, B1, B2 and B3) on the target DNA.

located outside the F2–B2 region.[46] In our study, we purchased the primer sets of maize CBH 351 for first screening (CaM03-5′ and CBH02-3′) and second screening (Cry9c-5′ and 35Ster-3′) from Nippon gene Co., Ltd. Japan. Primers for *Starch Synthase IIb* were also used to determine the inherent characteristics of the target DNA. The target DNAs used here were both the positive control plasmid (Nippon gene Co., Ltd. Japan) and the real sample. The LAMP reaction was carried out in a DS vial where the total reaction volume was 25 μL. To prepare the mixture, 1.6 μM each of the forward primer (FIP) and backward inner primer (BIP), 0.2 μM each of the forward outer primer (F3) and the backward outer primer (B3), 16 U of *Bst* DNA polymerase large fragment (New England Biolabs, Beverly, MA), 0.4 mM of each dNTPs, 2.5 μL of 10× Thermopol buffer (New England Biolabs, Beverly, MA), 3.0 mM of $MgSO_4$, 0.64 M of Betaine (Sigma-Aldrich) and 2.5 μL of target DNA were mixed. The mixture in the DS was incubated at 65 °C for 60 min using a conventional heating block, and was heated to ~80 °C to terminate the reaction.

DS measurements

LAMP on a DS (AZBIO Corp., Osaka, Japan) was performed on a Block Incubator (Astec, Fukuoka, Japan). The sensor is 6 cm in length, made of polypropylene which has several parts for amplification and detection in a single device. The DNA amplification part is where the LAMP reaction occurs. The detection layer is where H33258 and PBS reside to mix with amplified products. The mixing valve is opened after amplification so that the interaction phenomenon of DNA–PBS–H33258 can be measured by the DEP chip, which is connected with the electrochemical equipment. To minimize evaporation from the DNA amplification layer, 20 μL of mineral oil was added onto the LAMP solution. Fig. 2 and its corresponding legend describe how the amplicon can be formed and detected electrochemically with the use of the DS.

Procedure

All electrochemical studies were performed in conjunction with Linear Sweep Voltammetry (LSV) using an Autolab PGSTAT 12 electrochemical analysis system (Eco Chemie, The Netherlands) in connection with its General Purpose Electrochemical System (GPES) software. All experiments were carried out at the ambient temperature of the laboratory (23–27 °C). Measurements were the average of at least three repeated measurements. LSV was performed while scanning from −0.1 V to 1.0 V with a step potential of 0.00244 V and a scan rate of 100 mV/s. LAMP products were diluted to 4× with PBS, and then they were mixed with 20 μM of H33258. The changes in the anodic peak current responses were recorded and processed using the software Autolab PGSTAT 12.

Polymerase chain reaction (PCR) for CBH 351

To compare the current differences with its negative controls, 4× diluted LAMP and 2× diluted PCR product were analyzed successfully with the same target concentrations (Fig. SI-1†). PCR was performed with the isolated maize CBH 351 DNA. PCR amplification was performed in an automated thermocycler (Gene amplifier 9700, Applied Biosystem). Reactions were carried out in a total of 25 μL containing 3×10^5 to 3 copies/reaction template DNA and the deoxynucleotide triphosphate (dNTPs) each at 0.16 mM. The final concentration of $MgCl_2$ was 1.5 mM. 10× PCR gold buffer was supplied from Applied Biosystem, USA, which contained 150 mM Tris-HCl, pH 8.0 and 500 mM KCl. Each primer (at 0.6 mM) and 0.8 Units of AmpliTaq Gold DNA Polymerase (Applied Biosystem, USA) were added for each reaction prior to the PCR.

For efficient PCR, the samples underwent initial denaturisation at 95 °C for 10 min. For 40 repeating cycles, each cycle consisted of 95 °C for 30 s, then 30 s for annealing at 60 °C and an extension of 30 s at 72 °C. Final extension was set as 72 °C for 7 min. The absence of primer specificity and control amplicons in the same reaction was indicative of PCR amplification failure. Primer sequences were: CaM03-5′: 5′-CCTTCGCAAGACCCTTCCTCTATA-3′; CBH02-3′: 5′-GTAGCTGTCGGTGTAGTCCTCGT-3′.

Results and discussion

In our previous study, we developed an electrochemical biosensor that did not rely on the immobilization of probes on the electrode surface. The principle of that method was to quantify DNA at a minute level through its anodic current peaks.

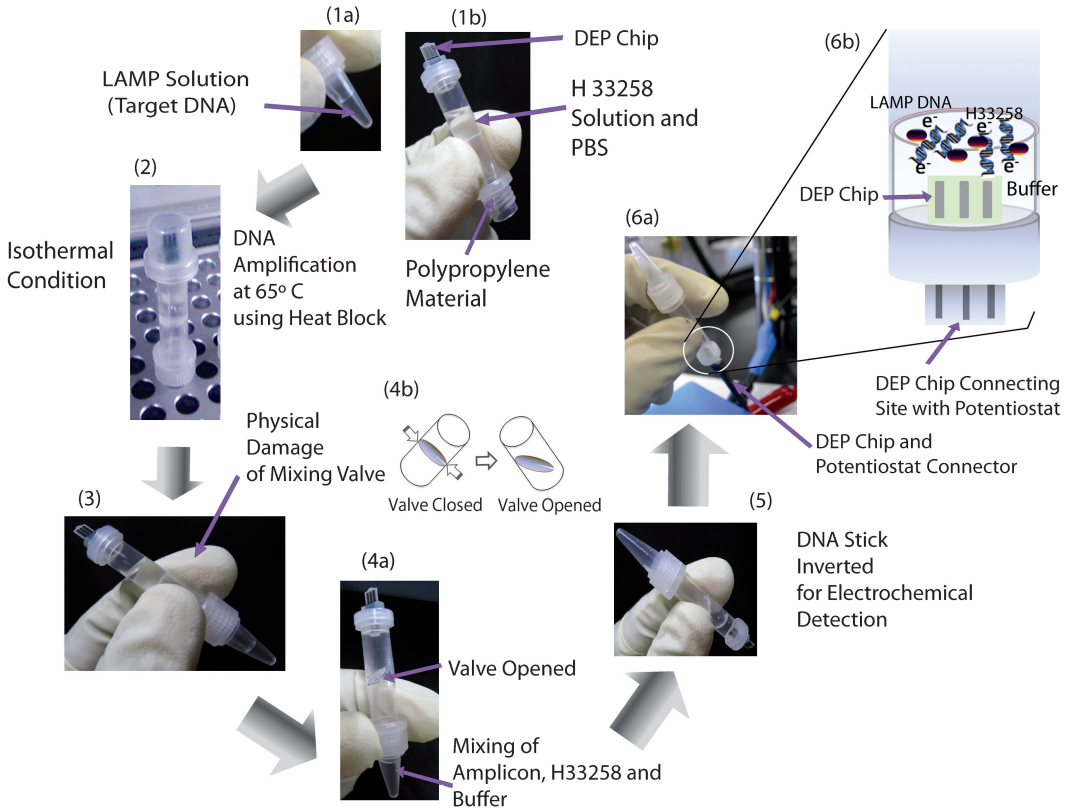

Fig. 2 Procedure for the detection of maize CBH 351 GMO using the electrochemical sensor (DS): (1a) LAMP solution (master-mix) is placed into the amplification part of the DS along with the target DNA; (1b) DS detection site where the H33258 and buffer mixture are added; (2) LAMP solution is placed on the heat block at 65 °C for DNA amplification; (3) valve is distorted to facilitate the mixing of fresh amplicon and H33258–buffer solution; (4a) mixing of amplicon, H33258 and buffer using mild hand shaking; (4b) it shows how the valve is opened in the DS; (5) the DS is inverted for electrochemical detection; (6a) it shows how the DS is connected to a potentiostat with the help of a connector; (6b) schematic diagram shows the DNA–H33258 interaction onto the DEP chip surface inside the DS made of polypropylene.

We observed that the anodic current decreases in proportion to the titration of dsDNA with H33258.[34,35] The DEP chip enabled us to detect the target DNA in a rapid and cost-effective manner. Here, for the first time, we studied the detection of CBH 351 maize GMO using LSV with H33258 with a concentration of 20 μM. The property of H33258 to bind with the DNA minor groove enabled us to detect loop amplicons using our electrochemical sensor system.[51–53] In our previous report[35] on PCR amplicons for the detection of single nucleotide polymorphism (SNP) of human samples without the use of a DS, we have applied varying accumulation time periods to the mixtures (*i.e.* PCR amplicons–H33258–buffer) onto the sensor surface and observed that the immediate measurement without any accumulation at room temperature were ideal for the detection of PCR amplicons. Here, in this study immediate measurements, without any accumulation of LAMP amplicon–H33258–buffer onto the chip surface were made for the detection of LAMP product. For optimization of the desired DNA binder, 4× diluted LAMP products were used in the presence of 10–50 μM of H33258 as shown in Fig. SI-2.† The best current difference was observed for 20 μM and as such it was used for further studies.

Besides, the linear relationship between the scan rate and the anodic peak current due to the presence and absence of LAMP products in the sensor was analyzed in Fig. 3. The size of the

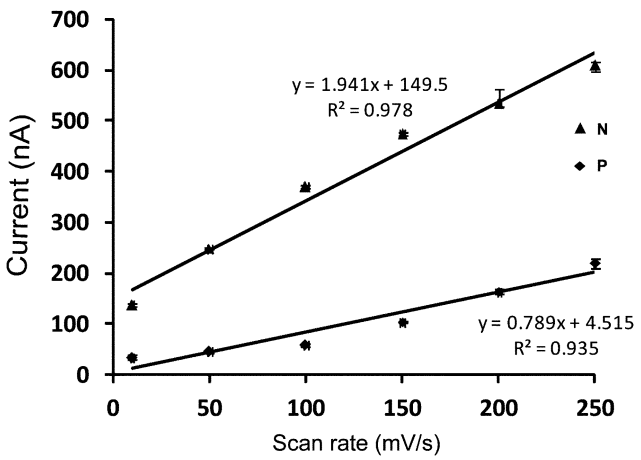

Fig. 3 Linear relationship between the scan rate and the anodic peak currents due to the presence (P) and absence (N) of LAMP product in the DNA stick. Scan rates were examined in the range of 10–250 mV/s. All experiments were carried out at ambient temperature (23–27 °C). Error bars indicate the standard deviation of at least three replicated measurements.

diffusion layer above the electrode surface becomes different depending upon the voltage scan rate used. In slow voltage scan the diffusion layer grows much further from the electrode in

comparison with a fast scan. Consequently, the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates. As the current is proportional to the flux towards the electrode, the magnitude of the current will be lower at slow scan rates and higher at high scan rates. Scan rates were examined in the range of 10–250 mV/s in our experiment. We observed the maximum ratio of I_N/I_P (I = current, N = negative samples, P = positive samples) for 100 mV/s. Therefore, in this study, a scan rate 100 mV/s was used.

The DS consisted of an amplification layer and a detection layer. These two layers are mechanically separated by a mixing valve, and the DEP chip is attached to the DS at its detection layer. This DEP chip needed to be connected with electrochemical equipment to measure the anodic signal. To prevent evaporation, the LAMP reaction mixture was covered with mineral oil at its surface. We used each DS only once for one measurement, and then disposed of it.

The reasons for using the DS are numerous. It is disposable, portable, contamination-free and inexpensive to manufacture. After each amplification step, the LAMP product was diluted with PBS and mixed with 20 μM of H33258 by physical distortion of the mixing valve. The DS inverted and mixed products were then measured efficiently at the detection layer side with the DEP chip. We used LSV as detection mode transducers where it showed efficient detection of LAMP products. All of our electrochemical results were in agreement with the available conventional detection method, gel electrophoresis. In our study, we also found that the anodic peaks were significantly correlated with the LAMP efficiency, *i.e.* the concentration of LAMP products, where the efficiency depended mostly upon the template DNA conditions (source of DNA, purity, *etc.*) which was in agreement with our previous work with the PCR products.[35,38] For the negative control, no template was used and thus it produced a higher anodic current.

We could monitor at certain time intervals (0, 20, 40 and 60 min) the maize CBH 351 LAMP products under isothermal conditions, where the electrochemical sensor detected the LAMP products as they were produced and amplified at 65 °C. In the negative control we used only ultra-pure water, instead of template DNA. Negative control sample without template DNA was not amplified, due to the absence of specific target DNA, but CBH 351 synthetic DNA was amplified in the presence of specific loop primers, and was measured by the DS (Fig. 4). Here, the template DNA concentration for the LAMP reaction was 1 pg/μL. We firmly concluded here that the LAMP product detection was possible at 20 min after the reaction started. This is due to the high quantity of amplicon produced within a short time. From 40 min, we observed a saturated condition of the amplified product, so the anodic peak remained the same up to 60 min of heating at isothermal conditions. Therefore, this result suggested that the method allowed much faster DNA quantification in ~20 min without the steps of DNA purification and immobilization.

Later, we performed atomic force microscopy (AFM) image analysis to observe whether any aggregations with H33258 molecules were formed or not (data not shown). As a specific ratio is necessary to observe the DNA–H33258 aggregation,[34] we could not observe any aggregation with this combination, so we can infer from this observation that it is the minor groove

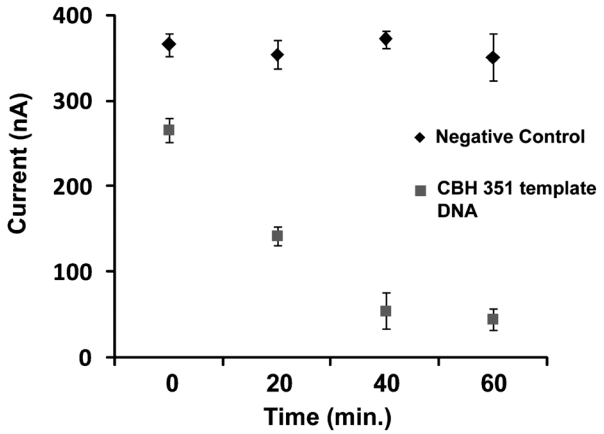

Fig. 4 Time-dependent monitoring of maize CBH 351 plasmid's LAMP amplicon from 0 to 60 min incubation time at isothermal conditions, 65 °C. Here, the template DNA concentration for the LAMP reaction was 1 pg/μL. Negative control samples were not amplified due to the absence of specific target DNA, but detection was possible from 20 min. Error bars indicate the standard deviation of at least three repeated measurements.

binding phenomenon of H33258 which led us to detect the presence of DNA by lowering the anodic signal.[52–54]

Yang and co-workers measured the size of H33258 as 2.12 nm in length, and 0.48 nm in width.[39] Major grooves of the DNA double helix are 1.2 nm in diameter and 0.85 nm in depth, while the minor grooves of DNA are 0.6 nm in diameter and 0.75 nm in depth. Since aromatic bases have van der Waals thicknesses of 0.34 nm and an 'ideal' DNA helix has a helical twist of 36° per base pairs, four or five base pairs were needed to react with H33258. So, it can be deduced that H33258 bound to the minor groove of DNA by geometrical requirement. They also suggested that H33258 binds tightly to minor grooves of fish sperm DNA and covers four base pairs. Pjura *et al.* and Teng *et al.* studied the crystal structure of the complex between a dodecamer d(CGCGAATTCGCG) and H33258 by X-ray diffraction analysis.[52,53] Their analysis suggests that the H33258 sits within the minor groove in the AT-rich region of the DNA double helix and covers four base pairs. All these are in agreement with our report here and electrochemical detection of loop amplicon in solution was possible without the phenomenon of DNA–H33258 aggregation.

To obtain the electrochemical behavior with various types of target amplicons of maize CBH 351, we detected and verified the synthetic, real and also the negative control samples. The inherent characteristics of maize CBH 351 were also confirmed by our sensor using the *starch synthase* (*ssIIb*) gene as target. In the negative control (NC; Fig. 5A), water was used instead of any template DNA. After mixing with H33258 and buffer, it produces the highest peak current due to H33258 oxidation. As shown in Fig. 5B and 5C, we detected and verified the synthetic maize CBH 351 target DNA as a template. In addition, we also detected 0.1% and 0.05% of CBH 351 real maize DNA (Fig. 5D and 5E). In this study, the inherent characteristic of maize was also confirmed while targets were both the synthetic and real samples. To confirm the inherent characteristic of the maize variety, the universally present 'internal standard' gene, *starch*

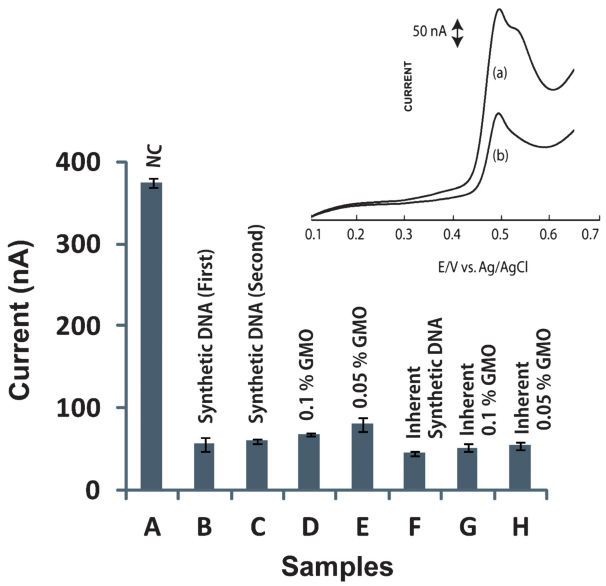

Fig. 5 LSV behavior of 20 μM H33258 at a 100 mV/s scan rate with an electrode area of 1.96 mm^2: (A) negative control (NC; no target DNA); (B) first screening of CBH 351 using synthetic DNA as a target (detection); (C) second screening of CBH 351 using synthetic DNA as a target (verification); (D) detection of 0.1% CBH 351 maize from real sample; (E) detection of 0.05% CBH 351 maize from real sample; (F) determination of inherent characteristics of synthetic DNA of maize CBH 351; (G) determination of inherent characteristics of 0.1% real sample; (H) determination of inherent characteristic of 0.05% real sample. The inset displays LSV in the absence (a), and presence (b) of 0.1% CBH 351 amplicon. The observed peak potential was 0.5 V.

synthase (*ssIIb*) was detected electrochemically from synthetic maize DNA (Fig. 5F), 0.1% real samples (Fig. 5G) and 0.05% real sample (Fig. 5H). In the study from Fig. 5B–H, LAMP reactions occurred successfully and a significant drop in the peak current intensities of H33258 oxidation was observed.

To obtain the detection limit of the DS, we prepared the real samples with dilutions from 0 to 3×10^5 copies/reaction. In our study we used a 60 min LAMP reaction since after 60 min the reagents are used up and eventually the LAMP reaction stops. Even though we did not conduct systematic experiments to see the effect of the length of the LAMP reaction on the detection limit, we have seen that, in the case of low template concentrations of target, if the reaction time is reduced, the detection becomes difficult (data not shown). After LAMP reaction in isothermal conditions LSV was used to determine the electrochemical detection limit which was 3×10^2 copies/reaction (Fig. 6). With 3×10^5 copies of target DNA the LAMP efficiency was quite high as confirmed by the lowest anodic peak current. With 3 and 30 copies/reaction of target DNA, the LAMP reaction was not possible due to the low copy number of target DNA. For both the primer combinations as evident from Fig. 6, 3×10^2 copies/reaction of target was the minimum level of template for detectable amplicon production. In the negative control (0 copies/reaction) no amplicon was produced or no contamination was observed as confirmed by the highest anodic peak current. Primer sets CaM03-5′, CBH02-3′ and Cry9c-5′, 35Ster-3′ were used here for the first and second screening of the specific GMO contents. Finally, In this paper we have successfully performed

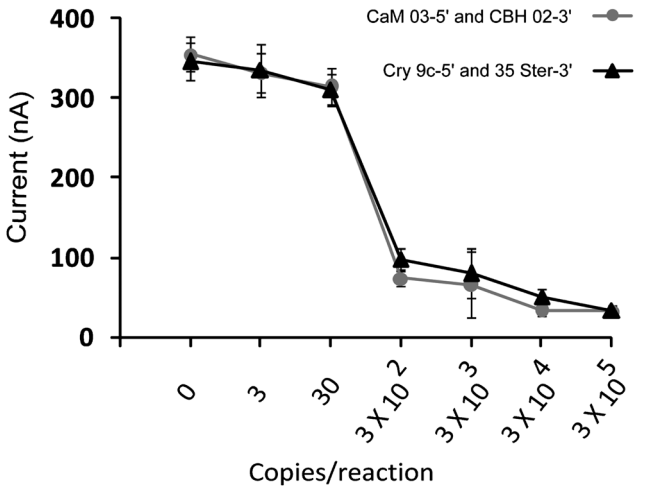

Fig. 6 Different target concentrations (0 to 3×10^5 copies/reaction) of GMO were used as templates for the LAMP reactions with two CBH 351 loop-primer sets: CaM03-5′ and CBH02-3′, and Cry9c-5′ and 35Ster-3′; the resulting amplicons were finally detected electrochemically using LSV. Error bars indicate the standard deviation of at least three repeated measurements. Currents were measured using the parameters as mentioned in the Experimental section.

the detection of maize GMO from unpurified LAMP product using our sensor.

Conclusions

Integration of isothermal amplification and subsequent analysis of unpurified amplicon using the DNA–H33258 interaction showed the proof-of-concept in this study. The purpose of using loop amplicons compared to PCR products was also mentioned here where different template concentrations (3 to 3×10^5 copies/reaction) were used. Optimization of the sensor with different H33258 concentrations and scan rates was also performed. To detect the loop amplicon in the shortest possible time, time-dependent monitoring was also tested and it was observed that within 20 min the GMO-specific amplicon can be detected. In the previous published report it was assumed that the DNA–H33258 aggregation phenomenon caused the reduction of peak current intensity onto the electrode surface. Contrastingly, without the aggregation with the H33258 molecule the unpurified amplicons can be detected due to DNA–H33258 minor groove binding, as confirmed by AFM analysis. The limit of detection of target GMO using our sensor was observed as 3×10^2 copies/reaction. Efforts are currently in progress in our laboratory to measure the amplicons with high sensitivity, in the shortest possible time scale and also in real-time using a micro-electromechanical (MEMS)/microfluidics system.

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