



Short communication

## Ultrasensitive electrochemical detection of *Bacillus thuringiensis* transgenic sequence based on *in situ* Ag nanoparticles aggregates induced by biotin–streptavidin system

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## ABSTRACT

A novel electrochemical biosensor was developed for detecting short DNA oligonucleotide of *Bacillus thuringiensis* (Bt) transgenic sequence based on Ag nanoparticle aggregates. To fabricate this DNA biosensor, the thiol-modified capture DNA (cDNA) was first anchored on gold (Au) electrode, and then the target DNA (tDNA) was hybridized with the immobilized cDNA. Subsequently, the probe DNA (pDNA) functionalized by biotinylated Ag nanoparticle was associated with the fixed tDNA, and the single Ag nanoparticle label was obtained (cited as SAg label). Finally, dissociative biotinylated Ag nanoparticle was bound to the resultant biotinylated SAg label assembled on Au electrode by virtue of bridge molecule streptavidin (SA) through biotin–SA specific interaction, which could lead to *in situ* aggregate of Ag nanoparticles on Au electrode and induce a novel tag including multiple Ag nanoparticles (cited as MAg tag). The novel tag exhibited excellent electroactive property in the solid-state Ag/AgCl process and was successfully applied to Bt transgenic sequence assay. A detection limit of 10 fM was achieved, which was improved by three orders of magnitude as compared to the SAg label. Furthermore, this novel DNA biosensor demonstrated a good selectivity towards tDNA.

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## 1. Introduction

The genetically modified (GM) crops engineered to express insecticidal proteins derived from bacterium *Bacillus thuringiensis* (Bt) can avaiably resist special insect pests and reduce the use of chemicals for crop protection, thus the growth of Bt crops has increased in an surprising rate. Since first commercially grown in 1996, the Bt crops have yielded billions of dollars of benefits to farmers (Hutchison et al., 2010). However, a series of problems particularly in food security and bio-security need to be taken into great consideration (Quist and Chapela, 2001; Kaplinsky et al., 2002). Therefore, it is necessary to establish certain simple, rapid and sensitive detection methods for monitoring Bt food and feed. Polymerase chain reaction (PCR) based methods are the first choice in the detection of GM organisms (Hernández et al., 2005). Despite being highly sensitive and stable, the PCR method mainly suffer from drawbacks of “high cost”, “high complexity” and “time consuming” (Zhu et al., 2008; Qiu et al., 2011). Hence it is urgent to establish a cost-effective, time-saving, reliable and rigorous monitoring system for Bt transgenic sequence.

At present, various methods have been developed for specific transgenic sequences detection based on electrical (Yang et al., 2007, 2008, 2009; Scarano et al., 2009) and optical (Gao et al., 2011) technology. Many electrochemical biosensors have been constructed and applied to transgenic sequences assay benefiting from their unique properties such as simplicity, speediness, low-cost and accuracy (Merkoçi et al., 2005; Sassolas et al., 2008). Especially, it is well recognized that the electrochemical biosensors involving nanoparticles are of particular interest because the nanoparticles represent unique structural, electronic and catalytic properties, allowing high sensitivity and high reaction rates for DNA analysis (Merkoçi et al., 2005; Castañeda et al., 2007). Some electrochemical biosensors including gold nanoparticles (Yang et al., 2007), multi-walled carbon nanotubes (Yang et al., 2009) and single-walled carbon nanotubes (Yang et al., 2008) have been contrived to detect specific sequences of PAT gene and NOS gene. However, there is no report on electrochemical DNA biosensors involving Ag nanoparticles for transgenic sequences detection in spite of their excellent electrochemical activity (Wang et al., 2002, 2003; Gao et al., 2008; Zhang et al., 2009; Li et al., 2010).

Herein, we present a direct electrochemical DNA biosensor for Bt transgenic sequences assay using solid-state Ag/AgCl process for the first time. Since streptavidin (SA) is a tetrameric protein containing four binding sites for biotin with extraordinarily high affinity ( $K_d = 10^{-15}$ ), *in situ* biotin–SA-induced Ag aggregates

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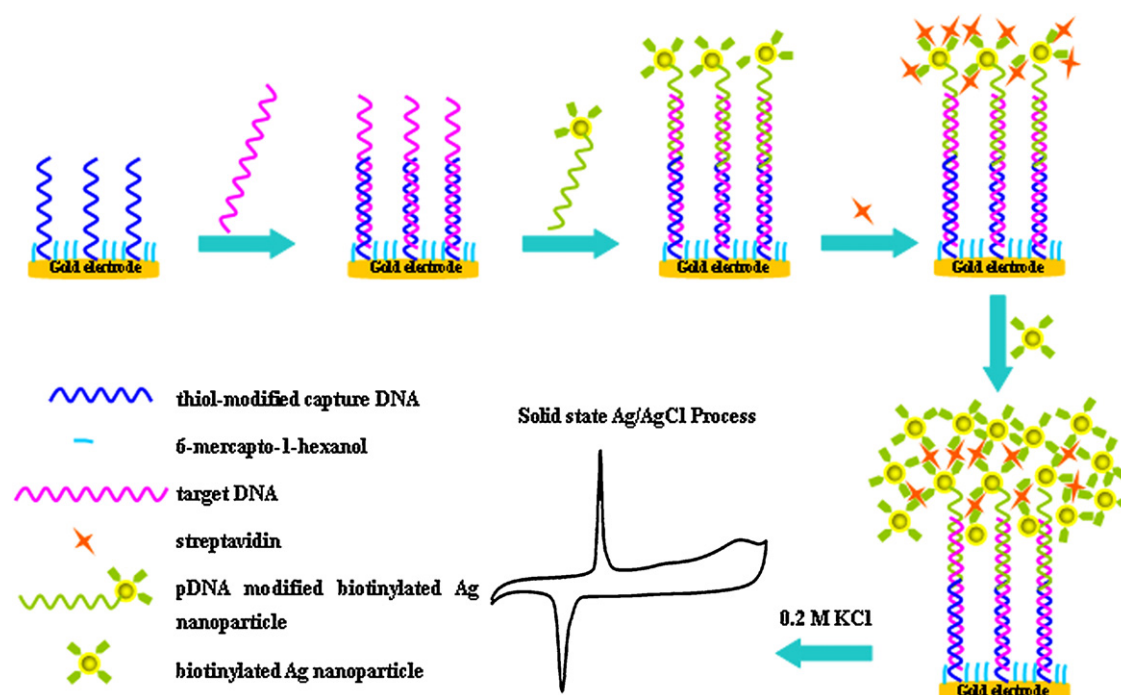


Fig. 1. Schematic image of the DNA sensing strategy.

tags (cited as MAg tag) on Au electrode were designed to realize sensitive, simple and rapid determination of DNA sequence in a wide range. The detection scheme is shown in Fig. 1. Biotinylated Ag nanoparticles modified by probe DNA (pDNA) were applied to bind with target DNA (tDNA) via sandwich hybridization. Then dissociative biotinylated Ag was bound to the single biotinylated Ag nanoparticle label (cited as SAg label) by virtue of bridge molecules SA, which could bring on *in situ* aggregate Ag nanoparticles on Au electrode. To our best knowledge, biotin–SA interaction induced *in situ* aggregate Ag tag has not been investigated on specific sequences detection yet. Finally, the novel tags induced by biotin–SA interaction were successfully applied to offer amplified readout by solid-state Ag/AgCl process. The results demonstrated that the detection sensitivity using this novel tags was improved by three orders of magnitude as compared to the single Ag nanoparticle label and a detection limit of 10 fM was achieved.

## 2. Experimental

### 2.1. Chemicals and materials

5'-Thiolated capture oligonucleotide (cDNA, sequence: 5'-HS-ATC CCT ATA CCC TCT-3'), tDNA (sequence: 5'-AGA GGG TAT AGG GAT TAC ACG CCA CTA CCA-3'), one base-mismatched tDNA (sequence: 5'-AGA GAG TAT AGG GAT TAC ACG CCA CTA CCA-3'), 5'-thiolated pDNA (sequence: 5'-HS-TGG TAG TGG CGT GTA-3') and biotinylated thiol oligonucleotide (sequence: 5'-HS-TTTTTT-biotinyl-3') were synthesized by AuGCT biotechnology Co. Ltd. (Beijing, China). 6-Mercapto-1-hexanol (MCH), ethylenediaminetetraacetic acid (EDTA), NaBH<sub>4</sub> and AgNO<sub>3</sub> were purchased from Aladdin Chemical Co. (Shanghai, China). All chemicals concerned were of analytical grade and all solutions were prepared with ultrapure water obtained from a Millipore water purification system ( $\geq 18 \text{ M}\Omega$ , Milli-Q, Millipore).

### 2.2. Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed with a CHI 660 electrochemical workstation (China, Chenhua). All experiments were carried out with a conventional three-electrode system. A modified Au electrode with 2-mm-diameter, a platinum wire and an Ag/AgCl (saturated KCl) electrode were used as the working electrode, counter electrode and reference electrode, respectively.

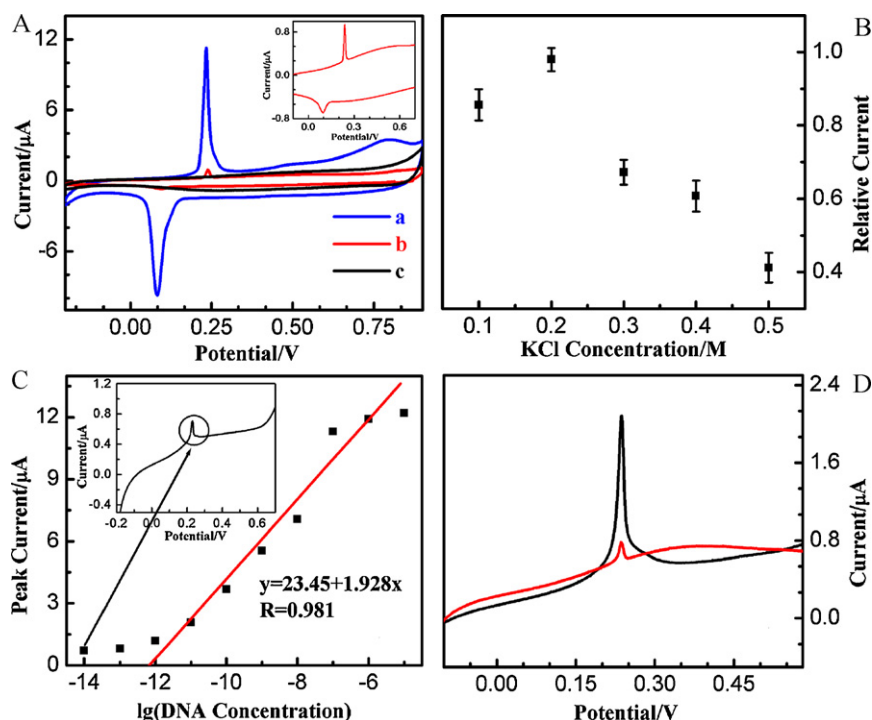
The absorption spectra were acquired on the Nicolet Evolution 300 UV–vis spectrometer (America, Thermo Nicolet) coupled with a 1.00 cm quartz cell. Transmission electron microscopy (TEM) images were obtained using a JEM-2010 transmission electron microscope (Japan, JEOL) operating at an acceleration voltage of 200 kV. The hydrodynamic diameters were measured with a Zetasizer Nano ZS90 DLS system (England, Malvern).

### 2.3. Preparation and modification of Ag nanoparticles

Ag nanoparticles were prepared by the reduction of AgNO<sub>3</sub> by NaBH<sub>4</sub> according to the literature with some modifications (Liu et al., 2006). The obtained solution was centrifugated at 8000 rpm and the precipitate was re-dissolved in water. Biotinylated Ag nanoparticles and pDNA modified biotinylated Ag nanoparticles were fabricated according to the literature with some modifications (Liu et al., 2006).

### 2.4. Electrode modification, DNA hybridization and detection

The whole DNA biosensor fabrication process was schematically demonstrated in Fig. 1. The detailed procedures for SAg label were listed in the Supplementary data. In order to amplify the current signal, the obtained electrode was subsequently incubated in 1  $\mu\text{M}$  SA in 0.1 M phosphate buffer (pH=8) for 1 h and SA was anchored to the biotinylated SAg label through biotin–SA specific interaction. Then, after thoroughly rinsing with ultrapure water, the modified gold electrode was immersed into 0.1 M phosphate



**Fig. 2.** (A) Typical voltammetric response of the (a and c) MAg tag and (b) SAg label in 0.2 M of KCl (a and b) with or (c) without hybridization with 0.1  $\mu\text{M}$  of target DNA. Inset: the amplification of curve b. (B) Effect of KCl concentration on the peak current normalized by peak height obtained in 0.2 M of KCl. (C) Dependence of the peak current on the concentration of complementary DNA. Inset: the voltammetric response of the DNA biosensor to 10 fM of complementary DNA present in the hybridization step. (D) Voltammetric responses of the DNA biosensor to 10 pM of (–) complementary DNA and (–) one base-mismatched DNA. The sensor consisted of a 2-mm-diameter Au electrode, scan rate: 100  $\text{mV s}^{-1}$ .

buffer (pH = 7.4) with 1  $\mu\text{M}$  biotinylated Ag for 1 h and the biotinylated Ag nanoparticles were bound to the SA assembled on the SAg label. Finally the prepared electrode was washed carefully with 10 mM of Tris-HCl/0.15 M of NaCl (pH 8.8) and with KCl consecutively.

The modified electrodes obtained during stepwise modification were electrochemically characterized using EIS and CV in 0.5 M KCl solution containing 5.0 mM  $[\text{Fe}(\text{CN})_6]^{3-}$ . Voltammetric responses of the DNA biosensors were recorded in 0.2 M KCl.

### 3. Results and discussion

#### 3.1. Characteristics of Ag nanoparticles and modified Ag nanoparticles

The UV-vis spectrum of pDNA modified biotinylated Ag nanoparticles at 390 nm was almost identical to that of Ag nanoparticles (Fig. S1), which indicated that no obvious aggregate came into being when Ag nanoparticles were modified by pDNA and biotin. However, the absorption spectrum of the conjugation between pDNA modified biotinylated Ag nanoparticles and biotinylated Ag nanoparticles through SA demonstrated a maximum wavelength of around 573 nm, which was red-shifted by 183 nm from that of Ag nanoparticles. The fact suggested an obvious aggregate between the two types of modified Ag nanoparticles through the linker.

TEM image showed that the single Ag nanoparticles were around 20 nm in diameter (Fig. S2A). An obvious aggregation was found when the two types of modified nanoparticle were linked by SA (Fig. S2B), demonstrating that two types of Ag nanoparticle could aggregate by the linker of SA. The hydrodynamic diameters also increased dramatically when the two types of modified nanoparticle were linked by SA (Fig. S3), which were in good agreement with the results of TEM, and again confirmed the two types of Ag nanoparticle could available aggregate by the linker of SA. These

facts indicated that *in situ* Ag aggregates on Au electrode could be obtained by using biotin-SA interaction.

#### 3.2. Electrochemical characteristics of biosensor

The performance of the working electrode during stepwise modification was evaluated by EIS and CV in 0.5 M KCl solution containing 5.0 mM  $[\text{Fe}(\text{CN})_6]^{3-}$  (Fig. S4). There are differences in the electron-transfer resistance ( $R_{\text{et}}$ ) upon the stepwise modification of the modified electrode (Fig. S4A). In comparison, the  $R_{\text{et}}$  of cDNA modified-Au electrode (curve b) is larger than that of bare electrode (curve a), mainly due to the electrostatic repulsion between negative charges of cDNA oligonucleotides backbone and electroactive probe  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . After hybridization of tDNA with cDNA, the negative charge at the electrode surface was essentially increased. As a result, a  $R_{\text{et}}$  (curve c) increased by 1.9 times than that of single stranded DNA (cDNA)-modified electrode. When pDNA was immobilized on the Au electrode, the largest  $R_{\text{et}}$  (curve d) was observed. Fig. S4B shows the CVs of  $[\text{Fe}(\text{CN})_6]^{3-}$  on the modified electrode at different stages. On the bare Au electrode, a well-defined redox peak pair was observed (curve a), showing the excellent electron-transfer kinetics of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The amperometric response decreased and the peak-to-peak separation was enlarged after different DNA oligonucleotides were immobilized on the Au electrode (curves b–d) step by step. The CV experiments for differently modified electrodes were in good agreement with the EIS results, confirming again the success of each assembly step.

#### 3.3. Detection of Ag nanoparticle labels using solid-state process

The typical solid-state voltammetric response of SAg label in 0.2 M of KCl is shown in Fig. 2A (curve b), and two well-defined current peaks can be observed (inset of Fig. 2A). In the forward scan, Ag was oxidized to  $\text{Ag}^+$ , and the electrogenerated  $\text{Ag}^+$  formed

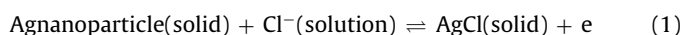
**Table 1**

Comparison of characteristics of the proposed method with previously published methods for transgenic sequences determination.

| Detection technique     | Liner range ( <i>M</i> )                     | DNA detection limits ( <i>M</i> ) | <i>R</i> | Reference             |
|-------------------------|--|-----------------------------------|----------|-----------------------|
| EIS                     | $1.0 \times 10^{-10}$ – $1.0 \times 10^{-5}$ | $2.4 \times 10^{-11}$             | 0.9937   | Yang et al. (2007)    |
| DPV                     | $1.0 \times 10^{-11}$ – $1.0 \times 10^{-6}$ | $2.6 \times 10^{-12}$             | 0.9951   | Yang et al. (2008)    |
| EIS                     | $1.0 \times 10^{-13}$ – $1.0 \times 10^{-7}$ | $2.7 \times 10^{-14}$             | 0.996    | Yang et al. (2009)    |
| QCM                     | $0$ – $5.0 \times 10^{-7}$                   | $2.5 \times 10^{-8}$              | 0.985    | Scarano et al. (2009) |
| DLS                     | $1.0 \times 10^{-13}$ – $5.0 \times 10^{-9}$ | $3.0 \times 10^{-14}$             | 0.9970   | Gao et al. (2011)     |
| Solid-state voltammetry | $1.0 \times 10^{-12}$ – $1.0 \times 10^{-6}$ | $1.0 \times 10^{-14}$             | 0.981    | This work             |

EIS: electrochemical impedance spectroscopy; DPV: differential pulse voltammetry; QCM: quartz crystal microbalances; DLS: dynamic light scattering.

insoluble AgCl on the electrode surface in the presence of  $\text{Cl}^-$ . In the backward scan, the solid AgCl on the electrode surface was reduced to Ag, and the  $\text{Cl}^-$  released into the solution (Zhang et al., 2009; Ting et al., 2009a,b). The overall process is represented by Eq. (1).



In order to improve the detection sensitivity, we contrived a novel MAg tag based on biotin–SA-induced *in situ* Ag aggregates to realize a sensitive, simple and rapid survey of DNA sequence in a wide range, which had not yet been reported for electrochemical assay of DNA targets. This novel MAg tag produced 12-fold amplification when compared to that of the SAg label (Fig. 2A, curve a), which can be attributed to the large aggregated nanostructure indicated in TEM image (Fig. S2B). Additionally, a controlled electrode was performed as the DNA biosensor fabricated by MAg tag only without hybridization with tDNA, and no distinct peak was observed (Fig. 2A, curve c). The outcome demonstrated that *in situ* aggregates amplification can dramatically improve the peak current without enhancing the background. The fact also indicated that the non-specific adsorption of Ag nanoparticle on Au electrode was slender and could be ignored. The reproducibility of the biosensor was also investigated with three biosensors prepared independently under the same experimental conditions. The variation coefficients obtained were 7.38% indicating fabrication reproducibility. Hence, the novel MAg tag can be used to quantify tDNA of Bt transgenic sequence.

It was anticipated from Eq. (1) that the characteristics of the solid-state Ag/AgCl process could be affected by the  $\text{Cl}^-$  concentration and the effect of KCl on oxidation peak current was investigated. The results revealed that a distinct oxidation peak could be observed in all solutions and reached the greatest when the concentration of KCl was 0.2 M (Fig. 2B). Therefore, 0.2 M KCl was chosen for this study.

### 3.4. Sensitivity of the DNA biosensor

In this system, the current signal is responsive to the amount of Ag nanoparticle bonded to the probe oligonucleotides hybridized with Bt oligonucleotides to form sandwich structure. Therefore, the quantity of Bt oligonucleotides is proportional to the peak current. Fig. 2C shows the correlation between the concentration of the Bt oligonucleotides and peak current of MAg tag. It could be seen that the peak current increased with the increase of Bt oligonucleotides in the range of  $10^{-14}$ – $10^{-5}$  M, and showed a linear response to the Bt oligonucleotides concentration in the wide range of  $10^{-12}$ – $10^{-6}$  M. The regression equation was  $Y = 23.45 + 1.928X$  ( $X: M$ ) with  $R = 0.981$ . And even when 10 fM of Bt oligonucleotides was present, the detected signal was still well distinguished (inset of Fig. 2C). This level of sensitivity was comparable to that of the non-PCR based method in previously published reports for transgenic sequences determination (Table 1).

### 3.5. Selectivity of the DNA biosensor

The selectivity of the present biosensor was investigated by using the pDNA probe labeled with aggregated Ag nanoparticles to hybridize with the same concentration of completely complementary tDNA sequences and one-base mismatched DNA sequences, respectively. As shown in Fig. 2D, a well-defined solid-state voltammetry signal of Ag was obtained for the complementary sequences, and only a very weak of signal emerged for one-base mismatched sequences. The result demonstrates that the proposed DNA electrochemical biosensor has a good selectivity.

## 4. Conclusion

A new amplification strategy based on the signal of DNA probe modified Ag nanoparticle aggregates through biotin–SA interaction was developed to determine the specific DNA sequence of Bt. The MAg tag was detected through the characteristic of solid-state Ag/AgCl redox process, which was subsequently used to quantify the amount of Bt transgenic sequence. A detection limit of 10 fM was achieved with this new DNA biosensor. The new electrochemical DNA biosensor not only exhibited excellent sensitivity, but also showed good selectivity. We believe that the preliminary work will promote further study on electrochemical DNA biosensor of transgenic sequence and impact actively on the applications of electrochemical DNA biosensor.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.042.

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