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An Efficient Electrochemical Biosensor for Silver Ion Detection Using Hydrogen Peroxide as a Redox Indicator

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Abstract. The strong and specific binding of two DNA cytosine bases by silver ion (C-Ag^+-C) was applied to develop an efficient electrochemical biosensor for the detection of silver ion in aqueous solution. As a redox indicator, the hydrogen peroxide worked to generate a readable electrochemical signal. Thiolated short oligonucleotide strands containing 5 cytosine bases served as probe and self-assembled via Au–S bonding on gold electrode. In the presence of Ag^+, the specific coordination between Ag^+ and cytosine bases resulted in more stable and porous arrangement of oligonucleotide strands. Hydrogen peroxide could adsorb onto the surface of gold electrode and produce an electrochemical signal. The cyclic voltammetry shows a linear correlation between the signal and the concentration of Ag^+ over the range 0-0.2 μM (R^2 = 0.9955) with a detection limit of 30 nM. The length of probe DNA has no significant impact on the sensor performance. This biosensor is simple, economical and reusable with good sensitivity and selectivity. We also validated the practicality for the determination of Ag^+ in real water samples.

Introduction

Silver ion (Ag^+) is toxic and harm environments and natural source. Therefore, monitoring Ag^+ is important for the environment and human health [1]. Based on the unique coordinate interaction between Ag^+ and biscytosine, plenty of electrochemical sensors for Ag^+ have been reported, presenting many advantages over traditional methods. Researchers have proposed modified fluorescent Ag^+ sensors, which are sensitive but also expensive [2,3]. Other electrochemical detection methods to improve sensitivity and signal-to-noise ratio were developed, including various indicators or auxiliaries, such as nanoparticles and biomolecules, which are effective but difficult or costly to prepare [4,5]. Hydrogen peroxide (H_2O_2) is strong oxidizing agent as well as a metabolin of many biomolecules [6]. So far, no one has used H_2O_2 as redox indicator for electrochemical detection. In this study, H_2O_2 is capable to transfer electrons on the surface of the electrode, provoking electrical signals. When thiolated poly-C DNA probes are self-assembled through Au-S bonding on gold electrode, the vertical-parallel ss-DNA strands effectively prevent the adsorption of H_2O_2 due to its oversize. By employing this principal, we demonstrated that this biosensor was not only efficient but also simple, economical and reusable.

Experimental

Materials. Thiolated poly-C oligonucleotides (C5: 5'-HS-CCCCCC; C9: 5'-HS-CTCTCTCTC; C18: 5'-HS-CTCTCTCCAACCTCTCTC) and Tris (hydroxymethyl) aminomethane were purchased from Sangon Inc. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) was purchased from J&K Scientific Ltd. (Guangzhou, China). H_2O_2 was obtained from Xilong Group Chemical Reagent Co.,Ltd. (Shantou, China). Silver nitrate, and other metal salts were obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). All solutions were prepared with high purity water (18 MΩ·cm^{-1}) from a high purity water preparation system (Development Center of Water Treatment Technology, Hangzhou, China).

Electrode preparation. Gold disk electrodes (2 mm in diameter, CH Instruments Inc., USA) were polished on microcloth with alumina suspensions (0.5 and 0.05 μm in diameter) for 5 min, followed by sonication in an ultrasonic bath successively with pure water, absolute alcohol, and pure water for 5 min. Then the electrodes were electrochemically cleaned in a fresh 0.5 M sulfuric acid solution by cycling the electrode potential between -0.3 V and +1.55 V at a scan rate of 0.1 V/s until a reproducible cyclic voltammogram was achieved to remove any residual contaminant. After rinsing and drying with nitrogen, the cleaned electrode was modified with probe DNA by incubation in a solution containing 2 M thiolated poly-C oligonucleotides at 4 ℃. Three lengths of poly-C oligonucleotides (C5, C9 and C18) were employed during sensor fabrication. The incubation time of the electrode ranged from 1 to 96 h. After DNA immobilization, the electrode was treated with 1 mM MCH solution for 1 h to obtain well-aligned DNA monolayers.

Electrochemical measurement. Electrochemical measurements were performed with an electrochemical workstation (CHI660C, CH Instrument, USA) with a 3.0 M KCl-Ag/AgCl as the reference electrode and a platinum (Pt) wire as the auxiliary electrode. A 10 mM Tris–Ac (Ac stands for acetic acid) buffer (pH 7.4) was adopted as the electrolyte. The modified electrode was immersed in $AgNO_3$ solutions of different concentration containing 5 mM H_2O_2 for 1 h, then washed with 10 mM Tris-Ac buffer (pH 7.4) and put in electrochemical cell. Cyclic voltammetry (CV) was carried out at a scan rate of 0.1 V/s between 0.2 V and -0.6 V. All experiments were performed independently at least 3 times.

Results and discussion

Cyclic voltammetry of H_2O_2 on bare and oligonucleotide-modified electrodes. The cyclic voltammetric behaviors of the sensing interface have been investigated. Within the negative scanning potential range, H_2O_2 can conduct redox reaction on the surface of the gold electrode. Fig. 1A shows the cyclic voltammograms of bare gold electrode in H_2O_2 with various concentrations. Within the scanning range (0.2 to -0.6 V), the current displayed an obvious increase with increasing concentration of H_2O_2. Fig. 1B showed the relationship between the concentration of H_2O_2 and the peak current. When H_2O_2 concentration increased from 0 to 5 mM, the current changed from -2.79±0.26 μA to -33.11±1.92 μA correspondingly. There was a linear relationship within the concentration range from 0-3 mM (R^2=0.9906). These data proved that H_2O_2 was capable to transfer electrons on the surface of the electrode, provoking electrical signals. Fig. 2A displayed the cyclic voltammograms and calibration curves of oligonucleotide-modified gold electrode. The CV current slightly increased with increasing concentration of H_2O_2. To illustrate the relationship clearly, the current variation $(I-I_0)/I_0$ was given, in which I_0 and I stand for the current to the potential of -0.6 V in the absence and presence of H_2O_2 (Fig. 2B). Compare with bare gold electrode, C5/MCH-modified electrode produced minor variation, while no evident change was observed for glassy carbon electrode. These results demonstrate that well-aligned DNA strands on the surface of gold electrode can effectively block the electron transference of H_2O_2. Thus H_2O_2 is applicable to be redox indicator for the electrochemical biosensor.

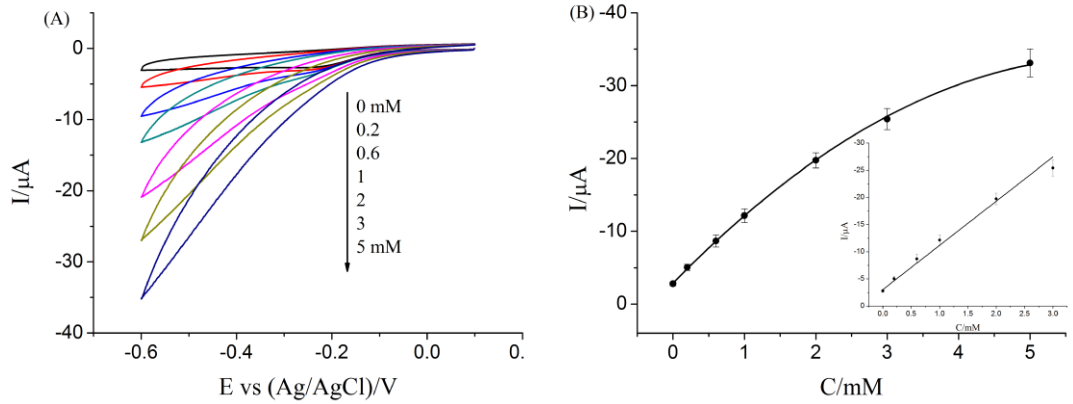

Figure 1. (A) Cyclic voltammograms of bare gold electrode in H_2O_2 with various concentrations. (B) A calibration curve of the peak current. Inset: the linearity of the peak current range from 0 to 3 mM.

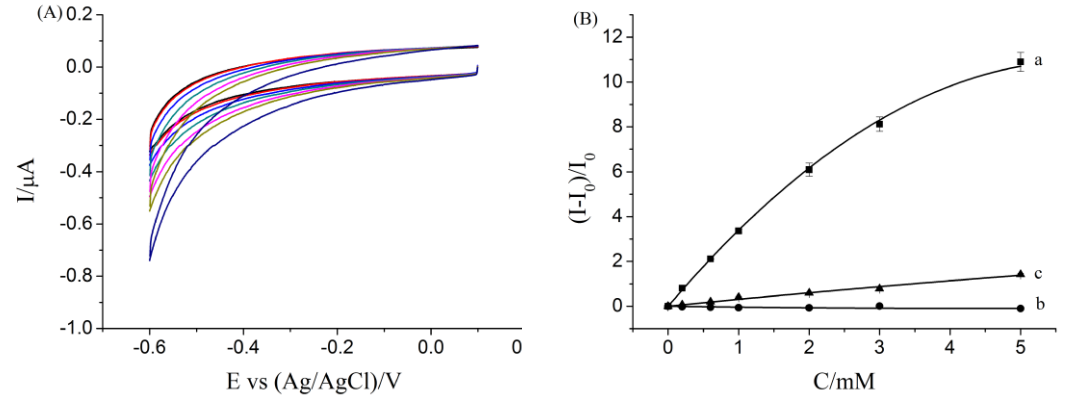

Figure 2. (A) Cyclic voltammograms of the C5/MCH modified electrode at different concentrations of H_2O_2 (0, 0.2, 0.6, 1, 2, 3 and 5 mM). (B) Current variation $(I-I_0)/I_0$ at different concentrations of H_2O_2 at -0.6 V for (a) bare gold electrode, (b) glassy carbon electrode and (c) C5/MCH modified electrode.

The principle of the electrochemical biosensor is depicted in Fig. 3. Thiolated poly-C DNA probes were self-assembled through Au–S bonding on gold electrode. After treatment of MCH, all the poly-C DNA strands were well aligned and nearly perpendicular to the surface of gold electrode [7]. In the absence of Ag^+, the vertical-parallel ss-DNA strands can effectively prevent the absorption of H_2O_2. Thus H_2O_2 can be easily removed by washing, resulting in weak electronic signal. In the presence of silver ions, their specific coordination with cytosine bases can change DNA steric structure. Single-stranded probe DNA strands are changed to double-stranded DNA by Ag^+, leading to accessible crevices in the self assembled monolayer (SAM). Therefore, H_2O_2 can pass through the slit of SAM system and be adsorbed on the surface of gold electrode owing to its high hydrophobic property. Therefore electron transfer (eT) between the H_2O_2 and the electrode surface occurs. After elution with cysteine solution, most of the silver ions are eluted, and the DNA strands return to the original state [8]. The electrode is regenerated and can be used again.

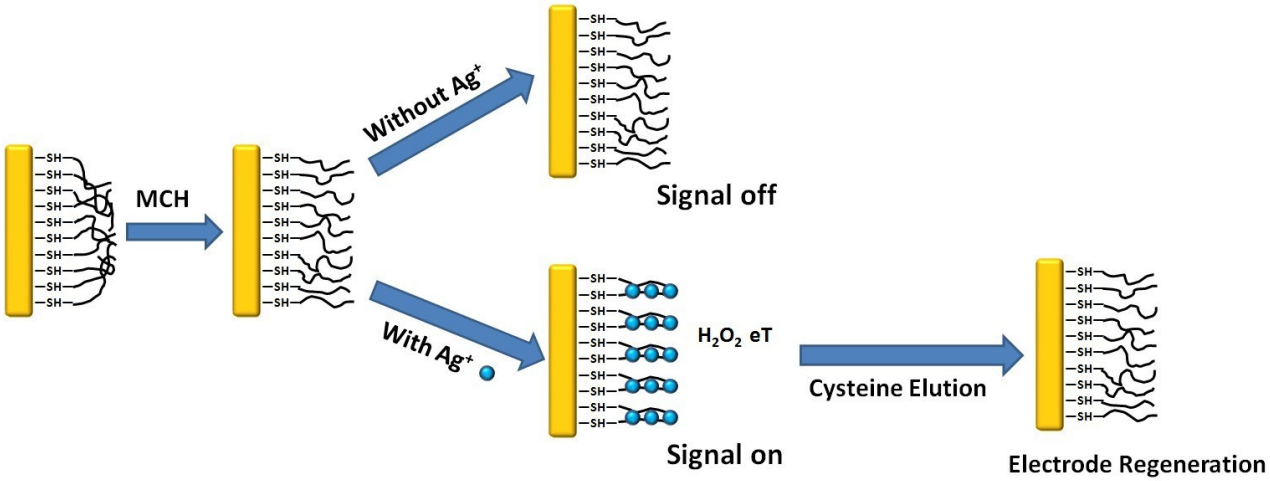

Figure 3. Schematic illustration for electrochemical detection of Ag^+ using H_2O_2 as a redox indicator and regeneration of the biosensor by cysteine elution. DNA sequence: C5. eT: electron transfer.

Sensitivity and selectivity of biosensor. After a 1 h treatment with various concentrations of Ag^+ in 10 mM Tris-Ac buffer (pH 7.4) containing 3 μM H_2O_2, the peak current variation $(I-I_0)/I_0$ increased with increased concentration of Ag^+. As illustrated in Fig. 3, the peak current is attributed to the adsorbed H_2O_2 molecules on the electrode surface. Inset of Fig. 4A showed the linearity of the relative CV peak current, ranging from 0 to 0.2 μM ($R^2 = 0.9955$). A detection limit of 30 nM could be obtained.

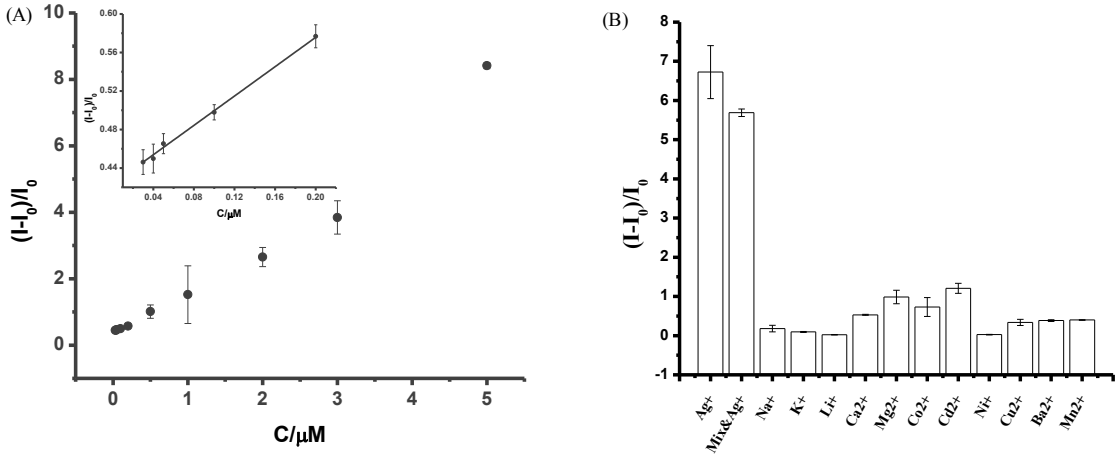

Figure 4. (A) Calibration curve for Ag^+ sensor. Inset: the linearity of the peak current range from 0 to 0.2 μM. (B) Peak current variations of various metal ions (1 mM), and a mixture of metal ions (1 mM each of Na^+, K^+, Li^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Cd^{2+}, Ni^+, Ba^{2+}, Cu^{2+}, Mn^{2+}, 5 μM Ag^+) and 3 μM H_2O_2. The probe DNA modified electrode was incubated in 2 μM C5 for 16 h.

The selectivity was investigated by recording the electrochemical signals of other metal ions such as Na^+, K^+, Li^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Cd^{2+}, Ni^+, Cu^{2+}, Ba^{2+} and Mn^{2+}. These metal ions showed quite weak signals compared with Ag^+ (Fig. 4B). This result indicated that our biosensor was highly selective owing to the specific coordination between C bases and silver ions. Besides, we performed experiment with real water samples, including tap water, lake water and seawater. No signals for these water samples were observed. Determination of Ag^+ concentration was performed by standard addition method. The results obtained from real water samples showed good recovery values (95.12–102.50%), confirming that the interferences in water samples could be almost neglected and the proposed sensor was applicable for practical Ag^+ detection (Table 1).

Table 1. Determination of Ag^+ in real water samples

Sample[a]	Ag^+ added (μM)	Ag^+ measured by this sensor (μM)	RSD (%)[c]	Recovery (%)
Tap water	0.2	0.190231	6.69	95.12
Lake water	0.2	0.204992	5.36	102.50
Seawater	0.2	0.195592	5.29	97.80

[a] All water samples were collected from Xiamen, China. [b] Mean of three measurements. [c] Relative standard deviation for n=3

Factors influencing the sensor performance. In order to optimize the signal gain of this biosensor, we studied the effect of H_2O_2 concentration, probe DNA length and incubation time of electrode on the sensor performance, which is represented by the relative intensity $(I-I_0)/I_0$. Fig. 5A showed that optimal results could be obtained at 3 μM H_2O_2. At low concentration of H_2O_2, the signal gain is low, whereas at high concentration of H_2O_2, the peak current of the sensor in the absence of Ag^+ increased, leading to decrease of $(I-I_0)/I_0$.

Three different lengths of thiolated poly-C oligonucleotides (C5, C9, and C18) were used during sensor fabrication. Thymine and adenine bases were introduced in C9 and C18 in order to avoid the *C*-C bonding formation in cytosine rich sequence [9]. The length of DNA probes had no significant influence on the sensor performance (Fig. 5B).

Fig. 5C showed that the ratios of $(I-I_0)/I_0$ increased along with the prolonged incubation time (1–96 h) of electrode. We presume that, over short incubation period, fewer oligonucleotides are immobilized on the electrode surface, thus the ss-DNA structure is flexible and the poly-C strands may not be well aligned and perpendicular to the surface of gold electrode, leading to an ineffective prevention to the absorption of H_2O_2. On the contrary, longer incubation time enables assembling oligonucleotides of a high density on the electrode, so the well aligned and perpendicular ss-DNA can effectively prevent the absorption of H_2O_2 in the absence of Ag^+ and give strong electrochemical signal gain when Ag^+ is added.

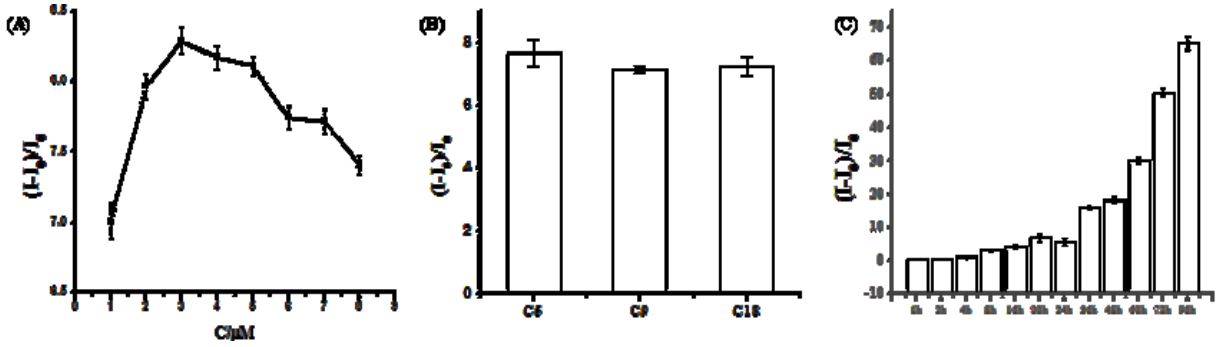

Figure 5. (A) $(I-I_0)/I_0$ of the sensor in various concentration of H_2O_2. DNA sequence: C5. (B) Peak current variation of the three sensors. Pairwise comparisons (C5 and C9, C5 and C18) were performed using Tukey's test (P>0.5). (C) Current variation at different incubation time (1-96 h). DNA Sequence: C5.

Reproducibility and regeneration. We determined the reproducibility of our biosensor. The relative standard deviations (RSDs) were <8%. As shown in Fig. 6A, the 7 days stability of the sensor was investigated. No significant change of current response was found (Tukey's test, $P > 0.5$). Therefore, this biosensor has high reproducibility and stability.

To regenerate our biosensor, we used cysteine to elute Ag^+ off the DNA strands. The elution is conducted by sinking the DNA-modified gold electrode into the cysteine aqueous solution (30 mM) for 1 h. The regeneration of C5-based biosensor is poor, we presume that the C5 probe contains 5 seriate cytosines which can coordinate with silver ions and form stubborn triplets. These triplets

might be difficult to unwind. C18 probe, which is comprised of three kinds of bases, obtained ideal regeneration rate of ~70% (Fig. 6B).

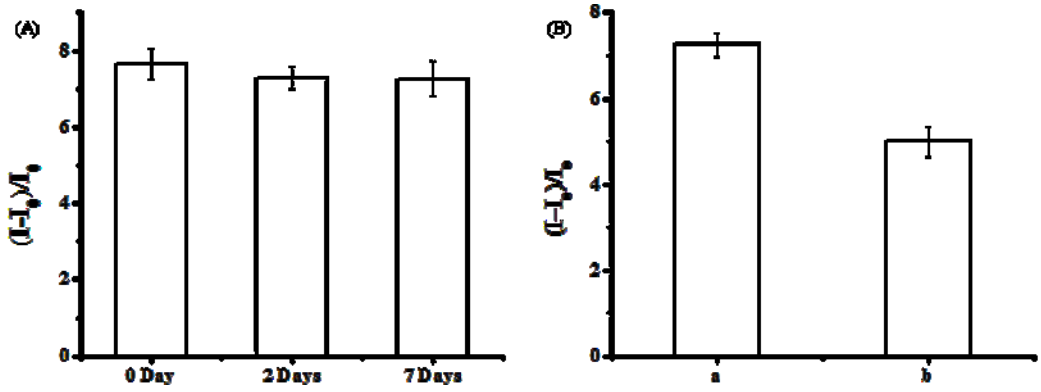

Figure 6. (A) Current variation $(I-I_0)/I_0$ of the biosensor in different periods from 0 to 7 days. (B) The current variation of biosensors of the first test (a) and the second test after elution by cysteine (b). DNA sequence: C5.

Conclusions

In conclusion, the paper focuses on the detection of silver ion in aqueous solution based on coordination of the Ag^+ by cytosine residues. In presence of Ag^+, the monolayer of oligonucleotide strands becomes more porous, so H_2O_2 as a redox mediator can more easily gain access to the electrode surface where it is detected by cyclic voltammetry. This method does not require costly equipment and sophisticated sample pretreatment. It is simple, efficient and rapid. It may have practical application of Ag^+ detection in environment and food.

Acknowledgement

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