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PII: S0039-9140(16)30027-3
DOI: http://dx.doi.org/10.1016/j.talanta.2016.01.026
Reference: TAL16278

To appear in: *Talanta*

Received date: 29 November 2015
Revised date: 8 January 2016
Accepted date: 12 January 2016

Cite this article as: Lu Hong, Jing Wan, Xiaojun Zhang and Guangfeng Wang, DNA-gold nanoparticles network based electrochemical biosensors for DNA MTase activity, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2016.01.026

DNA-Gold Nanoparticles Network Based Electrochemical Biosensors for DNA MTase Activity

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ABSTRACT

In this work, a highly sensitive electrochemical DNA methyltransferase (MTase) activity assay was fabricated with DNA-gold nanoparticles (Au NPs) network as signal amplification unit and an easy assembly method by the linkage of benzenedithiol bridge. By two complementary AuNPs modified single-stranded DNA, DNA-gold nanoparticles network was self-assembled. With the linkage of benzenedithiol bridge, the DNA network structure was immobilized on the surface of gold electrode through the covalent Au-S bond. In the presence of Dam MTase, the special sites of DNA-AuNPs network were methylated and could not be digested by restriction endonuclease Mbo I. Thus the loaded electrochemical indicator Methylene blue (MB) was MB molecules still remained on the DNA-Au NPs network. The electrochemical response depended on the methylated degree, which could be used to detect MTase activity. By the differential pulse voltammetry (DPV), it was demonstrated a linear relation-ship between the DPV response with logarithm of Dam concentration ranging from 0.075 to 30 U/mL, achieving a low detection limit of 0.02 U/mL. The use of benzenedithiol avoided the direct incubation of the solid electrode with the capture DNA probe under complex and harsh conditions. Therefore the immobilization of DNA-AuNPs network was easy to be carried out, which is favorable for the specially high stability and reproducibility of the electrochemical biosensor.

Keywords: Methylation; Methyltransferase activity; DNA-AuNPs network; Signal amplification

1. Introduction

DNA methylation, a major epigenetic modification of the genes, plays a significant role in many biological processes including transcription, genomic imprinting, cellular differentiation, chromatin structure and embryogenesis [1]. DNA methylation reaction occurs in both prokaryotes and eukaryotes with the help of DNA methyltransferase (MTase) which catalyzes a methyl group to transfer from the donor S-adenosyl-l-methionine (SAM) to the target adenine or cytosine, producing S-adenosyl-l-homocysteine (AdoHcy) and methylated DNA [2, 3]. The aberrant DNA methylation patterns may affect the normal cellular functions and change phenotypes expression of the cells [4], which are associated with several genetic diseases and various types of cancer [5-8]. Thus, it is crucial to develop sensitive and selective methods for quantitative detection of

DNA methylation and analysis of DNA MTase activity [9-12]. Conventional methods for the estimation of DNA MTase activity and detection of DNA MTase are mainly focused on radioactive labeling of [methyl-3H]-SAM, polymerase chain reaction (PCR)-based techniques, high-performance liquid chromatography (HPLC) and gel electrophoresis, etc [13-16]. However, most of these methods are time-intensive, DNA consuming, laborious treatment, or usually require isotope labelling. To overcome the above limitations, recently, novel fascinating strategies have been developed, including fluorescence [17-20], bioluminescence [21], electrochemistry [22-26], chemiluminescence [27,28] and electrogenerated chemiluminescence method [29, 30]. Although the luminescent approaches indeed improved the sensing performance and offered a high sensitivity, the demand of complicated processes and expensive analytical instruments limited their development and application in the assay of DNA MTase. Owing to the attractive advantages of convenience, sensitivity, fast response, low production cost and ease of miniaturization, the electrochemical technique has received an intensive attention in the detection of DNA methylation and assay of MTase activity [31, 32]. For instance, Liu et al. reported an electrochemical approach for DNA methylation detection and the MTase activity assay using an electroactive ferrocene acetic acid conjugated DNA probe coupled with a specific methylation responsive restriction endonuclease reaction [33]. Likewise, Su and colleagues proposed a methylene blue labelled single-stranded DNA as signal probe in the electrochemical assay of DNA methylation [34]. However, most of the electrochemical methods for the assay of MTase activity are usually involved with a key assembly step of the capture probe immobilized on the surface of the bare or the nanomaterials modified electrode [34-36]. It is noteworthy that the DNA incubation with the bare electrode is under harsh conditions including the control of the acidity, concentration and time. In order to effectively improve the efficiency and stability of the immobilization, it is essential to develop a novel assembling way to avoid the incubation of the capture probe with the electrode for the assay of MTase activity.

On the other hand, recently, isothermal amplification techniques, such as rolling circle amplification (RCA) [37], strand displacement amplification (SDA) [38], and nicking enzyme signal amplification (NESA) [39], network amplification are frequently used due to their wide variety of choices and significant signal amplification. Van Ness, J. et al applied the SDA-based colorimetric method for simple visualization of MTase activity [40]. However, the linear amplification characteristic of SDA and the poor detection limit of the colorimetric method unfavorably affect the detection sensitivity. The NESA-based method for MTase activitydetection exhibits improved sensitivity, but it requires the initial heating to denature the molecule beacon and thus is not truly isothermal [41]. Networks are monodispersed, three dimensional, hyperbranched, nanoscale architectures [42]. They not only possess numerous functionalized groups in the dendrimer structure for signal amplification but also involve easy controllable assembling process [43-45]. In the past few years, gold nanoparticles (Au NPs) with excellent biocompability [9,33] have been conjugated with functional DNA ingeniously and assemble the

DNA network structure [44], which can increase the loading of DNA or signal molecules to amplify the signal easily. Thus, it's promising to develop 3D DNA-Au NPs networks for DNA MTase signal amplified electrochemical biosensor.

Herein, we report a simple signal amplified electrochemical assay for MTase activity based on DNA-gold nanoparticles network. In order to successfully avoid the direct incubation step of the DNA capture probe with the electrode, benzenedithiol, was used as a linkage. Because the assembly of benzenedithiol is very easy and irrespective of the concentration and temperature [46], the linkage of benzenedithiol makes the DNA-AuNPs network could be self-assembled on the electrode under mild conditions with high efficiency. In the system the electrochemical signal was obtained by the loading of electrochemical redox hybridization indictors, the phenothiazine dye MB [47-49], on the DNA-AuNPs network after the treatment of MTase and digestion process. Thus, a sensitive electrochemical assay of MTase activity was developed with the DNA-Au NPs network signal amplified strategy by an easy benzenedithiol linked assembly method with high stability and reproducibility.

2. Materials and methods

2.1 Materials and reagents

Hydrogen tetrachloroaurate trihydrate (HAuCl43H2O) and tri (2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Aladdin (Shanghai, China). Dam MTase and Mbo I were supplied by New England Biolabs (Ipswich, MA). Methylene blue hydrate (MB), dithiothreitol (benzenedithiol) and Tris (hydroxy-methyl) aminomethane (Tris) were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). 6-Mercapto-1-hexanol (MCH), Sodium dodecyl sulfate (SDS) was purchased from Sigma-Aldrich (St. Louis, USA). All solutions were prepared with doubly distilled water. All oligonucleotide sequences were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The base sequences of oligonucleotides were as following. The recognition site for Dam MTase and Mbo I endonuclease were marked with italics.

DNA S1: SH-GTCTGATCCCTGTGTA

DNA S2: SH-TACACAGGGATCAGAC

DNA S3: SH-ACACACCAAACACACC

DNA S4: TACACAGGGATCAGAC

The oligonucleotides were dissolved in 10 mM Tris-HCl and 1 mM EDTA (Ethylene Diamine Tetraacetic Acid) buffer (pH 8.0) to the desired concentrations and stored at −20°C.

2.2 Apparatus

All electrochemical experiments were carried out using a CHI 660D electrochemical workstation (Shanghai Chen hua Instruments, China). A conventional three-component electrochemical cell was employed in all electrochemical measurements, with a bare or modified Au electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt wire as the auxiliary electrode. UV–visible absorption spectroscopy (U-2910

Spectrophotometer, Hitachi, Janpan) and scanning electron microscopy (SEM, Hitachi S4800) were used to investigate the morphology of Au NPs and DNA-Au NPs network.

2.3. Preparation of DNA-AuNPs bioconjugates and DNA–AuNPs network

In order to fabricate the DNA-AuNPs network, AuNPs were first prepared. Gold nanoparticles (16 nm) were prepared according to a classic citrate method [50]. Briefly, 50 mL of $HAuCl_4$ solution (0.01%, m/v) was boiled with vigorous stirring, and 2 mL of trisodium citrate solution (1%, m/v) was added quickly into the boiling solution. Then, the color of the solution changed from yellow to wine red, indicating that the Au NPs were formed. After left stirring and cooled to the room temperature, the resulting solution was stored in brown glass bottles at 4 ^{o}C for future use.

After that, DNA modified Au NPs were prepared according to the previously reported methods with slight modification [51]. Before S1 and S2 labelled on the Au NPs surface, thiolated DNA S1 and S2 were respectively activated by treatment with 100-fold excess TCEP at room temperature for 1h. Then, 15 μL 100 mM S1 and S2 were severally mixed with 500 μL Au NPs solution and incubated at 4 ^{o}C for 12 h. After incubation, 10 μL of 1% SDS was added to stabilize the Au NPs with shaking at room temperature for 1 h. Then the S1-Au NPs and S2-Au NPs were aged for 12 h by slowly adding 50 μL 0.5 M NaCl solution. To remove excess reagents, the solution was centrifuged at 10,000 rpm for 20 min. The wine red S1-Au NPs and S2-Au NPs precipitate were washed 3 times by centrifugation with 0.01 M Tris-HCI (pH7.4) , then resuspended in 0.5mL 0.01M Tris-HCI (pH7.4) for future use.

The formation of DNA–Au NPs network was carried out by the hybridization of S1-AuNPs and S2-AuNPs. It was conducted through mixing 250 μL of the prepared S1-AuNPs solution and 250 μL S2-AuNPs solution for 2 h at 37 ^{o}C.

2.4 Self-assembly of DNA-AuNPs network on a gold electrode

Firstly, the gold electrode (GE) (3mm diameter) was carefully polished to a mirror-like furnish with alumina powder of 0.3 and 0.05 mm, followed by sequential sonication for 10 min each in ultrapure water, ethanol and ultrapure water. Then it was dipped in freshly prepared piranha solution (98% H_2SO_4: 30% H_2O_2, 3:1 by volume) for 10 min and rinsed with ultrapure water thoroughly. The electrode was then scanned in 0.1 M H_2SO_4 between 0.2 V and 1.55 V at 100 mV/s until a reproducible cyclic voltammogram (CV) was obtained. Once completed, the electrode was washed with double distilled water and dried under nitrogen. Then, the gold electrode was incubated in 2 mM benzenedithiol for 12 h to form benzenedithiol/GE. After that, the electrode was treated with 500 μL the prepared DNA-Au NPs network solution for 10 h. Then the electrode was treated with 1 mM 6-mercaptohexanol solution for 10 min to block the remaining bare region and thoroughly rinsed with ultrapure water. Thus, DNA-AuNPs network/benzenedithiol/GE was prepared.

To confirm our strategy, a number of control experiments were carried out. The benzenedithiol/GE under the same conditions was prepared. S3 which can't hybridize with S1 completely was designed to prepare AuNPs-S1-S3/benzenedithiol/GE under the same incubation conditions except replacing S2 with S3. S4 with the same sequence as S2 but without thiol were designed to prepare AuNPs-S1-S4/benzenedithiol/GE with the same experimental process except replacing S2 with S4. Another way to assemble the Au-DNA network without the linkage of benzenedithiol (DNA-AuNPs network/GE) was carried out as follows. The cleaned electrode was first immersed in 400 μL Tris-HCl containing 15 μL capture DNA probe (S1), and incubated for 12 h at 37 ºC. During this process, the capture DNA probe was conjugated onto the gold electrode through the Au-S bond. After rinsing with distilled water, the modified gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris-HCl buffer, pH 7.4, for 60 min. Then the modified electrode was immersed in 400 μL S2-AuNPs solution and S1-AuNPs solution in turn.

2.5. Electrochemical detection of Dam MTase

For methylation, the electrode was incubated with 10 mL different concentrations of Dam MTase solution, which was performed in Dam MTase reaction buffer (50 mM Tris–HCl, 5 mM 2-mercaptoethanol, 10 mM EDTA, pH 7.4) containing 80 mM SAM and a varying amount of Dam MTase at 37 ^{o}C for 2 h strictly. After methylation, the electrode was washed thoroughly with ultrapure water to terminate the methylation reaction, and dried under nitrogen. Mbo I cleavage was performed at 37 ^{o}C for 2 h in Mbo I buffer (50 mM potassium acetate, 20 mM Tris–acetate, 10 mM magnesium acetate, 100 mg/mL BSA, pH 7.4) containing 50 U/mL Mbo I endonuclease. Thereafter the electrode was immersed in 10 mL of 20 mM Tris–HCl buffer (pH 7.4) containing 20 mM MB and 20 mM KCl to equilibrate for 5min before measurements. DNA-Au NPs network modified electrode without incubating with Dam MTase under the same conditions was prepared as the unmethyled DNA-AuNPs network for further control experiment.

2.6. The electrochemical experimental process

All of the electrochemical experiments were carried out at room temperature. Cyclic voltammetry was performed in Tris-HCl buffer solution (pH7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ within the potential range from 0.6 to -0.2 V using a step potential of 0.05 V at a scan rate of 50 mV/s. Electrochemical impedance spectroscopy (EIS) was performed in Tris-HCl (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and 0.1 M KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of 0.18 V. To obtain a stable and high signal, differential pulse voltammetry (DPV) measurement was conducted. Each electrochemical measurement was repeated at least 3 times under the same condition with the following parameters: initial potential, -0.50 V; final potential, 0.20 V; increment potential, 0.005 V; pulse amplitude, 0.05 s; pulse width, 0.05 s sample width, 0.0167 s; pulse period, 0.5 s; and quite time, 2 s.

3. Results and discussion

3.1 Principle of methyltransferase activity assay

Scheme 1 shows the working principle of the detection of MTase activity by the DNA-Au NPs network based signal amplified electrochemical biosensor. Dam MTase, catalyzing the N6-adenine methylation in the symmetric tetranucleotide 5'-G-A-T-C-3', was used as a model MTase [2]. The endonuclease Mbo I, which identifies the same site (a double-stranded DNA containing 5'-G-A-T-C-3' palindrome sequence) as Dam MTase, was employed to cleave the unmethylated sites on the double-stranded DNA. S1 and S2 were designed containing specific recognition sequences for Dam MTase and methylation-resistant endonuclease Mbo I. Firstly, thiolated DNA S1 and S2 were self-assembled onto Au NPs via Au–S bond to prepare S1-AuNPs and S2-AuNPs, respectively, followed by the hybridization with each other to form DNA-Au NPs network (part 1). In part 2, DNA-Au NPs network amplification unit was then successively brought onto the gold electrode surface through the linkage of benzenedithiol. In the absence of Dam MTase, the unmethylated DNA hybrid will be selectively digested by Mbo I and few electrochemical indicator MB molecules could be adsorbed on the electrode after the network was digested. Thus a relatively low electrochemical signal will be achieved. However, in the presence of MTase, after methylation event by Dam MTase, the Mbo I digestion would be blocked and the DNA-Au NPs network amplification units would remain on the electrode providing the inserting sites for the loading of large amounts of MB molecules, resulting in a high electrochemical signal. Therefore, Dam MTase activity and DNA methylation could be sensitively detected by electrochemical response of MB which can be accumulatedon DNA-Au NPs network after the recognition site was methylated by Dam MTase. In this paradigm, benzenedithiol was used to assemble the DNA-Au NPs network to simplify the incubation conditions and increase the stability and reproducibility of the biosensor. With benzenedithiol self-assembled onto the gold electrode, DNA-Au NPs network amplification unit could be successively conjugated onto the electrode by the covalent bond of –SH in benzenedithiol and Au atom in network. Harsh incubation of the capture probe with the electrode would be avoided. Thus an easily operated, signal amplified electrochemical approach for MTase activity was presented.

Scheme 1

3.2. Characterization

3.2.1. SEM and UV-vis Characterization of DNA–AuNPs network

To realize our design, one precondition for the amplification of the electrochemical signal was the formation of DNA-Au NPs network. To demonstrate this issue, we used the SEM to investigate Au NPs before and after the network formed. As seen from Fig. 1A, representative TEM image of Au NPs was shown. Fig. 1B shows the TEM image of DNA-Au NPs. In contrast, in Fig. 1C, most pure Au NPs aggregated together, Thousands of nanoparticles are linked by DNA to form a rigid network structure. The results revealed

that the hybridization reaction could be carried out successfully. The UV–vis absorption spectra were also used to characterize the Au NPs and DNA-Au NPs network. As shown in Fig. 1D,the characteristic absorption peak of gold nanoparticles was red-shifted from 520 to 523 nm due to the decoration of DNA on Au nanoparticles. When the S1-AuNPs was incubated withS2-AuNPs, the characteristic absorption peak was further shifted to 528 nm, indicating the hybridization of S1-AuNPs with S2-AuNPs. These results demonstrated the successful formation of DNA-Au NPs network.

Fig. 1

3.2.2. Electrochemical characterization of the biosensor

To verify the interface properties of the modified electrode, cyclic voltammetry was performed for each step of the working electrode modification process. As Figure 2A shows, a well-defined redox pair was observed, showing a fast electron-transfer kinetics of $Fe(CN)_6^{3-/4-}$ on the bare gold electrode (curve a). The current response decreased and the separation degree of two peaks was enlarged after the self-assembling process of benzenedithiol (curve b), caused by the electrostatic repulsion between the negatively charged benzenedithiol and $Fe(CN)_6^{3-/4-}$, indicating the successful immobilization of benzenedithiol. After the assembly of the DNA-AuNPs network, a large decrease in peak currents and enlarged peak-to-peak separation could be observed (curve d) maybe due to the large hindrance of DNA structure. Then, the electrode was treated with Dam MTase and Mbo I, the current response further decreased (curve e) resulting from the methylated effect. In the control experiment after the unmethylated DNA network modified electrode was treated with Mbo I, the current response increased (curve c) maybe due to the digestion of the DNA structure on the electrode. All these CV experimental results proved that the surface of gold electrodes have been successfully fabricated according to the principle scheme.

The EIS measurements, as an effective method for the investigation of the interface properties of modified electrodes, were performed for the study of each immobilization step [52]. The semicircle diameter of EIS, which is equal to the value of R_{et}, indicates the surface electron transfer resistance of the electrode. Fig. 2B showed the Nyquist plots of different modified electrodes in $Fe(CN)_6^{3-/4-}$ probe solution. The bare gold electrode exhibited a very small semicircle domain (curve a), attributing to a free electron-transfer process. When benzenedithiol was self-assembled onto the gold electrode, Ret increased (curve c). It was also found that the network modified electrode led to a significant increase in R_{et} (curve d). With the DNA-Au NPs network modified electrode was treated with Dam MTase for the methylation, the Ret increased a little (curve e). Furthermore, the prevention to the digestion of Mbo I has no obvious effect on the R_{et} (curve f). However, the R_{et} decreased (curve b) when the unmethylated DNA network modified electrode was treated with Mbo I, which could cleave the dsDNA. The changes in EIS were in agreement with those in the CV method, which further demonstrated the success of each modification step.

Fig.2

3.3. Feasibility investigation of the electrochemical biosensor

Differential pulse voltammetry was further used to investigate the feasibility of the electrochemical assay. The DNA-Au NPs composites on the electrode surface were firstly self-assembled and then methylated with Dam MTase, cleaved with Mbo I endonuclease, further loading MB signal molecules; finally, the current response of MB was recorded by DPV. Fig. 3A shows the DPV curves of different electrodes. The bare GE showed almost no oxidation peak (curve a in Fig. 3A). After the DNA-Au NPs networks methylated with Dam MTase and further treated with Mbo I, there was still no obvious change. However, a strong current peak was observed with MB further loaded on the network (curve d in Fig. 3A). The response was almost unchangeable compared withDNA-AuNPs network/benzenedithiol/GE loading MB molecules without the treatment of Dam MTase and Mbo I (curve c in Fig. 3A), implying that as we designed, DNA-Au NPs network has been successfully methylated by Dam MTase and then the digestion of the Mbo I was blocked by the methylated sites which kept the network structure. On the contrast, after the unmethylated network was treated with Mbo I endonuclease and MB loading solution, an inconspicuous oxidation peak was obtained (curve b in Fig. 3A). It was suggested that because Mbo I can specifically recognize the site of 5'-GATC-3' digesting the dsDNA, the network was digested and only few MB molecules could be loaded.

To investigate our proposal, the control experiment was carried out under the same conditions except with S2 replaced by S3 which can't hybridize with S1. As shown in Fig. 3B, there was no obvious response in the resulting DPV because the DNA-AuNPs network (curve a) can't be formed with AuNPs-S1 and AuNPs-S3(curve b) and thus MB indicator can't be loaded. The signal amplified role of DNA-AuNPs network/GE (Fig. 3Ccurve a) was investigated through the comparison of DNA-AuNPs network with AuNPs-S1-S4/benzenedithiol/GE (curve b). S4 has the same sequence as S2 but without thiol labelled. It could hybridize with S1 but can't form the network. As shown in Fig. 3C, the DPV response has an evident decrease on the AuNPs-S1-S4/benzenedithiol/GE after it was treated with the same process, which further proved the essential role of DNA-AuNPs network for the signal amplification. In order to prove the role of benzenedithiol, control experiment without benzenedithiol was carried out as shown in Fig. 3D. In the absence of benzenedithiol under the same conditions, the DPV shows no obvious response which resulted from the unsuccessful self-assembly of DNA-Au NPs network, implying that benzenedithiol is indispensable in our assay.

Fig.3

3.4. Dam MTase detection

The current signal was directly related to the quantity of methylated duplex target sequence 5'-GGCC-3', which was methylated by Dam MTase. The optimization assay was carried out to obtain the optimized conditions for the detection of Dam MTase as shown in Fig. S1. Then, under the optimal conditions, a series of current signals was obtained by exposing the fabricated sensor to series of Dam MTase solution with different concentrations under the same optimized

experimental conditions. As shown in Fig. 4A, with Dam MTase's concentration ranging from 0 to 40 U/mL, the current signal increased gradually. The results shown in the inset of Fig. 4B revealed a good linear relationship between the DPV peak current and the logarithm of concentrations of the Dam MTase in the range of 0.05–30 U/mL, with a correlation coefficient (R) of 0.999 and detection limit of 0.02 U/mL. The regression equation could be expressed as I (μA) = 0.64387+ 0.12125lg[C] where I is the peak current and C is the concentration of Dam MTase. As compared with other reported work in Table S1, the detection limit and linear range was favourable or compared to other methods.

Fig.4

3.5. The stability and selectivity of DNA methylation

To study the stability of our sensor, we investigated the DPV responses of the sensor as a function of time, the result tells us the current still remains about 90% in 96 h which can buttress the stability of our sensor (Fig. 5). We suggested the good stability is attributed to the benzenedithiol linkage, which is free from the effect of incubation conditions. On the contrary, if we used S1 as a capture probe to assemble DNA-Au NPs network on the electrode. As Fig. 5 shows, its stability is not a quarter as good as the use of benzenedithiol linkage we designed.

Fig.5

Next, we assessed the selectivity of our newly developed strategies, by treating the biosensor with solutions containing different DNA MTases. HaeIII MTase and M.SssI MTase were selected to evaluate the specificity of the biosensor. HaeIII MTase [53] modifies the internal cytosine residues in the sequence of symmetric tetranucleotide 5’-GGCC-3’, and M.SssI MTase methylates [54] the cytosine residues in the double-stranded symmetric 5’-CCGG-3’sequence. As shown in Figure S2, the catalytic current of Dam MTase was about 7 and 8 times higher than that of HaeIII and M.SssI, respectively. This is evidently due to the fact that the CpG dinucleotide site in the Mbo I endonuclease recognition sequence (5’-GATC-3’) cannot be methylated by the HaeIII and M.Sss I cytosine MTase. Consequently, the dsDNA-Au NPs network was cleaved off from the electrode surface in the Mbo I incubation step. Therefore, the proposed assay exhibited good selectivity toward Dam MTase over HaeIII MTase and M.Sss I MTase. Further, we compared the effect of Dam MTase with that of three other proteins BSA, tyrosinase and Klenow polymerase under identical conditions. As shown in Fig. 6, in the presence of Dam MTase, the current signal was remarkably increased. On the other hand, there was no current signal observed when other three proteins were used, respectively. These results indicated outstanding specificity and selectivity against other proteins of this biosensor.

Fig. 6

3.7. Analysis of complex biological samples

To evaluate the practical applicability of the proposed method in complex biological samples,

10% diluted human serum samples spiked with different concentrations of Dam MTase were investigated. As listed in Table 1, the recoveries of the three samples including 0.1U/mL, 1.0U/mL and 10 U/mL Dam MTase were 107%, 102%, and 99.6% with RSD of 3.8%, 4.2% and 2.4%, respectively. These results indicated that the proposed method held great potential for the accurate quantification of Dam MTase in complex biological samples

Table 1

4. Conclusions

Described here is a signal-on electrochemical assay for the sensitive detection of DNA methyltransferase activity. The present study has introduced method using benzenedithiol to combine electrode and DNA-Au NPs network which avoided the harsh incubation of electrode with DNA solution. Taking the signal amplification of DNA-Au NPs network and the electroactivity of MB that combined with dsDNA, a relatively lower detection limit of 0.02 U/mL and a wider linear range from 0.05 to 30 U/mL were achieved. Compared to other electrochemical methods, our method was time-efficient, simple, selective and sensitive. Widely accessible approaches, like the electrochemical assay presented here, will be necessary to meet this demand for cancer detection and treatment.

conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

This work was financially supported by the projects (21371007) from National Natural Science Foundation of China, Anhui Provincial Natural Science Foundation (1208085QB28), Anhui Provincial Natural Science Foundation for Distinguished Youth (1408085J03), Natural Science Foundation of Anhui (KJ2012A139) and the Program for Innovative Research Team at Anhui Normal University.

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Figure Captions

Scheme 1 The schematic representation of the electrochemical method for the assay of MTase activity using DNA-Au NPs network amplification.

Fig. 1. TEM images of Au NPs (A), ssDNA-AuNPs(B),DNA-Au NPs network (C); (D) UV–vis absorption spectra of Au NPs (curve a), DNA S1-AuNPs (curve b)and DNA-Au NPs network (curve c).

Fig.2. (A) Cyclic voltammetry ofbare GE (a),benzenedithiol/GE (b)DNA-AuNPs network/benzenedithiol/GE treated with Mbo I (c), DNA-AuNPs network/benzenedithiol/GE (d), DNA-AuNPs network/benzenedithiol/GE treated with Dam MTase and Mbo I (e); (B) Nyquist plots obtained from different electrodes of bare GE (a), DNA-AuNPs network/benzenedithiol/GE treated with Mbo I (b), benzenedithiol/GE (c), DNA-AuNPs network/benzenedithiol/GE (d), AuNPs network/benzenedithiol/GE treated with Dam MTase (e), AuNPs network/benzenedithiol/GE treated with Dam MTase and Mbo I (f)in5mM $[Fe(CN)_6]^{3-/4-}$ solution

Fig.3. DPV response of (A) (a) bare Au electrode, (b) DNA-AuNPs network modified electrode treated with Mbo I, (c) DNA-AuNPs network modified electrode, (d) the modified electrode treated with Dam MTase and Mbo I. Data are recorded in the 0.1 M Tris-HCl buffer (pH 7.3) containing 0.3 M NaCl within the range of 0.1 Hz to 100 kHz, the amplitude of the alternate voltage was 5 mV, (B) AuNPs-S1-S3/benzenedithiol/GE, (C) AuNPs-S1-S3/benzenedithiol/GE and (D) GE modified with DNA-Au NPs network without benzenedithiol for the assay of MTase activity

Fig.4. (A) DPV response of different concentrations of Dam MTase. The curves from bottom to the top were obtained with different activities: 0.05, 0.75, 2.5, 5, 10, 20, 30, 40U/ml. The LOD base on S/N = 3 is 0.12 U/ml. (B) The response curve obtained with different concentrations of Dam MTase. Inset showed linear relationship between the current and the concentrations of the Dam MTase from 0.05U/mL~30U/mL.

Fig.5. DPV response of different incubation time of DNA methylation: 2h, 6h, 12h, 24h, 36h, 96h with benzenedithiol (A) or capture DNA (B)

Fig.6. Selectivity studies. (1) Dam MTase, (2) BSA, (3) tyrosinase, (4) Klenow polymerase, and (5) a mixture of the four proteins. (BSA)=5μM, the concentration of other three enzymes was 50 U/mL.

Table 1. Detection of DNA MTase in human serum samples using the proposed method.

Scheme 1

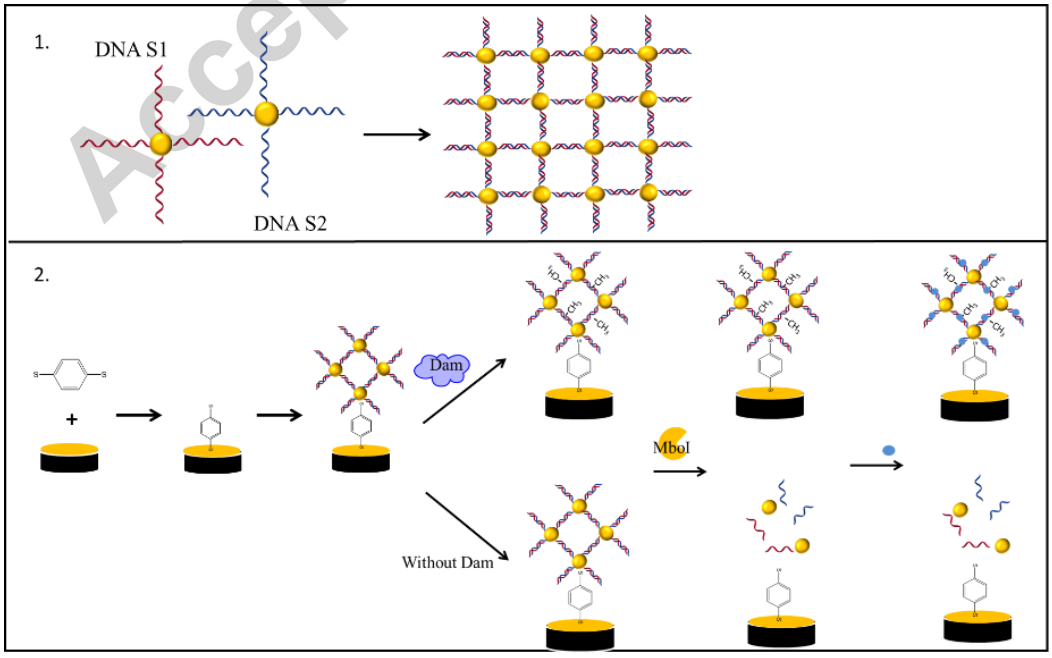


Fig. 1

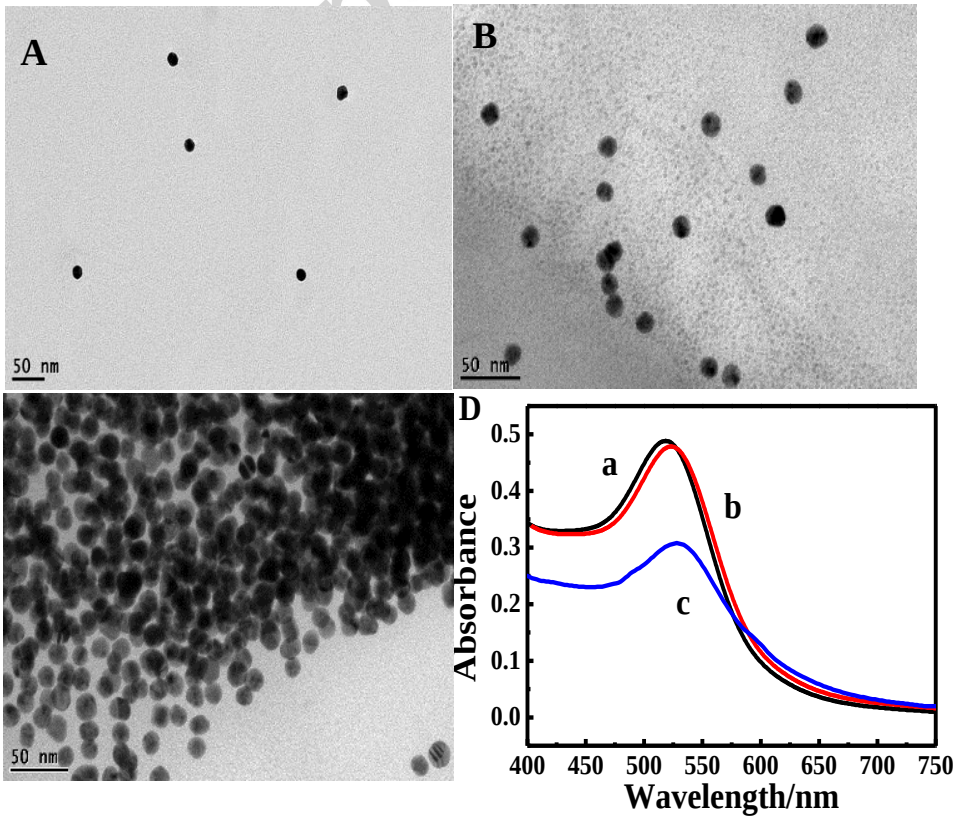


Fig. 2

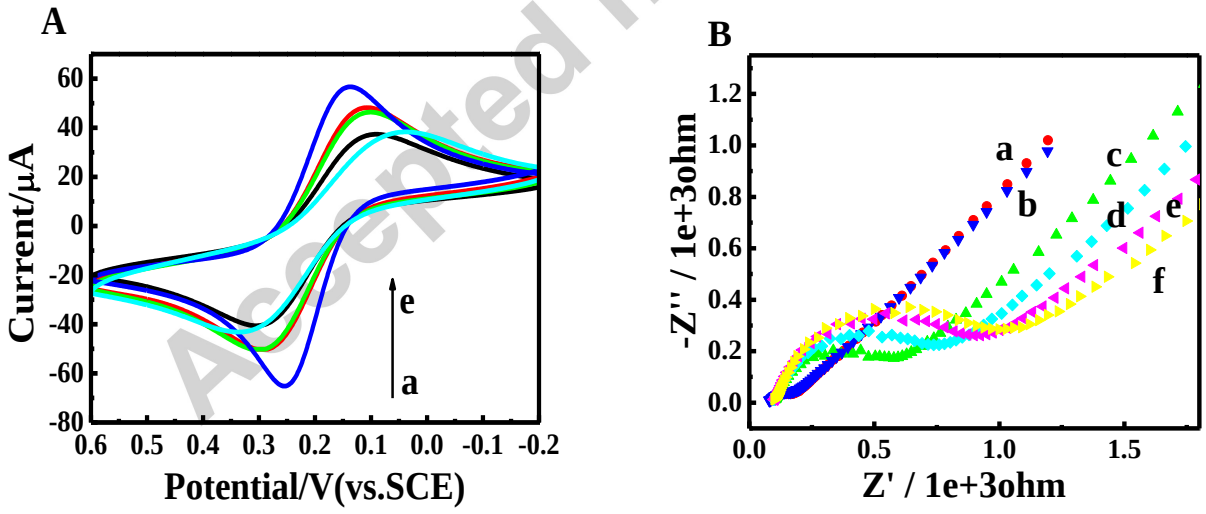


Fig.3

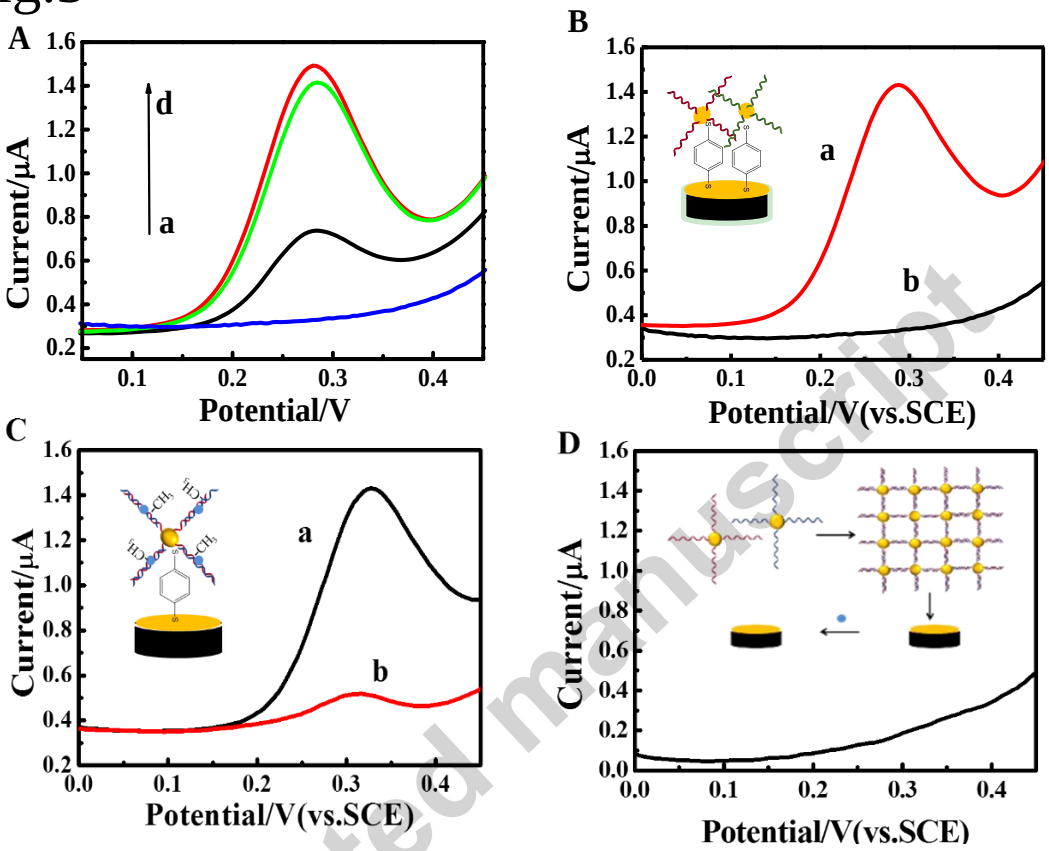


Fig.4

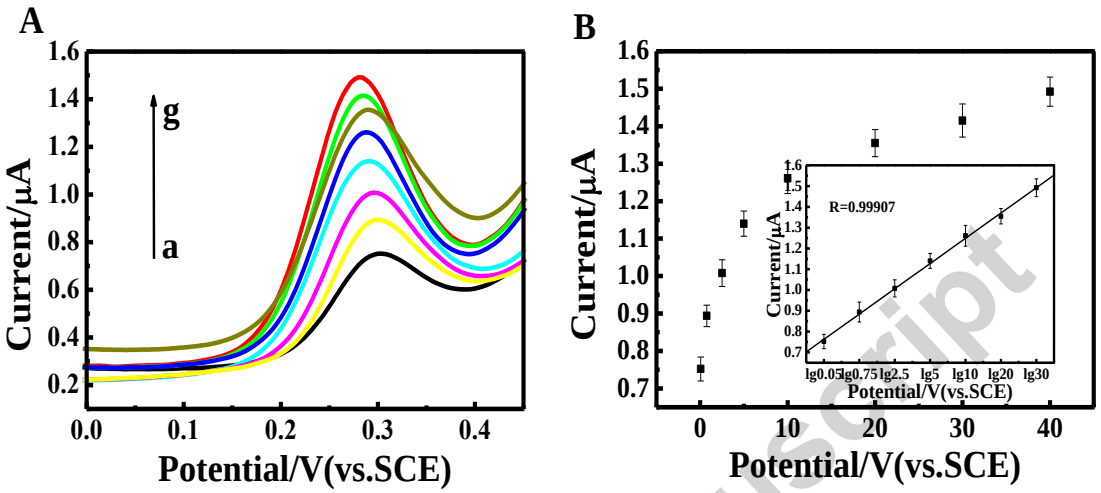


Fig.5

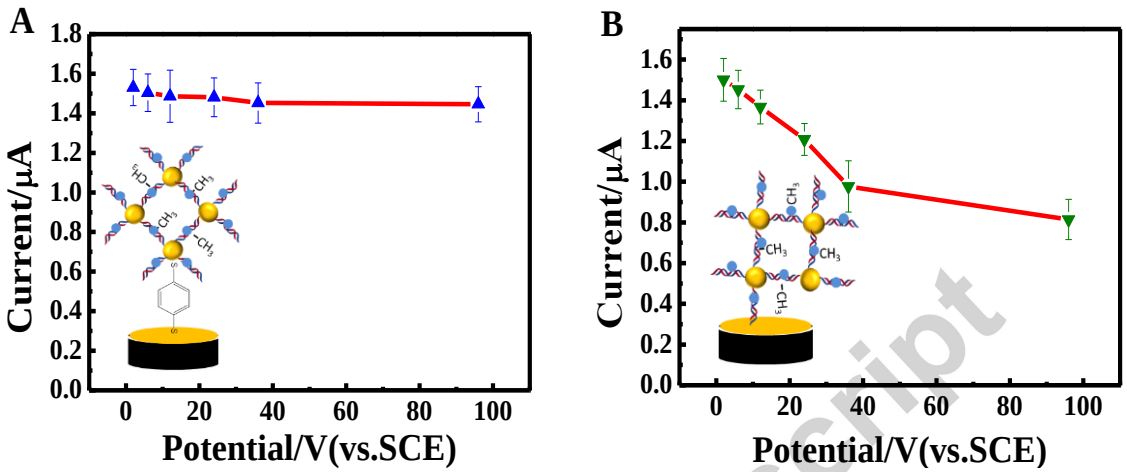


Fig.6

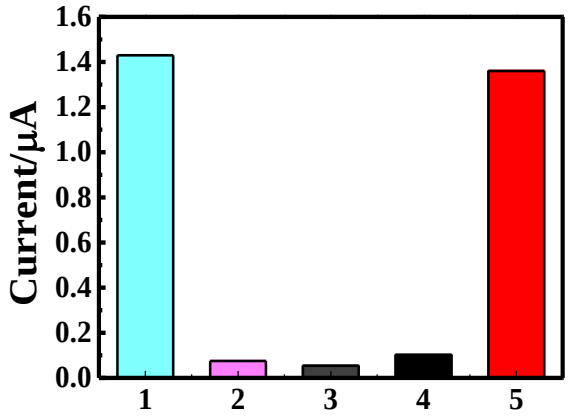
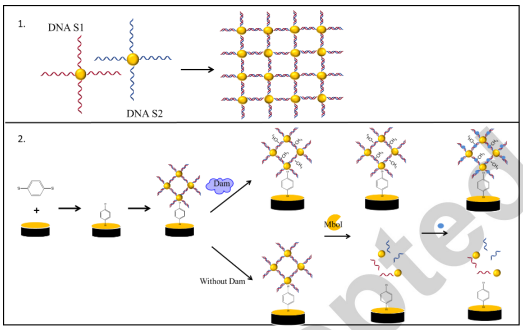


Table 1.

Sample Number	Added(U/mL)	Found (U/mL)	Recovery (%)	RSD (%, n=3)
1	0.10	0.107	107	3.8
2	1.0	1.02	102	4.2
3	10	9.96	99.6	2.4

Table of entry



Benzenedithiol Linked DNA-Gold Nanoparticles Network Based Electrochemical Biosensors for DNA MTase Activity

Highlight

1. DNA-gold nanoparticles network was as signal amplification unit.
2. An easy assembly method by the linkage of benzenedithiol bridge.
3. It avoided the direct incubation of the solid electrode with the DNA.
4. A highly sensitive DNA methyltransferase activity assay was fabricated.