Biosensors and Bioelectronics 66 (2015) 431–437

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel GMO biosensor for rapid ultrasensitive and simultaneous detection of multiple DNA components in GMO products ☆

Lin Huang [a], Lei Zheng [a], Yinji Chen [b], Feng Xue [a], Lin Cheng [a], Samuel B. Adeloju [a,c,*], Wei Chen [a,**]

[a] School of Biotechnology & Food Engineering, Key Laboratory of Functional Materials and Devices of Anhui Province, Hefei University of Technology, Hefei 230009, PR China
[b] National Engineering Laboratory of Food Storage and Transportation, Nanjing University of Finances and Economics, Nanjing 210023, PR China
[c] School of Chemistry, Monash University, Clayton, Vic 3800, Australia

ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form
17 November 2014
Accepted 1 December 2014
Available online 3 December 2014

Keywords:
Electrochemical biosensor
Genetically modified organism
Rapid detection
Simultaneous and multiple detection
Food safety

ABSTRACT

Since the introduction of genetically modified organisms (GMOs), there has been on-going and continuous concern and debates on the commercialization of products derived from GMOs. There is an urgent need for development of highly efficient analytical methods for rapid and high throughput screening of GMOs components, as required for appropriate labeling of GMO-derived foods, as well as for on-site inspection and import/export quarantine. In this study, we describe, for the first time, a multi-labeling based electrochemical biosensor for simultaneous detection of multiple DNA components of GMO products on the same sensing interface. Two-round signal amplification was applied by using both an exonuclease enzyme catalytic reaction and gold nanoparticle-based bio-barcode related strategies, respectively. Simultaneous multiple detections of different DNA components of GMOs were successfully achieved with satisfied sensitivity using this electrochemical biosensor. Furthermore, the robustness and effectiveness of the proposed approach was successfully demonstrated by application to various GMO products, including locally obtained and confirmed commercial GMO seeds and transgenetic plants. The proposed electrochemical biosensor demonstrated unique merits that promise to gain more interest in its use for rapid and on-site simultaneous multiple screening of different components of GMO products.

1. Introduction

The use of genetically modified organisms (GMOs) for food production has attracted considerable interest and attention since first introduced for commercial planting. More than 190 GMO events of 25 different crops have been developed and approved for commercialization worldwide and about 170 million hectares of genetic modified (GM) crops have been planted globally and the growth is still increasing at a rate of more than 10% each year (James, 2013; Wei et al., 2013; Zhang et al., 2013). However, concerns about the impact of GMOs on the environment and human health are also growing (Laura, 2003; Eric et al., 2012). This has already led to the introduction of a series of strict legislations and regulations in many countries and regions, including European Union (EU), USA, and Japan, to ensure good management of the use of GMOs and the labeling of GMO derived food and other products (Zhang et al., 2013; Gruere et al., 2009). The successful achievement of these tasks requires suitable and highly effective analytical methods.

Currently, the widely accepted gold standard identification methods for GMOs in foods and other products are DNA-based polymerase chain reaction (PCR), real-time PCR (RT-PCR) and antibody-based enzyme-linked immunosorbent assay (ELISA) (Peano et al., 2004; Jiang et al., 2009; Monaghan et al., 2008; Samson et al., 2013; Xu et al., 2006; Saiki et al., 1985). However, with the rapid growth in GMO products globally, the detection of GMOs has become increasingly challenging with a considerable increase in the number of samples requiring testing and labeling. Furthermore, GMO diagnostics also present several other challenges. A very important one of these is the great number of DNA targets that should be screened for all possible GMOs that are available (Novak et al., 2009). The traditional PCR-based methods can only detect the various DNA components by using rather expensive instruments that are operated by highly trained personnel and,

☆Research ID: www.researcherid.com/rid/F-4557-2010.

* Corresponding author at: School of Biotechnology & Food Engineering, Key Laboratory of Functional Materials and Devices of Anhui Province, Hefei University of Technology, Hefei 230009, PR China.
** Corresponding author.
E-mail addresses: sam.adeloju@monash.edu (S.B. Adeloju), chenweishnu@163.com (W. Chen).

http://dx.doi.org/10.1016/j.bios.2014.12.005
0956-5663/

thus, greatly limiting the practical application of such approach for in-field and on-site identification of GMOs. Furthermore, multiple detections of different DNA components can only usually be realized by using RT-PCR. (Grohmann et al., 2009; Arun et al., 2013; Ghedira et al., 2009) Evidently, there is still an urgent need for new detection methods that are easy to operate, more efficient and can achieve high throughput with simultaneous multiple components detection of GMOs.

In recent years, some strategies have been developed for rapid and ultrasensitive detection of GMOs with very good performance (Zhu et al., 2008; Huang et al., 2013a,b; D'Agata et al., 2010; Guo et al., 2009; Zhe et al., 2008; Gao et al., 2011). For example, Huang et al. (2014) used the loop-mediated isothermal amplification (LAMP) method to detect the phytase gene in the GMO maize by visual observation. Han et al. (2013) developed a suspension array based method for rapid identification of GMO maize by using a fluorescent reporter. Also Jiang et al. (2014) demonstrated the application of G-quadruplex DNAzyme probe for label free and sensitive visible detection of GMOs. These developments have already resolved the issues of rapid detection of GMOs to some extent. However, none has so far achieved simultaneous multiple detection of GMOs. A potential solution for achieving this goal lies in the use of electrochemical biosensor with multicomponent detection capability.

In the past two decades, the use of electrochemical biosensors has emerged as an important and powerful analytical tool for DNA detection because of their relatively high sensitivity, selectivity and low cost. It may also possible by judicious design and construction of electrochemical biosensor to extend this approach to achieve simultaneous detection of multiple DNA components in GMO products. However, to our knowledge, this has never been considered.

In this study, we report a novel electrochemical biosensor for simultaneous multiple DNA component detection of GMOs, based on the use of different redox tags for signal reporting. More importantly, further improvement of the sensitivity of the biosensor was accomplished by employing the enzyme EXO III-based catalytic cleavage and gold nanoparticle-based probe immobilization for a two-round signal amplification. Under optimized conditions, rapid and simultaneous detection of multiple DNA components of GMOs was realized with the biosensor. Furthermore, a set of samples containing different ratio of GMO materials and different categories of GMOs were also successfully used to demonstrate the ability of the proposed electrochemical biosensor to achieve rapid and sensitive simultaneous screening of their GMO contents. The rapid identification of DNA components of GMOs in these complex matrices is particularly attractive and offers a great promise for rapid and on-site screening of multiple components of GMOs.

2. Experimental

2.1. Reagents

Chloroauric acid ($HAuCl_4$) was obtained from J&K Chemicals. Mercaptohexanol (MCH), tris(2-carboxyethyl) phosphine hydrochloride(TCEP) and exonuclease III (Exo III) were from Sangon (Shanghai, China).

The genetic modified (GM) and non-genetic modified (non-GM) soybean and rice samples were both kindly supplied by Jiangsu and Anhui Entry–Exit Inspection and Quarantine Bureau, China. All samples used were checked for the presence/absence of GM events before use in this study.

Three recognition probes against three different DNA components of GMO were all synthesized and purchased from Shanghai Sangon Biological Engineering & Technology Co. Ltd., China. And the detailed sequence information of the three recognition probes were all listed in detail in Supporting information. The methylene blue (MB-) and ferrocene (FC-) labeled signal probes were also purchased from the same company. The anthraquinone (AQ-) modified ssDNA signal probe was prepared in our laboratory according to a previously reported method (Abi and Ferapontova, 2012).

The buffer solutions used in this work were as follows: (a) stock buffer was 10 mM phosphate buffer solution (PBS, pH 7.4) which contained 1 M NaCl, and (b) hybridization buffer was 10 mM phosphate buffer solution (PBS, pH 7.4) which contained 1 M NaCl and 0.1 mM $MgCl_2$. Electrochemical impedance spectroscopy (EIS) measurements were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ which contained 0.1 M KCl.

2.2. DNA extraction from GMOs

DNA of GMOs was extracted by using superparamagnetic nanoparticles (SPMN) as the separation substrates, which were prepared in our laboratory as previously reported (Chen et al., 2006; Zhao et al., 2012). The SPMNs based method for genomic material extraction and purification was adopted in this study. The soybean sample was first ground to a powder after treatment with liquid nitrogen. SDS buffer (700 μL) at 65 °C and RNase (5 μL) were added to the soybean powder (50 mg) and the mixture was maintained at 65 °C for 30 min after vigorous vortexing. It was then centrifuged at 12,000 rpm for 5 min at 4 °C and the supernatant was transferred to another clean tube which contained PEG/NaCl solution. The functionalized magnetic nanoparticles (MNPs) were added to portions of this solution at the same concentration to separate and purify DNA samples. After 5 min, the MNPs were collected under an applied magnetic field and rinsed three times with ethanol to elute the genomic DNA, which was then dispersed in Tris–EDTA (TE) buffer. The extracted DNA was analyzed by UV/vis spectrophotometry and agarose electrophoresis. The results obtained were further confirmed by PCR.

2.3. Preparation of single-strand oligonucleotide (ssDNA) probe-gold nanoparticle (GNP) conjugates

The immobilization of ssDNA probe on GNP was according to our previously reported procedure (Huang et al., 2013a,b; Wu et al., 2012). Firstly and briefly, 50 mL of 0.01% aqueous $HAuCl_4$ solution was heated to boiling and 1 mL of 1% sodium citrate was added rapidly with vigorous stirring. After a red wine colored solution was formed, the reaction solution was kept heating and stirring for another 10 min and then cooled down to room temperature for future use. Then, the single strand signal DNA was immobilized onto the prepared GNPs. Three different signal DNA were first activated by Tris(2-carboxyethyl)phosphine (TCEP) at 37 °C for 60 min. Then, these activated signal DNAs were incubated with GNPs at room temperature for 60 min and the resulting solution was further blocked with dATP. Subsequently, the mixture was aged with NaCl in a stepwise process to a final concentration of 0.14 M of NaCl. Finally, the mixture was centrifuged to remove the unbound signal DNA and the remaining pellet was collected and re-suspended in the PBS.

2.4. Construction of sensing interface and signal amplification strategy

The first step in the construction of the sensing interface is the immobilization of the capture probe according to our previously published methods (Huang et al., 2013a,b; Wu et al., 2012; Xue et al., 2013). Prior to the modification of the capture DNA, the gold electrode was polished progressively with 1, 0.3 and 0.05 μm

alumina slurry to a mirror-like surface finish. Then, the polished electrode was treated with piranha solution (98% H_2SO_4/30% H_2O_2 =3:1) for 10 min and rinsed with double-distilled water. Then, the electrode was cleaned ultrasonically in ethanol for 5 min and with double-distilled water for 5 min. Finally, the electrode was electrochemically cleaned in 0.5 M H_2SO_4 to remove any remaining impurities. 5 μL of stem-loop shaped capture probe was dropped on the cleaned electrode surface and incubated at 37 °C for 2 h.

20 μL mixture of target DNA at varying concentration and EXO III (10 U) was dropped onto the modified electrode surface and incubated at 37 °C for 40 min. The electrode was then rinsed with water twice to remove residual mixture. Finally, three GNP-labeled signal DNA probes were kept at 37 °C for 1 h.

EIS measurements were performed in the potassium ferricyanide solution (AC voltage amplitude, 5 mV, the voltage frequency ranged from 0.1 Hz to 10^5 Hz). The redox current of FC, MB and AQ were measured using DPV from −0.7 to 0.5 V (Increment *E* 0.004 V, amplitude 0.05 V, pulse width 0.05 s and pulse period 0.2 s).

3. Results and discussion

3.1. Design of signal amplified sensor for multiple DNA detection in GMOs

Previous studies on GMOs detection have mainly focused on PCR based gold protocols, which can often only be carried out in professional laboratories by experienced personnel. However, there have been numerous reports on the development of electrochemical sensors for DNA detection (Ronkainen et al., 2010; Hvastkovs and Buttry, 2010). Our present research aims to extend the development and use of electrochemical sensor for rapid and simultaneous multiple DNA component detection of GMOs to enable wider use and accessibility.

As illustrated by the scheme in Fig. 1, to achieve this goal, the different stem-loop shaped recognition probes against different targets were first immobilized onto the electrode surface. The loop was opened in the presence of target DNA sequence and a double stranded DNA was formed. This double stranded DNA was hydrolyzed with the aid of the Exo III enzyme to produce a ssDNA on the surface of the electrode and release the target DNA into the solution. These released target DNA then re-opened the remaining stem-loop capture DNA on the electrode and subsequently initiated the next round recognition induced loop opening. In this way, even trace amount of target DNA could induce the ssDNA probe on the electrode surface for further signal labeling and reading. This first step of the scheme is referred to as the "First round signal amplification" process.

In the second round bio-bar code related signal amplification, as shown in the scheme in Fig. 1, the GNPs was adopted for signal amplification in the labeling and acquiring of signal for detection. Consequently, the target analyte-induced autonomous cross-opening of hairpin combines with different signal reporter system. Overall, three pairs of immobilization probe and signal producing probe were conjugated to the GNPs through the stable Au–S bond. The three GNPs-signal DNA conjugates were then used for signal labeling on the basis of the first-round enzymatic amplification. Due to the high surface area of the GNPs, the final signal of the electrochemical detection was further enhanced when compared with that of the single ssDNA label approach (Chen et al., 2009; Nam and Mirkin, 2004). Based on this designed sensing interface and the dual signal amplification strategy, the proposed platform can be readily applied to the detection of multiple DNA components in GMO.

3.2. Optimization of biosensor for detection of multiple DNA components of GMOs

To achieve optimum performance of the electrochemical biosensor for multiple DNA component detection of GMOs, various parameters were optimized. EIS was employed to monitor the step-by-step modification of the electrode. The magnitude of the electron transfer resistance (Ret) on the electrode surface was

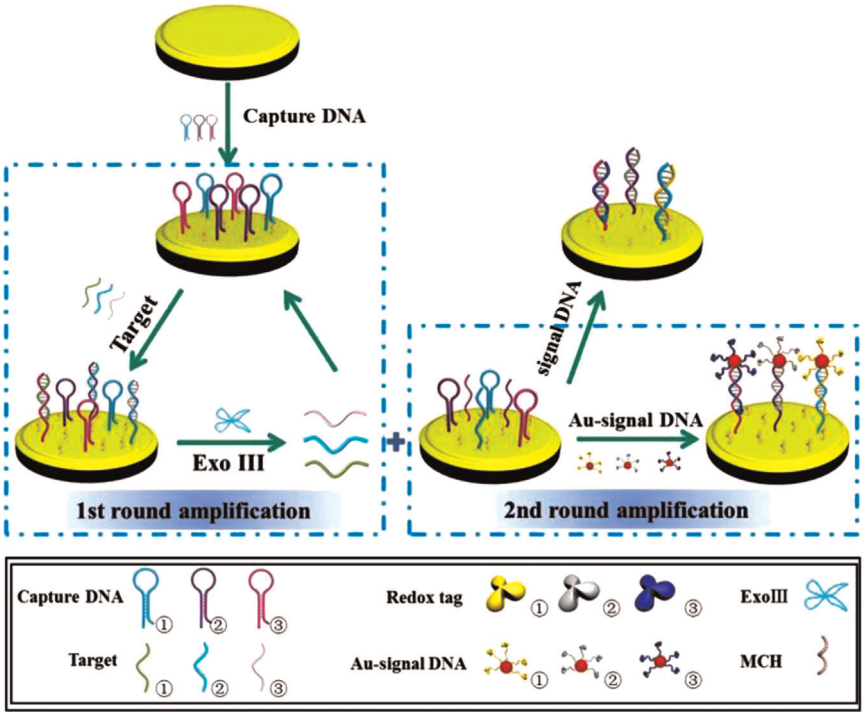

Fig. 1. Schematic illustration of the steps involved in the construction of the signal amplified electrochemical biosensor for simultaneously detection of multiple DNA components of GMOs.

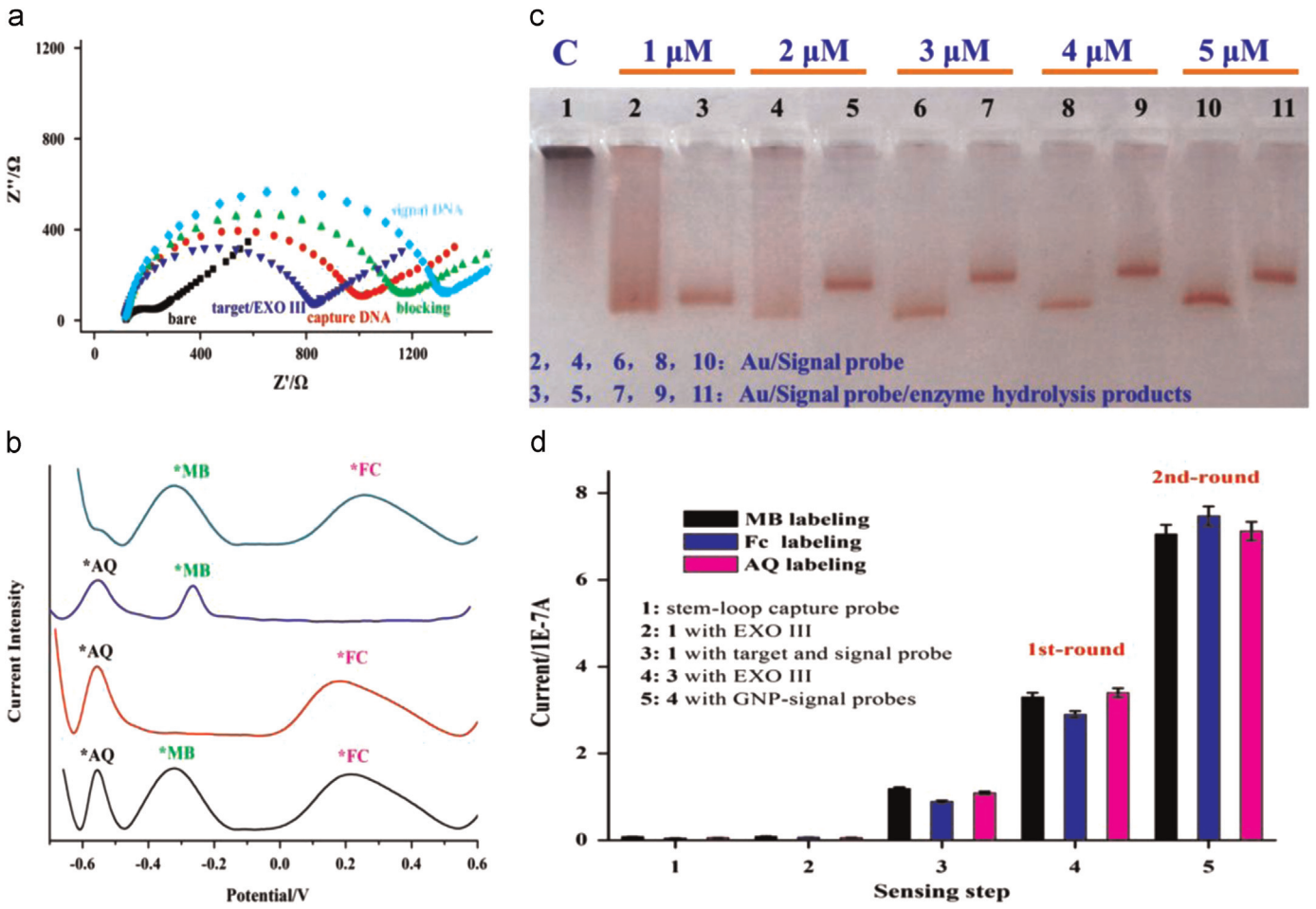

Fig. 2. Optimization of electrochemical sensor for simultaneous detection of multiple DNA components. Influence of (a) sensing process on the interface, (b) simultaneous detection by multiple labeling redox tags, (c) optimization of signal probe on gold nanoparticles and (d) signal response obtained with the electroactive tags at each step in the sensing process.

greatly influenced by the surface properties of the electrode. The probe density on the surface of the electrode was investigated according to the EIS results, and finally, 1.5 μM of the capture probe was adopted as the optimal immobilization concentration for sensing (See details in Supporting information). Meanwhile, EIS was also used to monitor each step of the electrode fabrication and the sensing process. Fig. 2a shows that the Ret value increased with the immobilization of capture probe and blocking of the surface due to the inhibited electron transfer effect. On the other hand, after recognition of target DNA and treatment with EXO III, the Ret value decreased and was obviously smaller than that obtained for immobilization of the capture probe. This dramatic change in the Ret value is a direct evidence of the hydrolysis effect of EXO III, which hydrolyzed the dsDNA into the shorter ssDNA as we designed. Briefly, after the recognition of the target DNA, the double stranded DNA formed on the electrode surface acts as the substrate of EXO III. With ongoing hydrolysis reaction in the presence of EXO III, the dsDNA was subsequently converted to the short ssDNA for hybridization with the signal probe on the electrode. Obviously, the remnant ssDNA is shorter than the original stem-loop like capture probe and, therefore, accounts for the dramatic decrease of the Ret value to even smaller than that of capture probe. Finally, with the hybridization with signal DNA, new dsDNA structures were formed again and the Ret value recovers and increased again due to the poor electron transfer efficiency of the formed dsDNA. These EIS results have already clearly demonstrated that the designed sensing strategy for target DNA is feasible. Another important factor requiring consideration in the electrochemical sensing strategy is the hydrolysis reaction time of the EXO III. As illustrated in Fig S1b, the sensing response increased with increasing reaction time of EXO III, indicating the incompleteness of the hydrolysis reaction in the sensing system. An optimum sensing response was obtained with the use of a reaction time of 40 min and further increase beyond this reaction time did not improve the sensing response. A reaction time of 40 min was therefore adopted as the optimal culture time for EXO III.

3.3. Choice of electroactive redox tags

As the focus of this research is to achieve simultaneous multiple component detection of GMOs without repeated detection steps, it was necessary to choose different active redox tags carefully for simultaneous multiple components detection to avoid cross interferences. Consequently, three different redox tags chosen as the labeling tags for three different components of GMOs were anthraquinone (AQ), methylene blue (MB) and ferrocene (Fc). As shown in Fig. 2b, it was obvious that simultaneous detection was easily accomplished when any two types of the electrochemical active tags was used. The signals of both tags were easily differentiated from each other without any overlap or interference. These results demonstrate that the three chosen electrochemical active tags can be adequately used for simultaneous detection of multiple components in GMOs.

The optimization of the different electroactive tags (MB-, Fc- and AQ-) labeled signal probes was also undertaken to determine their required optimum concentrations for immobilization on the surface of the gold nanoparticles (See detailed results in SI). The determined optimum concentrations from these investigations were 2, 2 and 3 μM for MB-, Fc- and AQ-labeled probes, respectively.

3.4. Influence of signal amplification process on sensing signal

The amount of signal amplification probe on gold nanoparticles for the second-round amplification was also investigated. As shown in Fig. 2c, compared with the control group in lane 1, the stability of the gold nanoparticles were improved after modification with signal amplification probe due to the protection effect of the ssDNA on GNPs. Also with the increase of signal amplification probe for modification, the modified GNPs were increased accordingly and came to the saturation at 3 μM in lane 6. Furthermore, all signal amplification probe modified GNPs at different ratios (lanes 2, 4, 6, 8 and 10) could effectively hybridize with residual ssDNA produced by the EXO III catalytic hydrolysis (lanes 3, 5, 7, 9 and 11). These results confirmed the successful conjugation of signal amplification probes on GNPs for following signal enhancement research.

Under the optimized conditions, the sensing signal obtained in each step and during the signal amplification process were carefully investigated and compared to gain a good understanding of the whole electrochemical sensing strategy in Fig. 2d. Evidently, when only capture probe was immobilized on the electrode surface, no signal probe can be attached and, therefore, no electrochemical signal was obtained. Also, when EXO III was added after sensing **step 1**, no electrochemical response was obtained either. On the 3' end of the stem-loop capture probe, there is an extended single stranded DNA which could not be hydrolyzed by EXO III, thus restraining the immobilization of the signal probe. Therefore, the electrochemical response obtained in sensing **step 2** was also negligible. Afterward, when multiple DNA targets of GMOs were added into the sensing system, the target DNA preferably hybridized with the capture probe and induced the conformational change from stem-loop to linear structure of the original capture probe. Then the signal probe hybridized with the free ssDNA on the one end of the dsDNA at a ratio of 1 to 1 for the resulting signal. Therefore, in sensing step 3 in Fig. 2d, the analytical response obtained with the presence of target ssDNA of GMOs was easily observed compared with those obtained in sensing steps 1 and 2.

The use of EXO III as an effective reagent for signal amplification was further investigated. EXO III is a highly efficient 5' to 3' cleavage enzyme, catalyzing the removal of 5'-mononucleotides one at a time from one strand of 5'-double-strand DNA to generate signal-stranded DNA and mononucleotides. EXO III was added in the sensing step 3, the new formed dsDNA structure was hydrolyzed in the presence of EXO III, leaving a residual ssDNA on the electrode surface for further immobilization of the signal probe. In theory, only one target ssDNA in the sensing system, many residue ssDNA could be produced through the cycle hydrolysis effect, which is termed as the *1st round* signal amplification process. Therefore, in sensing step 4, as shown in Fig. 2d, in the presence of three different DNA targets, all electrochemical responses from the different redox tags were evident and were obviously enhanced compared with that of the sensing step 3. Furthermore, colloidal gold nanoparticles were applied as the platform for immobilization of signal probes. This enabled further enhancement of the electrochemical response due to the high surface area of the nanomaterials, as evident in the *nd round* signal amplification of the electrochemical sensing strategy.

Fig. 2c and d shows that the sensing response in the sensing step 5 was considerably more improved compared to those obtained in sensing steps 1–3 of the 1st round amplification. Obviously, the sensing responses of the three redox tags were all improved simultaneously in sensing step 5 and, notably, only one type of enzyme was used for all signal improvements. This also avoids the likelihood of non-specific reaction during the hydrolysis and simplifies the detection processes. In summary, all the results in Fig. 2 demonstrate that: (1) multiple DNA components of GMOs can be successfully simultaneously detected by using three different redox tags and (2) the sensitivity of the biosensor can be improved through the use of the proposed two-round signal amplification strategy.

3.5. Influence of target DNA ratio

Further interrogation of the multiple detection strategy was accomplished by investigating the influence of different target DNA ratio in the analyte. The three ratios considered were 10:1:10, 1:1:1, and 1:10:1. The results in Fig. 3a show that regardless of the ratio of the three different target DNA, the proposed electrochemical biosensing strategy can differentiate the specific target qualitatively. To enable quantitative analysis of the different DNA components of the GMOs, calibration curves for the different components were constructed. As shown in Fig. 3b, the calibration curves for specific DNA components demonstrate that detection can be realized from 0.1 pM to 10 nM with the different redox tags.

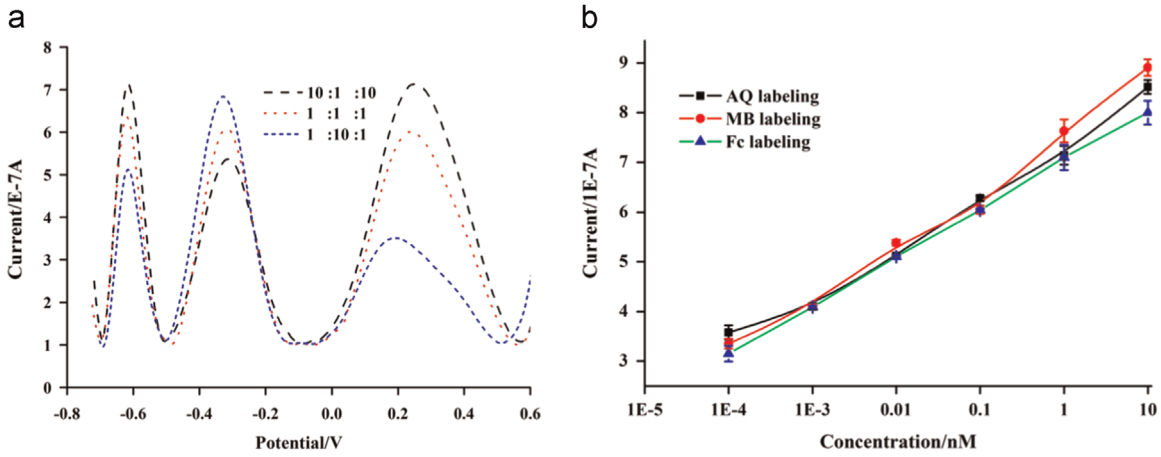

Fig. 3. Simultaneous detection of (a) multiple DNA components of different ratio and (b) the calibration curves obtained with different electroactive tags.

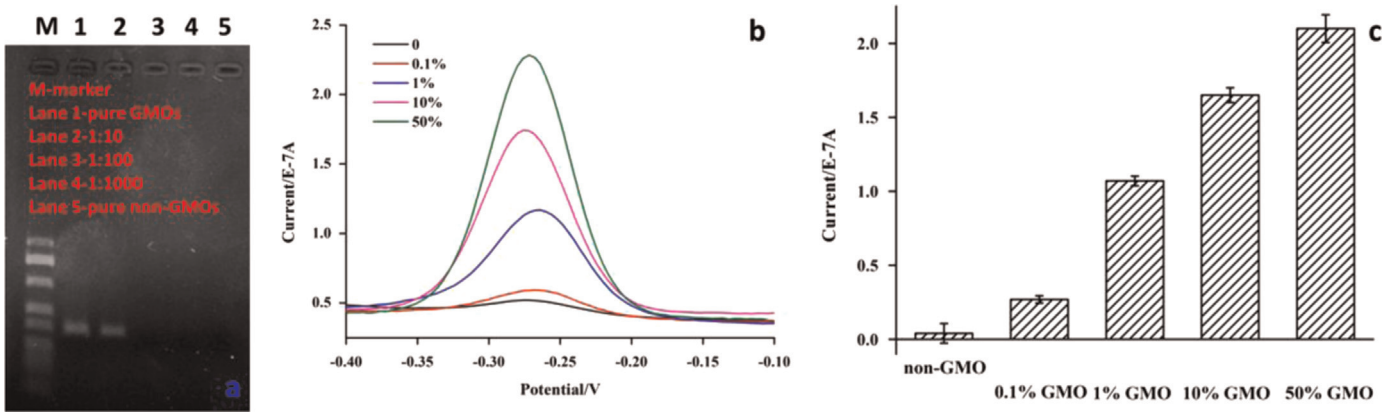

Fig. 4. GMO detection by (a) PCR, (b) and (c) with fabricated electrochemical biosensor.

3.6. Application to GMO samples

The extracted genomic samples from the confirmed positive GMOs samples (See the PCR results of the positive GMOs samples in SI) were sonicated for 5 min and used directly for detection with the proposed electrochemical biosensor. A series of DNA fragment samples used, containing 0.1–100% genomic DNA from GM soybean, were prepared by mixing the genomic DNA from GM soybean with appropriate aliquots of genomic DNA extracted from non-GM soybean. All the GMO samples with different spiked ratio were first analyzed and confirmed with traditional PCR. The PCR detection results of the positive spiked genomic samples of GMOs are shown in Fig. 4a. From these results, it was obvious that the genomic samples from pure GMOs were amplified and the target band was observed at 197 bp (lane 1 in Fig. 4a). With the genomic samples extracted from 1:10 GMOs mixed materials, the target GMOs component was also be amplified successfully (lane 2 in Fig. 4a). However, with further decrease in the GMO components in the mix samples (1:100 and 1:1000), the target DNA component of GMOs could not be amplified by the traditional PCR (lanes 3 and 4 in Fig. 4a). Meanwhile, the proposed biosensor was also used to detect the same extracted genomic samples from GMOs at different spiked ratios. Fig. 4b and c shows that even with the GMOs spiked with non-GMOs at the ratio of 1:1000, the proposed signal amplified electrochemical biosensor successfully differentiate it from the pure non-GMOs, thus, demonstrating the excellent sensitivity of the proposed electrochemical biosensor. Evidently, with increased spiked ratio of GMOs in the mixture, the detection with the biosensor becomes more obvious and easier to distinguish. According to the international standard (REF), only a GMO detection sensitivity of 1% is required, which is much higher than the minimum of 0.1% achieved with the proposed electrochemical biosensor.

Furthermore, it is possible to apply the proposed signal amplified electrochemical biosensor to the detection of other GMOs by simply replacing the sequence of the capture probe on the electrode surface. The GMO soybean and rice samples from Anhui and the confirmed GMO *Arabidopsis thaliana* samples from our university (See the PCR confirmation results in Supporting information) were all tested with the proposed signal amplified electrochemical biosensor. The detection results in Fig. 5 show that three different GMO plants were well differentiated by the proposed electrochemical sensor even at the international standard allowance value (1%). However, even at the same ratio values, there were only little differences in sensing sensitivity which could be attributed to the different probe length and to the different target GMOs and different hybridization dynamic process. Furthermore, all results shown in Fig. 5 clearly demonstrate a successful application of the proposed electrochemical biosensor for rapid and simultaneous screening of GMOs.

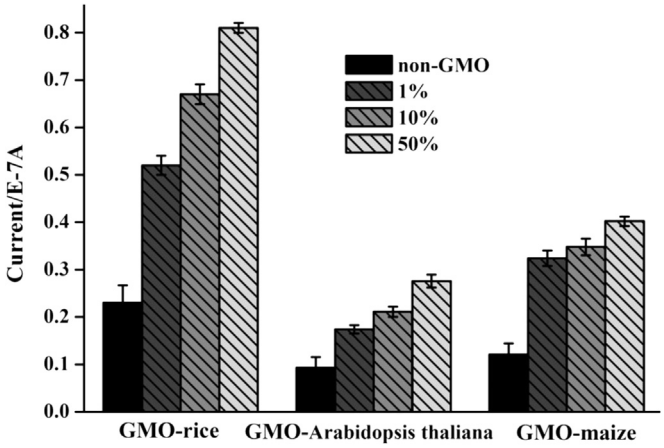

Fig. 5. Detection of different GMOs by the proposed electrochemical biosensor.

4. Conclusion

We have successfully demonstrated the development of an electrochemical biosensor for simultaneous and sensitive detection of multiple DNA components in GMO products. The rapid detection and signal amplification were achieved by utilizing electroactive redox tags together with the enzyme EXO III and gold nanoparticle based probe immobilization in a two-round signal amplification strategy. Firstly, the adopted three different distinguishable electroactive redox tags were effective for simultaneous detection of multiple DNA components of GMO. Also, the adoption of both the enzymatic signal amplification and GNPs based signal amplification strategy led to considerable improvement in the sensitivity of the developed sensor. Under optimized conditions, this electrochemical biosensor achieved simultaneous detection of multiple DNA components of GMOs with relatively high sensitivity. Furthermore, the electrochemical biosensor was also successfully applied to the detection of different GMOs. The biosensor can detect as low as 0.1% GMO contents which is 10 times lower that the 1% GMO detection sensitivity set for the international standard (Wei et al., 2013; European Commission Regulation, 2003; Notification 2000-31, 2000), and comparable to surface plasmon resonance based instrumental methods (D'Agata et al., 2010) and other representive method summarized in Table S1. The proposed electrochemical biosensor therefore provides a suitable tool or rapid high throughput and sensitive on-site screening of GMOs.

Acknowledgement

This work is financially supported by the NSFC, China Grant of 21475030 and 31301460, the 12th Five Years Key Programs (2012BAK08B01-2, 2012BAK17B10, SS2012AA101001), the Science and Technology Research Project of General AQSIQ (201210127, 201310135), National and Zhejiang Public Benefit Research Project (201313010, 2014C32051) and the Fundamental Research Funds for the Central Universities (2013HGCH0008).

Appendix A. Suplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.12.005.

References

Abi, A., Ferapontova, E.E., 2012. J. Am. Chem. Soc. 134, 14499–14507.
Arun, O.O., Yilmaz, F., Muratoglu, K., 2013. Food Control 32, 525–531.
Chen, W., Jiang, Y., Ji, B.Q., Zhu, C.Q., Liu, L.Q., Peng, C.F., Jin, M.K., Qiao, R.R., Jin, Z.Y., Wang, L.B., Zhu, S.F., 2009. Biosens. Bioelectron. 24, 2858–2863.
Chen, W., Shen, H.B., Li, X.Y., Jia, N.Q., Xu, J.M., 2006. Appl. Surf. Sci. 253, 1762–1769.
D'Agata, R., Corradini, R., Ferretti, C., Zanoli, L., Gatti, M., Marchelli, R., Spoto, G., 2010. Biosen. Bioelectron. 25, 2095–2100.
Eric, S., Peter, A.H., Hans, B., Jaap, B., Bryan, S.G., Michiel, R., Oliver, S., Brajesh, K.S., Debora, C.M.G., 2012. Ecol. Indic. 14, 107–133.
European Commission Regulation (EC) 1829/2003 AND 1830/2003., 2003. Off. J. Eur. Communities: Legis. 268, 1-28.
Ghedira, R., Papazova, N., Vuylsteke, M., Ruttink, T., Taverniers, I., Loose, M.D., 2009. J. Agric. Food Chem. 57, 9370–9377.
Grohmann, L., Brunen-Nieweler, C., Nemeth, A., Waiblinger, H.U., 2009. J. Agric. Food Chem. 57, 8913–8920.
Gruere, G.P., Carter, C.A., Hossein Farzin, Y., 2009. Rev. Int. Econ. 17, 393–408.
Gao, J.C., Yang, L.T., Chen, L.L., Morisset, D., Li, X., Pan, L.W., Zhang, D.B., 2011. Anal. Chem. 83, 1579–1586.
Guo, L.H., Yang, H.H., Qiu, B., Xiao, X.Y., Xue, L.L., Kim, D.H., Chen, G.N., 2009. Anal. Chem. 81, 9578–9584.
Han, X.Q., Wang, H.Y., Chen, H.J., Mei, L., Wu, S.Q., Jia, G.J., Cheng, T., Zhu, S.F., Lin, X. M., 2013. Biosens. Bioelectron. 49, 360–366.
Huang, H., Jin, L., Yang, X., Song, Q.X., Zou, B.J., Jiang, S.W., Sun, L.Z., Zhou, G.H., 2013a. Biosens. Bioelectron. 42, 261–266.
Huang, L., Wu, J.J., Zheng, L., Qian, H.S., Xue, F., Wu, Y.C., Pan, D.D., Adeloju, S.B., Chen, W., 2013b. Anal. Chem. 85, 10842–10849.
Huang, X., Chen, L.L., Xu, J.M., Ji, H.F., Zhu, S.F., Chen, H.J., 2014. Food Chem. 156, 184–189.
Hvastkovs, E.G., Buttry, D.A., 2010. Analyst 135, 1817–1829.
Jiang, X.H., Zhang, H.M., Wu, J., Yang, X., Shao, J.W., Qiu, B., Lin, Z.Y., Chen, G.N., 2014. Talanta , http://dx.doi.org/10.1016/j.talanta. 2014.05.002.
James, C., 2013. International Service for the Acquisition of Agri-biotech Application: Ithaca, NY.
Jiang, L., Yang, L., Zhang, H., Guo, J., Mazzara, M., Van den Eede, G., Zhang, D.B., 2009. J. Agric. Food Chem. 57, 3525–3532.
Laura, R., 2003. Anal. Chem. 75, 394A–396A.
Monaghan, E.K., Venkatachalam, M., Seavy, M., Beyer, K., Sampson, H.A., Roux, K.H., Sathe, S.K., 2008. J. Agric. Food Chem. 56, 765–777.
Nam, J.M., Mirkin, C.A., 2004. J. Am. Chem. Soc. 126, 5932–5933.
Notification 2000-31, Ministry of Agriculture and Forestry of Korea: Seoul. 2000.
Novak, P.K., Gruden, K., Morisset, D., Lavrac, N., Stebih, D., Rotter, A., Zel, J., 2009. J. AOAC Int. 92, 1739–1746.
Peano, C., Samson, M.C., Palmieri, L., Gulli, M., Marmiroli, N., 2004. J. Agric. Food Chem. 52, 6962–6968.
Ronkainen, N.J., Halsall, H.B., Heineman, W.R., 2010. Chem. Soc. Rev. 39, 1747–1763.
Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., Arnheim, N., 1985. Science 230, 1350–1354.
Samson, M.C., Gulli, M., Marmiroli, N., 2013. Food Control 30, 518–525.
Wei, J.J., Li, F.W., Guo, J.C., Li, X., Xu, J.F., Wu, G., Zhang, D.B., Yang, L.T., 2013. J. Agric. Food Chem. 61, 11363–11370.
Wu, J.J., Chu, H.Q., Mei, Z.L., Deng, Y., Xue, F., Zheng, L., Chen, W., 2012. Anal. Chim. Acta 753, 27–31.
Xu, J., Miao, H.Z., Wu, H.F., Huang, W.S., Tang, R., Qiu, M.Y., Wen, J.G., Zhu, S.F., Li, T., 2006. Biosens. Bioelectron. 22, 71–77.
Xue, F., Wu, J.J., Chu, H.Q., Mei, Z.L., Ye, Y.K., Liu, J., Zhang, R., Peng, C.F., Zheng, L., Chen, W., 2013. Microchim. Acta 180, 109–115.
Zhang, M., Liu, Y.N., Chen, L.L., Quan, S., Jiang, S., Zhang, D.B., Yang, L.T., 2013. Anal. Chem. 85, 75–82.
Zhao, Y., Hao, C.L., Yong, Q.Q., Qu, C.L., Chen, W., Peng, C.F., 2012. Int. J. Food Sci. Technol. 47, 910–917.
Zhu, D.B., Tang, Y.B., Xing, D., Chen, W.R., 2008. Anal. Chem. 80, 3566–3571.
Zhe, D.B., Tang, Y.B., Xing, D., Chen, W.R., 2008. Anal. Chem. 80, 3566–3571.