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Sensitive electrochemical detection of hydroxyl radical with biobarcode amplification

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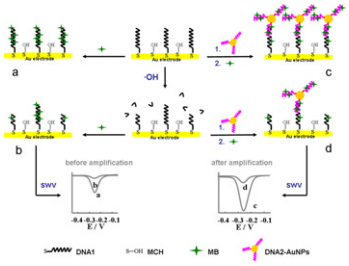
HIGHLIGHTS

- A DNA-based biosensor for amplified electrochemical detection of •OH was developed.
- The sensor shows good analytical performance for sensitive detection of •OH.
- The biosensor has potential application in the evaluation of antioxidant capacity.

GRAPHICAL ABSTRACT



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ABSTRACT

By using biobarcode nanoparticles, we have successfully constructed a DNA-based biosensor for amplified electrochemical detection of hydroxyl radical (•OH). Thiolated DNA1 (SH-DNA1) was firstly immobilized on the planar gold (Au) electrode. •OH generated from Fenton reaction could induce serious oxidative damage of the DNA layer adsorbed on the electrode surface, which was monitored by an intercalating probe, methylene blue (MB). In order to enhance the sensitivity of the biosensor, DNA2-functionalized Au nanoparticles (DNA2-AuNPs) were used to amplify the response signal. The developed DNA-based biosensor could detect •OH quantitatively with wide linear range up to 10 mM and low detection limit of 3 μM, and exhibited satisfactory selectivity. On the other hand, this electrochemical biosensor could have potential application in the evaluation of antioxidant capacity.

1. Introduction

Many metals are considered as essential trace elements and must be present in the human body with low concentration for normal cellular function. However, high concentrations of metals in the body are thought to be deleterious. Some transition metals have been confirmed to be human carcinogens or possibly carcinogenic to humans. It was reported that these metal ions induced their toxic effects primarily through their ability to produce reactive oxygen species (ROS) [1]. Among them, iron is the most abundant transition metal in biological systems. Fe^{2+} has been found to react with H_2O_2 to produce the extremely reactive hydroxyl radical (•OH), the so-called Fenton reaction [2]. •OH, belonging to the most reactive one of ROS, is primarily responsible for cellular disorders and cytotoxic effects that can be traced back to the oxidative damage to DNA [3,4], RNA [5], proteins [6,7], or lipids [8]. It is well known that, under normal physiological conditions, •OH is in a rather low physiological concentration. However, in response to traumatic brain injury ischemia-reperfusion, hypoxia, and environmental stresses, the concentration of •OH may increase significantly. So, it is highly desirable to develop a simple, rapid and sensitive method to detect •OH with a wide dynamic linear range.

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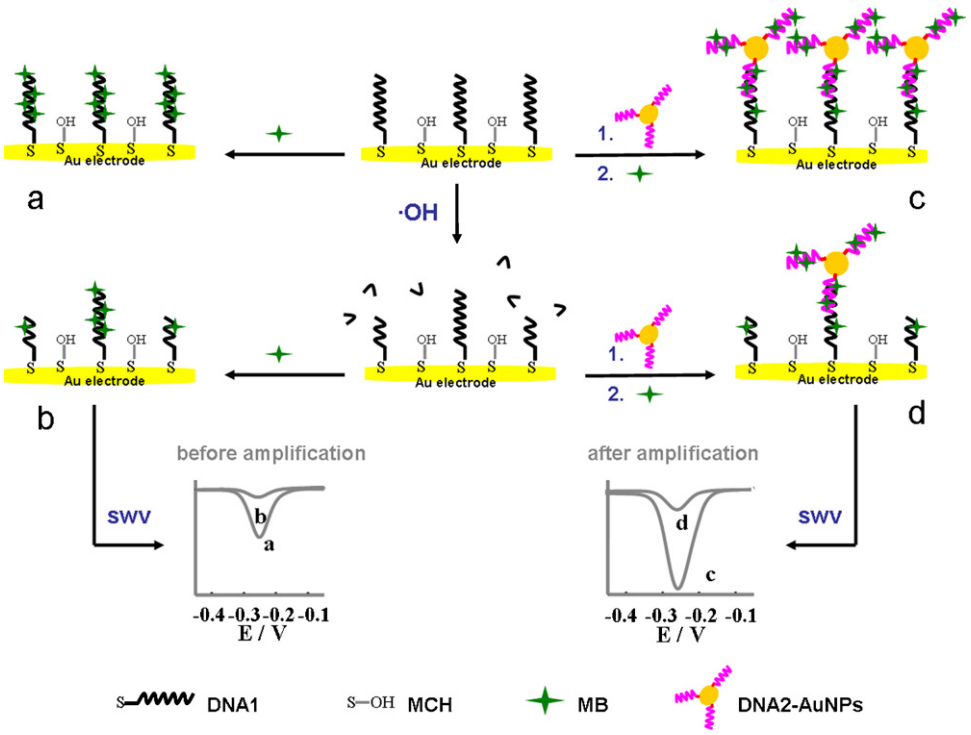

Fig. 1. Schematic illustration of the biobarcode amplified DNA-based electrochemical biosensor for •OH detection.

Due to the key role of DNA in life processes, ROS-mediated damage of DNA has received more and more attention [9–11]. Up to now, numerous powerful methods related to the oxidative damage of DNA have been reported, including fluorescence [12], capillary zone electrophoresis [13], single cell gel electrophoresis [14], HPLC-ECD [15–17], GC/MS [18,19], ^{32}P-postlabeling [20], colorimetric [21] and photoelectrochemical [1,22] methods. All these methods are laborious, time-consuming, or involve high-cost equipments. In contrast, electrochemical methods have received great interests because of their simplicity, fast response, relatively cheap cost, high sensitivity and low power requirement [23,24]. Recently, Barroso [25] modified carbon paste electrode with adenine-rich oligonucleotide (dA_{21}) to construct an electrochemical sensor for the determination of total antioxidant capacity in beverages, based on the oxidative damage of dA_{21} by •OH. Liu et al. [26] presented a TiO_2/ITO electrode in which the TiO_2 layer played the role of photoanode for •OH generation and DNA oxidation damage was evaluated by monitoring the electrochemical reduction current of the intercalated redox probe (methylene blue, MB). Wang and Jiao [27] developed an electrochemical biosensor to evaluate the DNA damage by incorporating the mixture of MCNTs and Fe@Fe_2O_3 nanonecklace into a membrane containing poly(dimethyldiallylammonium chloride) (PDDA) and intact DNA, and •OH was produced by electro-Fenton process. However, the object of these electrochemical strategies is the evaluation of the damage degree of DNA or the capacity of antioxidant, little attention has been paid to develop the electrochemical DNA biosensors for sensitive detection of •OH which is very important for human health.

Herein, a simple strategy based on a Fenton-mediated DNA damage system for highly selective and sensitive electrochemical detection of •OH was demonstrated. To improve the sensitivity and lower the detection limit of this biosensor, DNA functionalized AuNPs were used as the signal amplifier [28,29]. As shown in Fig. 1, a mixed layer of thiolated DNA1 and 6-mercaptohexanol (MCH) was firstly self-assembled onto a planar Au electrode. When the modified electrode was immersed into a Fenton solution containing Fe^{2+} and H_2O_2, DNA1 on the electrode surface was oxidatively damaged by •OH. This phenomenon could be monitored by the electrochemical probe (MB), and used to sensitively detect •OH. The response signal can be further amplified by DNA2-AuNPs. The resulted electrochemical biosensor (DNA2-AuNPs/MCH/DNA1/Au electrode) showed a wide linear range up to 10 mM with a detection limit of 3 μM, and satisfactory selectivity.

2. Materials and methods

2.1. Chemicals

Two oligonucleotides used in this paper were purchased form Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. and purified by PAGE method. Sequences of these two oligonucleotides are listed as follows: DNA1: 5′-SH-$(CH)_6$-GG**T CCG CTT GCT CTC GC**-3′; DNA2: 5′-SH-$(CH)_6$-CGG **GCG AGA GCA AGC GGA**-3′. DNA1 contains complementary sequence (bold) to DNA2. MCH was purchased from Sigma. MB was from Tianjin Hengxing Chemical Reagent manufacturing Co. Ltd. H_2O_2 (30%, w/v) was from Xilong Chemical Co. Ltd. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Beijing Solarbio Science and Technology Co. Ltd. All other chemicals were of analytical grade and used without further purification. Aqueous solutions used throughout were prepared with ultra pure water (>18 MΩ cm) obtained from a Millipore system.

On the other hand, phosphate buffer solution containing 20 mM NaCl (PBS, 50 mM, pH 7.4) was used throughout, except the specific statement. $^1O_2$ was obtained by the addition of NaClO to H_2O_2 (10:1 mol mol^{-1}) and KO_2 in ultra pure water was used to generate $O_2^{\bullet -}$.

2.2. Apparatus

The morphology of the DNA2-AuNPs modified electrode was investigated by field emission scanning electron microscopy (JSM 6700, Joel, Japan). All electrochemical measurements were

performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a planar Au electrode (2 mm in diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All experiments were carried out at room temperature.

2.3. Preparation of AuNPs and DNA2-functionalized AuNPs

AuNPs with the diameter of about 13 nm were prepared by the reduction of $HAuCl_4$ in citrate solution according to the literature [30]. Briefly, 5 mL of 38.8 mM sodium citrate was added to 50 mL of 1.0 mM $HAuCl_4$ refluxing solution under stirring, and the mixture was kept boiling for another 15 min. The solution color turned to wine red, indicating the formation of AuNPs. Then, the solution was cooled down to room temperature with continuous stirring. The prepared colloid AuNPs were stored in brown glass bottles at 4 °C.

The DNA2-functionalized AuNPs (DNA2-AuNPs) were prepared as follows: 1.5 mL of the above AuNPs solution was mixed with the thiolated DNA2 (1 OD) overnight and then slowly brought to 10 mM phosphate buffer containing 0.1 M NaCl (pH 7.0) and allowed to be incubated for 40 h. Subsequently, the DNA2-AuNPs conjugate was collected by centrifugation at 16,000 rpm for 30 min. The red precipitate was washed, centrifuged, and dispersed in 1.5 mL of 25 mM Tris–HCl buffer solution containing 0.3 M NaCl (pH 8.2) and stored at 4 °C.

2.4. Preparation of the electrochemical DNA-based sensor

Prior to DNA immobilization, the gold electrode was carefully polished to mirror-like surface with 0.5 and 0.05-μm alumina slurries, followed by ultrasonication in ultra pure water and methanol. The electrode was then pretreated electrochemically in 0.5 M H_2SO_4 by potential cycling in the potential range of −0.3 to 1.5 V at a scan rate of 100 mV s^{-1} until the cyclic voltammogram characteristic for clean Au electrode was obtained. Then, the Au electrode was washed thoroughly with copious amount of ultra pure water and dried under nitrogen gas.

The immobilization of thiol-terminated DNA1 was carried out as follows: 10 μL of 1.0 μM DNA1 solution (in 10 mM Tris–HCl buffer, 1 M NaCl, pH 7.4) was dropped onto the surface of the cleaned Au electrode and incubated for 16 h, and a self-assembled monolayer (SAM) of DNA1 was formed on the electrode surface. Then, the electrode was incubated in 1 mM MCH solution for 1 h to remove nonspecific DNA adsorption on the electrode surface. The MCH/DNA1/Au electrode was rinsed thoroughly with ultrapure water and dried with a stream of nitrogen. For signal amplification, the MCH/DNA1/Au electrodes treated or untreated by Fenton solution were further modified with DNA2-AuNPs. 20 μL of DNA2-AuNPs solution was dropped on the surface of the MCH/DNA1/Au electrode and incubated for 2 h to carry out the hybridization between DNA2 and DNA1. After every step, electrode was rinsed with washing buffer (10 mM Tris–HCl, pH 7.4) and dried under a stream of nitrogen prior to electrochemical measurement. To monitor each immobilization step, the electrochemical impedance measurements were performed in 0.1 mol L^{-1} KCl aqueous solution with 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ as the probe.

2.5. Electrochemical detection of •OH

The DNA modified electrode was immersed in Fenton solution (an aqueous solution containing $FeSO_4$ and H_2O_2 at the molar ratio 1:6, pH 3.0) for 1 h. The DNA strand was attacked by •OH which was generated by the Fenton reaction. Then, the electrode was taken out, rinsed with ultra pure water and immersed in 50 mM PBS buffer (pH 7.4) with 20 μM MB for 5 min. After accumulation of MB, the electrode was rinsed with 50 mM PBS buffer for three times. The square wave voltammetry (SWV) of the electrode was recorded in 50 mM PBS (pH 7.4) and the SWV response differences between the electrodes treated and untreated with Fenton solution depended on the •OH concentration. Each measurement was repeated at least three times.

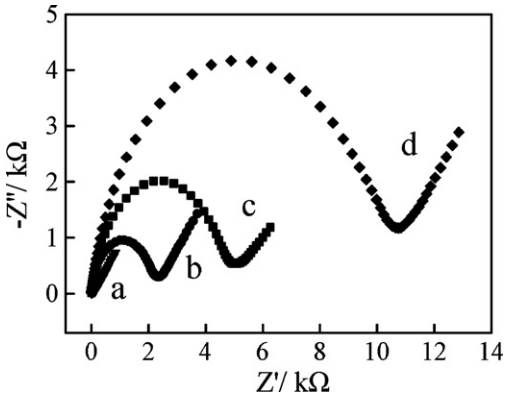

Fig. 2. Electrochemical impedance spectra (Nyquist plots) of the bare Au (a), DNA1/Au (b), MCH/DNA1/Au (c), DNA2-AuNPs/MCH/DNA1/Au (d) electrodes in 0.1 M KCl aqueous solution containing 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ as the redox probe.

3. Results and discussion

3.1. Characterization of the modified electrode

Fig. 2 shows the electrochemical impedance results of the bare Au (a), DNA1/Au (b), MCH/DNA1/Au (c) and DNA2-AuNPs/MCH/DNA1/Au (d) electrodes. In the Nyquist plots of impedance spectra, a linear section at the lower frequencies is attributed to a diffusion-limited process, and a squeezed semicircle portion observed at higher frequencies would correspond to the electron-transfer limited process. The increase of the diameter of the semicircle reflects the increase of the interfacial charge-transfer resistance (R_{et}) [31,32]. It is noted that the bare Au electrode shows a very small semicircle domain (R_{et} = 150 Ω, Fig. 2, curve a), indicating a very fast electron-transfer process of $[Fe(CN)_6]^{3-/4-}$ [31]. The self-assembly layer of negatively charged DNA1 on the Au electrode surface effectively repels the $[Fe(CN)_6]^{3-/4-}$ anions and thus leads to an enhanced electron-transfer resistance (R_{et} = 2400 Ω, Fig. 2, curve b) [33]. It is also found that the assembly of MCH on the DNA1-modified electrode leads to a significant increase in R_{et} (from 2400 to 5100 Ω, Fig. 2, curve c). After hybridization with DNA2-AuNPs, there is a large increase in R_{et} (from 5100 Ω to 11,000 Ω, Fig. 2, curve d), implying that the introduction of DNA2-AuNPs greatly inhibits the electron transfer of the redox probe on the electrode surface [30]. All these experimental results demonstrate that the sensing interface has been fabricated successfully.

3.2. SWV detection of •OH without AuNPs Amplification

The detection of •OH was carried out on the MCH/DNA1/Au electrode by using MB as the probe without DNA2-AuNPs amplification. MB can bind into DNA by electrostatic interaction with negatively charged backbone (phosphate groups) of DNA, direct interaction with guanine bases or intercalation between G–C base sequences [34,35]. Fig. 3 shows the reduction currents of MB at the MCH/DNA1/Au electrodes before (a) and after (b) incubation with Fenton reagent (1 mM Fe^{2+} and 6 mM H_2O_2) for 1 h. It is noted that a remarkable reduction peak can be observed at −0.252 V on the MCH/DNA1/Au electrode (Fig. 3, curve a), while very small

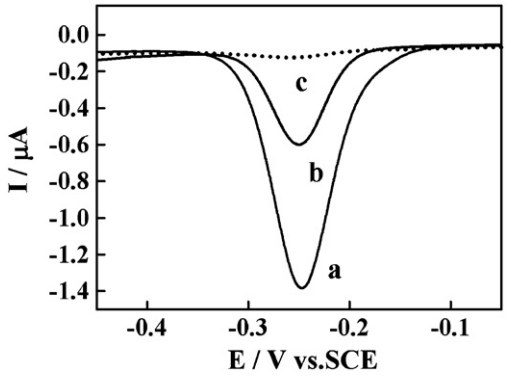

Fig. 3. SWV curves of MB in 50 mM PBS (pH 7.4) obtained on the MCH/DNA1/Au electrodes before (a) and after (b) incubation with Fenton solution (1 mM Fe^{2+} and 6 mM H_2O_2). The dotted line (c) presents the SWV curve obtained on the MCH/Au electrode.

reduction peak appears on the MCH/Au electrode (Fig. 3, curve c). This suggests that the reduction peak should result from the MB molecules bound in the DNA1 layer. On the other hand, after the MCH/DNA1/Au electrode is treated with 1 mM Fe^{2+}/6 mM H_2O_2 Fenton solution, the reduction peak current of MB decreases from −1.305 to −0.508 μA (Fig. 3, curve b). In order to further investigate the role of Fe^{2+} or H_2O_2, the following control experiments were carried out: the MCH/DNA1/Au electrode was treated with 1 mM Fe^{2+} or 6 mM H_2O_2 solution, then the reduction current of MB on the electrode was recorded. It is noted that no decrease of the reduction peak current can be observed obviously (data not shown). These results indicate that the above decrease of the reduction peak current of MB molecules should result from the serious oxidative damage of DNA by •OH, and the biosensor (MCH/DNA1/Au electrode) may be used to detect the concentration of •OH.

Fig. 4(A) shows the SWV current responses of MB at the MCH/DNA1/Au electrodes after treated with different concentrations of •OH. It is well known that the concentration of •OH is equal to that of Fe^{2+} in the Fenton solution. Herein, the concentration of •OH is expressed as that of Fe^{2+} approximatively. It is observed clearly that the reduction peak current of MB decreases with the increase of the concentration of •OH. Fig. 4(B) shows more clearly the relationship between the difference of the reduction peak current of MB and the concentration of •OH. It is noted that a linear range is from 50 μM to 10 mM with a linear correlation $R = 0.9961$ and the detection limit is 25 μM.

3.3. SWV detection of •OH with AuNPs amplification

It is expected that the application of DNA-functionalized AuNPs can further enhance the sensitivity of the present sensing strategy. Therefore, an electrochemical biosensor with DNA2-AuNPs amplification was fabricated. Fig. 5 shows the SWV current responses of MB at the DNA2-AuNPs/MCH/DNA1/Au (a) and MCH/DNA1/Au (b) electrodes. It can be observed that the peak current of MB enhances obviously after the MCH/DNA1/Au electrode is modified with DNA2-AuNPs. In addition, by integration of the area of the reduction current peak, the charge of the surface-bound MB can be calculated. It is noted that the charge of the surface-bound MB on the DNA2-AuNPs/MCH/DNA1/Au electrode is 2.17×10^{-7} C, 2.3 times higher than that on the MCH/DNA1/Au electrode (9.33×10^{-8} C). This quantitatively substantiates that the amount of the surface-bound MB on the DNA2-AuNPs/MCH/DNA1/Au electrode is much higher than that on the MCH/DNA1/Au electrode and implies the role of DNA2-AuNPs as the signal amplifier. It is believed that the introduction of DNA2-AuNPs is effective to improve the sensitivity of this electrochemical biosensor in the detection of •OH.

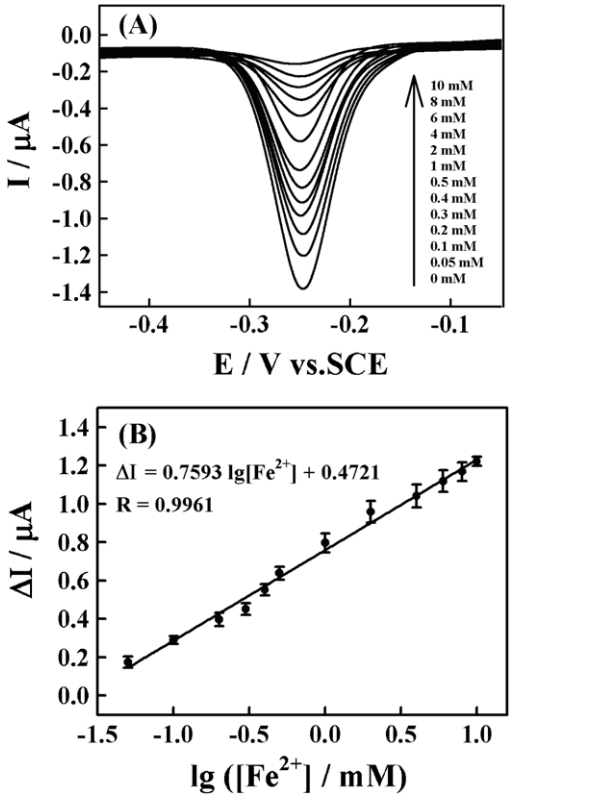

Fig. 4. (A) SWV curves of MB in 50 mM PBS (pH 7.4) obtained on the MCH/DNA1/Au electrodes after treatment in Fenton solutions with different concentration of Fe^{2+}. (B) The linear fit plot of current difference (ΔI) as a function of the logarithm of Fe^{2+} concentration. $\Delta I = I - I_0$, I_0 and I are the peak currents of MB obtained on the MCH/DNA1/Au electrodes before and after treated with Fenton solutions with different concentration of Fe^{2+}, respectively.

The amplifying effect of DNA2-AuNPs in the detection of •OH is further confirmed directly by the morphology analysis of the electrode. Fig. 6(A) shows the scanning electron microscopy (SEM) images of the DNA2-AuNPs/MCH/DNA1/Au electrode. It is noted that lots of AuNPs with diameter of about 13 nm are distributed on the electrode surface. However, when the MCH/DNA1/Au electrode was treated with Fenton solution and then to hybridize with DNA2-AuNPs, the amounts of AuNPs on the electrode surface decreases obviously (Fig. 6(B)). The SEM results indicate clearly that the DNA1 strand on the MCH/DNA1/Au electrode is damaged by •OH from Fenton reaction, resulting in less DNA2-AuNPs hybridized with DNA1. Because DNA2-AuNPs can bind lots of MB molecules, the

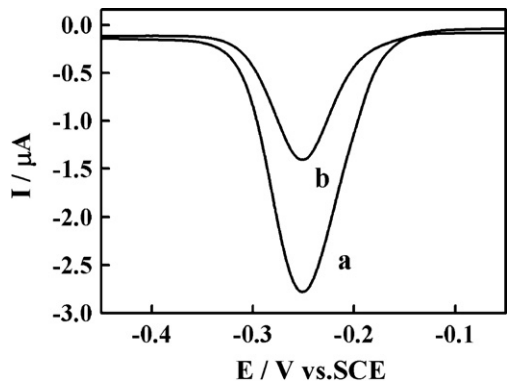

Fig. 5. SWV curves of MB in 50 mM PBS (pH 7.4) obtained on the DNA2-AuNPs/MCH/DNA1/Au (a) and MCH/DNA1/Au (b) electrodes.

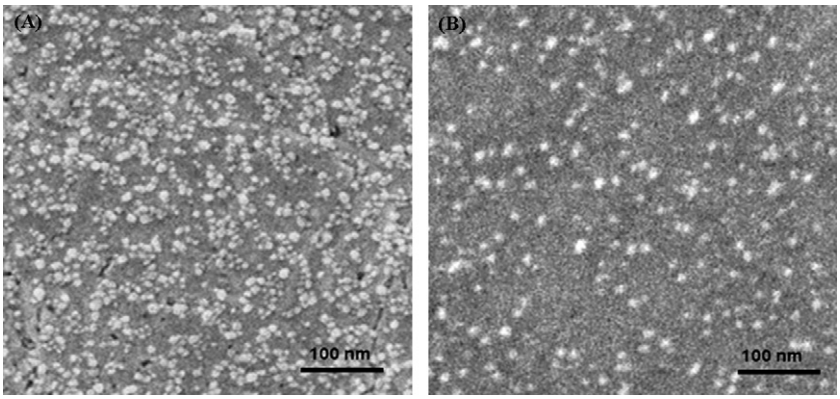

Fig. 6. SEM images of the MCH/DNA1/Au electrodes treated without (A) and with (B) Fenton solution (1 mM Fe^{2+} + 6 mM H_2O_2) and then hybridized with DNA2-AuNPs.

sensitivity of the MCH/DNA1/Au electrode for •OH detection should be improved obviously by using DNA2-AuNPs as an amplifier.

Fig. 7 shows the reduction current responses of the DNA2-AuNPs/MCH/DNA1/Au electrodes to different concentration of •OH. The linear response range is from 5 μM to 10 mM (R = 0.9940) (Fig. 7(B)), which is much wider than that obtained on the MCH/DNA1/Au electrode and some reported fluorescence strategies [12,36,37]. On the other hand, the detection limit is 3 μM, which is 8 times lower than that on the MCH/DNA1/Au electrode, and also much lower than the detection limit of 1 mM [22] and 10 μM [1] obtained on some photoelectrochemical biosensors.

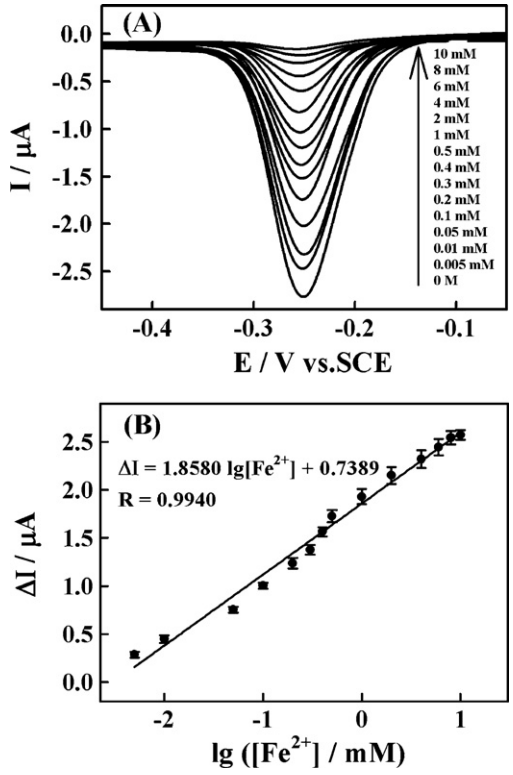

Fig. 7. (A) SWV curves of MB in 50 mM PBS (pH 7.4) obtained on the DNA2-AuNPs/MCH/DNA1/Au electrodes after treatment in Fenton solutions with different concentration of Fe^{2+}. (B) The linear fit plot of current difference (ΔI) as a function of the logarithm of Fe^{2+} concentration. $\Delta I = I - I_0$, I_0 and I are the peak currents of MB obtained on the DNA2-AuNPs/MCH/DNA1/Au electrodes before and after treated with Fenton solutions with different concentration of Fe^{2+}, respectively.

3.4. Selectivity, reproducibility and stability of the DNA2-AuNPs/MCH/DNA1/Au electrode

On account of the existence of competing ROS, the selectivity of the DNA2-AuNPs/MCH/DNA1/Au electrode for •OH detection has also been evaluated. From Fig. 8, it is noted that 15.7, 4.2 and 5.7% current responses are obtained for 10 mM superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and single oxygen ($^1O_2$) relative to 1 mM •OH, respectively. These indicate that the proposed strategy has a satisfactory selectivity.

The reproducibility of the DNA2-AuNPs/MCH/DNA1/Au electrode was investigated. Five modified electrodes were used to detect •OH and the relative standard deviation is 3.8%. Furthermore, the DNA2-AuNPs/MCH/DNA1/Au electrode was stored in ultra pure water at 4 °C for two weeks and the response current of MB has no significant change. These demonstrate that the developed electrochemical DNA-based biosensor has satisfactory reproducibility and stability.

3.5. Potential application in the evaluation of antioxidant capacity

AA, one of the effective hydroxyl radical scavengers, plays a great important role in protecting DNA from oxidative damage [38] and is used as the model to investigate the potential application of the DNA2-AuNPs/MCH/DNA1/Au electrode in the evaluation of antioxidant capacity. The corresponding results are shown in Fig. 9. As shown above, the response current of the electrode decreases obviously when the electrode is treated with Fenton solution (Fig. 9, curve c). However, when 5 mg L^{-1} AA exists in the 1 mM Fenton

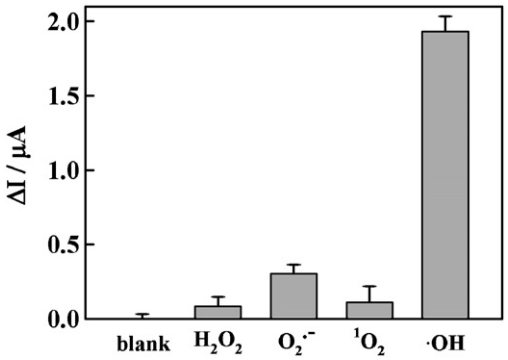

Fig. 8. The selectivity of the DNA2-AuNPs/MCH/DNA1/Au electrode. $\Delta I = I - I_0$, I and I_0 are the peak currents of MB obtained on the DNA2-AuNPs/MCH/DNA1/Au electrodes treated and untreated with different ROS, respectively. Concentration of •OH was 1 mM, and that of the other ROS was 10 mM.

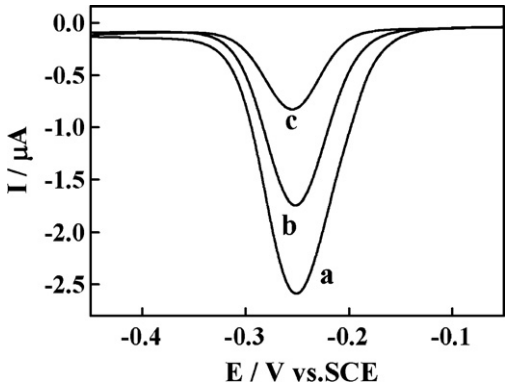

Fig. 9. SWV curves of MB in 50 mM PBS (pH 7.4) obtained on the DNA2-AuNPs/MCH/DNA1/Au electrodes untreated (a) and treated with 1 mM Fenton reagent (Fe^{2+}/H_2O_2 1:6 mol mol^{-1}) in the presence (b) and absence(c) of 5 mg L^{-1} AA.

solution, the response current of the electrode recovers obviously (Fig. 9, curve b). This implies that the proposed electrode has potential application in the evaluation of antioxidant capacity.

4. Conclusion

A DNA-based biosensor for amplified electrochemical detection of •OH was developed by using DNA2-AuNPs as the amplifier. The electrochemical DNA biosensor exhibits excellent analytical performance, such as wide linear range (from 5 μM to 10 mM), low detection limit (3 μM) and good selectivity. Moreover, the proposed strategy demonstrates the potential application in the evaluation of antioxidant capacity. We expect this strategy will have important applications in a wide range of biological and biomedical filed, as well as in the high-throughput screening of antioxidants.

Acknowledgments

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