Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences Supplement (2009) S56–S59
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Amperometric hydrogen peroxide biosensor based on immobilization of DNA-Cu(II) in DNA/chitosan polyion complex membrane

GU Tingting[1,*], LIU Ying[1], ZHANG Jing[1], HASEBE Yasushi[2]

1. School of Chemical Engineering, University of Science and Technology Liaoning, Anshan 114044, China. E-mail: gtt@ustl.edu.cn
2. Department of Life Science & Green Chemistry, Saitama Institute of Technology, Fukaya 369-0293, Japan

Abstract

DNA/chitosan polyion complex membrane was used as a support for immobilization of electrocatalytic species-copper ions, which specifically bound to dsDNA and catalyzed the hydrogen peroxide reduction. The polyion complex membrane consisted with DNA-Cu(II) complex and chitosan was prepared on a glassy carbon electrode (GCE). Electrochemical measurements of the DNA-Cu(II)/chitosan membrane-modified GCE revealed that the copper ion embedded in the DNA/chitosan layer exhibited good electrochemical behaviors. The DNA-Cu(II)/chitosan/GC electrode showed an excellent electrocatalytic activity for the H_2O_2 reduction. Even in the presence of dissolved oxygen, the sensor exhibited highly sensitive and rapid (response time, about 6 s) response to H_2O_2. The steady-state cathodic current responses of the sensor obtained at –0.2 V versus Ag/AgCl in air-saturated 100 mmol/L phosphate buffer (pH 5.0) increased linearly up to 10 mmol/L with the detection limit of 3 μmol/L. Effects of applied potential and buffer pH upon the response currents of the sensor were investigated for an optimum analytical performance. Ascorbic acid and glucose almost have no interference to measurement of H_2O_2. In addition, the sensor exhibited good reproducibility.

Key words: hydrogen peroxide biosensor; DNA/chitosan; DNA-Cu

Introduction

Hydrogen peroxide is a practically important analyte in many fields, including food, chemical, pharmaceutical, clinical, laboratory industry, and environmental analyses etc. Many conventional methods such as titrimetry, UV-Vis spectrophotometry, chemiluminescence and electrochemistry have been developed for determination of hydrogen peroxide. However, some of these methods involve complicated procedures and suffer from various interferences. Amperomeric biosensor is especially promising due to attractive features involving simple reliable, rapid, cheaper, and highly sensitive for hydrogen peroxide determination in health care, control of industrial processes and environmental monitoring (Luan *et al*., 1998; Lei and Deng, 1996; Bartlett *et al*., 1996). On the other hand, the optimal operational conditions of an enzyme-based amperomeric hydrogen peroxide biosensor are generally limited by the properties of native enzymes, so it is important to fabricate new "artificial enzyme" to instead of them. DNA-Cu complex has been reported to exhibit an enzyme-mimic catalytic activity, and a polyion complex membrane composed of DNA and poly(allylamine) (PAA) immobilization of electrocatalytic element-copper ion has been developed for hydrogen peroxide sensing (Gu and Hasebe, 2006; Hasebe and Gu, 2005).

Chitosan is a polymeric material, biodegradable, nontoxic and has been used as a cholesterol-lowering agent and as a wound-healing product (Illum, 1998). Because of its cationic nature, chitosan can form polyion complex with DNA. Chitosan has widely been investigated for the purpose of non-viral gene delivery in the form of DNA-chitosan complexes or as nanoparticles (Maytal *et al*., 2004; Dastan and Turan, 2004; Rikimaru *et al*., 2003). In this study, DNA/chitosan polyion complex membrane was used as a support for immobilization of electrocatalytic species-copper ions, which specifically bound to dsDNA and catalyzed the hydrogen peroxide reduction. Electrochemical measurements were used to study the electrocatalytic activity of DNA-Cu(II) complex which immobilized into DNA/chitosan polyion complex membrane on electrode surface. Further a highly functional hydrogen peroxide biosensor was developed using a DNA-Cu(II)/chitosan complex as sensing layer.

1 Materials and methods

1.1 Reagents and chemicals

Double-stranded DNA (from Herring Sperm, < 50 bp) was obtained from Sigma, USA. Chitosan was purchased from Sinopharm Chemical Reagent Co., China. All the other chemicals were of analytical grade and used without further purification. Twice-distilled water was used for preparation of buffer and standard solutions. A standard solution of H_2O_2 was prepared daily with buffer.

* Corresponding author. E-mail: gtt@ustl.edu.cn

1.2 Preparation of DNA-Cu(II)/chitosan/GC electrode

A glassy carbon (GC) electrode (3 mm diameter; Lanlike Chemistry and Electron High Technology Co., China) was polished with 0.05 μm alumna slurry, rinsed with water, sonicated in water for 2 min, and dried. The DNA-Cu(II) immobilized electrode was prepared as follows: 0.5 mL of 1 mg/mL dsDNA, 0.5 mL of 3 mmol/L $CuCl_2$ was mixed for 1 h to form DNA-Cu(II) complex; then 40 μL of DNA-Cu(II) complex and 20 μL of 0.5 mg/mL chitosan were placed on electrode surface. The electrode was kept for 1 d under a 500-mL beaker at room temperature. The electrode was stored in the refrigerator at 4°C when not in use. Before electrochemical measurements, the electrode was immersed in 100 mmol/L phosphate buffer for 10 min.

1.3 Electrochemical measurements

All electrochemical experiments were carried out with a three-electrode system comprising a DNA-Cu(II)/chitosan/GC electrode as a working electrode, an Ag/AgCl (sat. KCl) reference electrode and a platinum wire auxiliary electrode (1 mm diameter). A 100 mmol/L phosphate buffer (pH 5) was used as an electrolyte solution. Cyclic voltammetry and constant potential amperometry was performed with an electrochemical analyzer (CHI823, Shanghai Chenhua Instrument, Inc., China). All measurements were done at room temperature.

1.4 Scanning electron microscope (SEM) and atomic force microscope (AFM) analysis

The SEM analysis of DNA-Cu(II)/chitosan polyion complex membrane was performed with a JEOL-6480LV microscope (JEOL, Japan) at 15.0 kV. AFM measurements were carried out using a scanning probe microscope (MicroNano AFM-III, Shanghai Zuolun Micronano Equipment Co., China) in contact mode with CSC12 cantilever. DNA-Cu(II)/chitosan polyion complex membrane was prepared as follows: 60 μL of aqueous solutions containing DNA-Cu(II) complex and chitosan were placed on a glass slide, and dried in air for 1 d. Prior to SEM analysis, ca. 10 nm of carbon film was coated onto the samples with ion coater. The DNA/chitosan film was also prepared as a control in a similar manner.

2 Results and discussion

2.1 Structures of DNA/chitosan and DNA-Cu/ chitosan surface

Figure 1 shows the SEM views of the DNA/chitosan film and DNA-Cu/chitosan film surfaces. The DNA-chitosan film shows a smooth surface, which is different with the disperse-distributed small spots of DNA-PAA film (Gu and Hasebe, 2004). In contrast, the DNA-Cu/chitosan film shows a reticular and rough surface. From AFM image, the thickness of the DNA-Cu/chitosan film was about 500 nm.

2.2 Electrochemical behaviors of DNA-Cu/chitosan/GCE

Figure 2a shows the cyclic voltammogram of a DNA-Cu/chitosan membrane immobilized glassy carbon (GC) electrode. A Cu(II)/Cu(I) redox couple at the potential about –0.125 and –0.005 V with $E^{1/2}$ of –0.065 V were observed in the potential range between –600 and 500 mV (vs. Ag/AgCl) in 100 mmol/L phosphate buffer (pH 7.0), which was similar with that obtained by DNA-Cu/PAA/GCE (–0.064 V) (Gu and Hasebe, 2006; Hasebe and Gu, 2005). These cyclic voltammograms were stable during the repeated potential sweeps and the peak currents linearly increased with increasing in square root of the scan rate, which indicates that the electrode reaction of copper ion within the DNA/chitosan layer on the GC electrode is mainly controlled by a diffusion control process.

Figures 2b and 2c presented the cyclic voltammograms of DNA-Cu complex and $CuCl_2$ in a bare GC electrode. A Cu(II)/Cu(I) redox couple was obtained at 0.0175 ($E^{1/2}$) and 0.1635 V ($E^{1/2}$) for DNA-Cu complex and $CuCl_2$, respectively. These results show that copper(II) ion exhibited well defined redox peaks, and DNA-Cu complex exhibited good electrochemical behaviors on GC

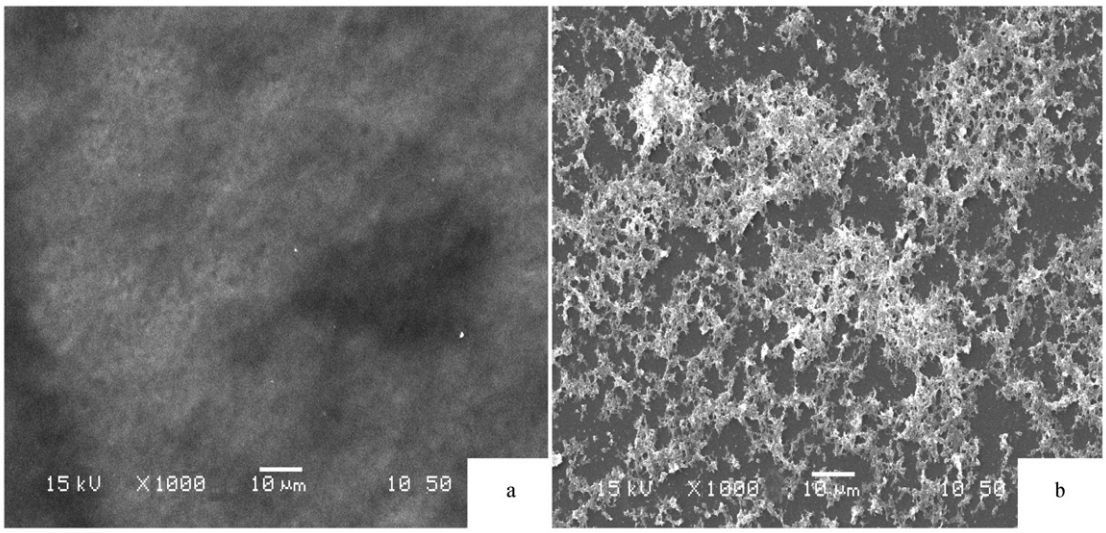

Fig. 1 SEM images of DNA/chitosan film (a) and DNA-Cu/chitosan film (b).

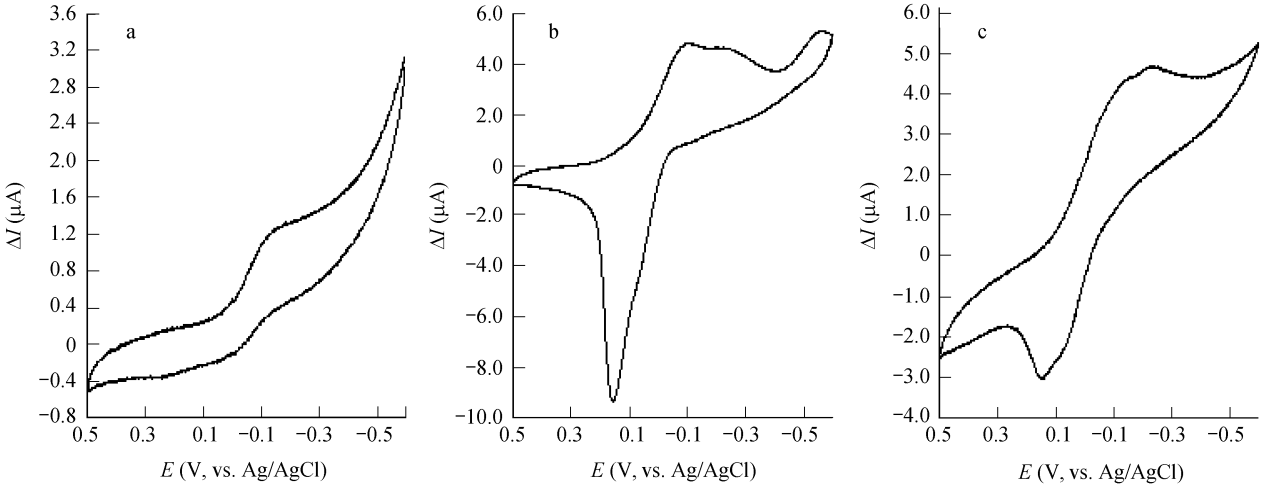

Fig. 2 Cyclic voltammograms of the DNA-Cu(II)/chitosan/GC electrode (a) in 100 mmol/L phosphate buffer (pH 7.0) and bare GC electrode in 100 mmol/L phosphate buffer (pH 7.0) containing DNA-Cu(II) complex (b) ($CuCl_2$, 0.1 mmol/L; DNA, 1 mg/mL) and 0.1 mmol/L $CuCl_2$ (c).

electrode. Copper(II) ions were known to interact with DNA by coordinate binding at GC base pair and phosphate site. A larger and stable anodic peak of DNA-Cu complex was observed in Fig. 2b, which is quite different from the result in the literature (Gu and Hasebe, 2006). It suggested that the electron transfer was improved by the short DNA (<50 bp) on the electrode surface.

Further, the cyclic voltammogram of DNA-Cu/chitosan membrane was greatly changed by the addition of H_2O_2 with markedly increase in cathodic current and completely disappearance in anodic current, indicating electrocatalytic reduction of H_2O_2. It revealed that the copper ion embedded in the DNA/chitosan layer exhibits good electrochemical behaviors and an excellent electrocatalytic activity for the H_2O_2 reduction. These voltammetric changes are attributed to the following catalytic schemes.

$$\text{DNA-Cu(II)/chitosan} + e^- \longrightarrow \text{DNA-Cu(I)/chitosan} \quad (1)$$

$$\text{DNA-Cu(I)/chitosan} + 1/2H_2O_2 \longrightarrow \text{DNA-Cu(II)/chitosan} + OH^- \quad (2)$$

2.3 Hydrogen peroxide sensor

The steady-state cathodic current responses of the sensor obtained at –0.2 V versus Ag/AgCl in air-saturated 100 mmol/L phosphate buffer (pH 7.0). Figure 3 shows a typical current-time curve for successive addition of 10 μmol/L H_2O_2 observed at applied potential of –0.20 V (vs. Ag/AgCl). When aliquot of hydrogen peroxide was added into the buffer electrolyte solution, the current obtained by DNA-Cu(II)/chitosan film immediately changed and reached to another steady-state current at about 6 s. As a control, a small response with higher base line and no response with lower base line were obtained respectively by Cu(II)-chitosan film and DNA-Cu(II) or DNA-chitosan film. Effects of applied potential and buffer pH upon the response currents of the sensor were investigated. The response current slightly increased with negative shifting the potential from –0.10 to –0.20 V, and the current approached a plateau at –0.20 V. The effect of pH on the response was examined in the pH range from 5.0 to 9.0. Higher response was observed in acidic pH, and the response gradually decreased with increase of pH. Thereby, potential of –0.20 V and buffer pH 5.0 were selected for an optimum analytical performance. Figure 4 shows the calibration graphs of the DNA-Cu(II)/chitosan/GC electrode at –0.20 V vs. Ag/AgCl in air-saturated 100 mmol/L phosphate buffer (pH 5.0). The response current to H_2O_2 increased linearly up from 10 μmol/L to 10 mmol/L with the detection limit of 3 μmol/L. In this study, glucose and ascorbic acid almost has no interference to measurement of H_2O_2, and the interference by glucose and ascorbic acid may be neglected. In addition, operational stability of the sensor was checked by repetitive measurements of 10 μmol/L H_2O_2. The current responses were retained more than 90% of the initial response after repeating 20 times.

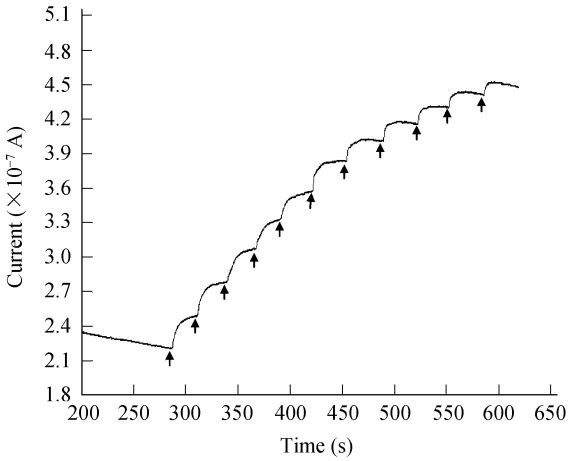

Fig. 3 Typical current-time curve for successive addition of 10 μmol/L H_2O_2 at applied potential of 0.20 V (vs. Ag/AgCl) in air-saturated 100 mmol/L phosphate buffer (pH 5.0).

3 Conclusions

DNA/chitosan polyion complex membrane was used as a support for immobilization of electrocatalytic species-

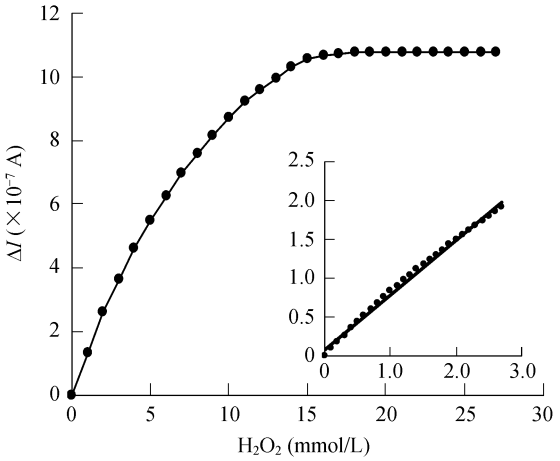

Fig. 4 Calibration graphs of the DNA-Cu(II)/chitosan/GC electrode at –0.2 V (vs. Ag/AgCl) in air-saturated 100 mmol/L phosphate buffer (pH 5.0).

copper ions. Electrochemical measurements of the DNA-Cu(II)/chitosan membrane-modified glassy carbon electrode revealed that the copper ion embedded in the DNA/chitosan layer exhibited good electrochemical behaviors. A high functional hydrogen peroxide biosensor was developed using a DNA-Cu(II)/chitosan complex as sensing layer. The sensor exhibited highly sensitive, with the linear range from 10 μmol/L to 10 mmol/L and rapid response to H_2O_2 with the detection limit of 3 μmol/L. Ascorbic acid almost has no interference with measurement of H_2O_2. In addition, the sensor exhibited good reproducibility and stability.

Acknowledgments

This work was supported by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry. The authors would like to thank Shanghai Zhuolun MicroNano Equipment Co., Ltd. for AFM analysis.

References

Bartlett P N, Birkin P R, Palmisano F, Benedetto G D, 1996. A study on the direct electrochemical communication between horseradish peroxidase and a poly(aniline) modified electrode. *Journal of the Chemical Society, Faraday Transactions*, 92: 3123–3130.

Dastan T, Turan K, 2004. *In vitro* characterization and delivery of chitosan-DNA microparticles into mammalian cells. *Journal of Pharmaceutical Science*, 7: 205–214.

Gu T, Hasebe Y, 2006. DNA-Cu(II) poly(amine) complex membrane as novel catalytic layer for highly sensitive amperometric determination of hydrogen peroxide. *Biosensors and Bioelectronics*, 21(11): 2121–2128.

Gu T, Hasebe Y, 2004. Peroxidase and methylene blue-incorporated double stranded DNA-polyamine complex membrane for electrochemical sensing of hydrogen peroxide. *Analytica Chimica Acta*, 525(2): 191–198.

Hasebe Y, Gu T, 2005. DNA-Cu(II) compolex as a novel electrocatalyst for a hydrogen peroxide sensor. *Journal of Electroanalytical Chemistry*, 576(1): 177–181.

Illum L, 1998. Chitosan and its use as a pharmaceutical excipient. *Pharmaceutical Research*, 15(9): 1326–1331.

Lei C, Deng J, 1996. Hydrogen peroxide sensor based on coimmobilized methylene green and horseradish peroxidase in the same montmorillonite-modified bovine serum albumin-glutaraldehyde. *Analytical Chemistry*, 68(19): 3344–3349.

Luan C, Yang F, Lei C, Deng J, 1998. A reagentless amperometric hydrogen peroxide biosensor based on covalently binding horseradish peroxidase and thionine using thiol-modified gold electrode. *Journal of Electroanalytical Chemistry*, 455(1-2): 121–125.

Maytal B B, Krista E M, Kees L F, Hans E J, Gerrit B, Tom H O, Annemieke G, 2004. Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of *Mycobacterium tuberculosis*. *Vaccine*, 22(13-14): 1609–1615.

Rikimaru S, Wakabayashi Y, Nomizu M, Nishi N, 2003. DNA-chitosan bilayer membrane as a bi-functional biomedical adhesive. *Polymer Journal*, 35: 255–260.