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CHEMICAL & BIOSENSORS

Amperometric Biosensor for Hydrogen Peroxide Based on Myoglobin Doped Multiwalled Carbon Nanotube Enhanced Grafted Collagen Matrix

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Abstract: A reagentless H₂O₂ sensor based on the direct electron transfer of myoglobin (Mb) doped in multiwalled carbon nanotubes enhanced grafted collagen matrix is proposed. The formal potential of the immobilized Mb was -0.358 V with a surface coverage of 4.0×10^{-10} mol cm⁻². The electrode process was surface-controlled with an electron transfer rate constant of 9.7 s⁻¹. The proposed biosensor displayed

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an excellent electrocatalytic response to the reduction of H₂O₂ with a linear range from 0.6 to 39.0 μM. Owing to the good biocompatibility and high enzyme loading of the matrix the biosensor exhibited acceptable stability and reproducibility.

Keywords: Biosensor, multi-walled carbon nanotubes, grafted collagen, myoglobin, hydrogen peroxide

INTRODUCTION

Carbon nanotubes have attracted much attention due to their unique mechanical, chemical, and electronic properties since 1991 (Iijima 1991). Multiwalled carbon nanotubes (MWNTs) have been extensively employed as electrode materials for investigation of bioelectrochemical reactions (Britto et al. 1996; Zhao et al. 2003) and electrochemical detection (Li et al. 2003; Wu et al. 2002). Since the surface of functionalized MWNTs contains abundant -COOH and -OH (Liao et al. 2004), they can be anchored with ultrasonic vibration into polymer matrix for different purposes by covalent or hydrogen bond without need of peptide coupling agent (Koshio et al. 2001). This work anchored MWNTs into grafted collagen matrix to form hybrid nanocomposites for both strengthening the mechanical properties and improving the electrical conductivity and thermal stability of the tri-helix scaffold of grafted collagen. Collagen is one of the biopolymers most extensively used to construct functionalized hybrid structures due to its abundant -OH and -NH₂ groups. By grafting collagen molecule with some lipophilic materials such as methyl methacrylate (MMA) the noticeable improvement in biocompatibility of collagen has been obtained (Se and Aoyama 2004). Here the formed nanoparticles hybrid matrix retained the biocompatibility of the grafted collagen for immobilization of biomolecules and the unique electronic properties of MWNTs for direct electrochemical study of the immobilized proteins. Owing to the commercial availability and known structure, myoglobin (Mb) was used as an ideal model protein in this work.

The immobilization of proteins on electrode surfaces has been achieved by incorporating them in polymer or hydrogel films (Shen et al. 2002), surfactants (Mimica et al. 2001), lipids (Huang et al. 2005), or nanoparticles matrices (Liu et al. 2003). However, few researchers pay their attention to the biocompatibility and biosensing application of hybrid nanocomposites. The presence of MWNTs in the enhanced tri-helix scaffold of grafted collagen increased the loading of Mb and accelerated the electron transfer of the immobilized Mb, which were significant for the preparation of relatively sensitive reagentless biosensor for hydrogen peroxide.

The accurate determination of hydrogen peroxide is of great importance because it is an essential mediator in food, pharmaceutical, clinical, industrial

and environmental analyses. Many electrochemical biosensors for hydrogen peroxide based on electron transfer mediators or redox polymers (Garguilo et al. 1993; Razola et al. 2003; Florescu and Brett 2004), direct electron transfer of proteins (Xiao et al. 1999; Fan et al. 2000; Xu et al. 2006) and the catalytic activity of carbon nanotubes (Kurusu et al. 2006) have been developed. The biosensors based on the direct electron transfer of proteins have attracted considerable attention, because the design of these biosensors is simple without the help of mediator. In this paper the Mb immobilized in MWNTs enhanced tri-helix scaffold of grafted collagen matrix showed fast direct electron transfer and excellent electrocatalytic activity to the reduction of hydrogen peroxide, leading to good analytical performance for electrochemical detection of hydrogen peroxide. The proposed hydrogen peroxide biosensors possessed high sensitivity with a linear range of 0.6 to 39.0 μM , which was much better than the range of 10 to 100 μM based on the direct electron transfer of Mb in SDS film (Fan et al. 2000) and even the limit of detection of 7.4 μM for a mediator-based sensor (Ricci et al. 2003). Owing to the introduction of both collagen and MWNTs, the biosensor exhibited low detection limit, good affinity, operational convenience and storage stability.

EXPERIMENTAL

Chemicals

Horse heart myoglobin (No. M-1882, type III) was purchased from Sigma and used as received. 2.0 mg ml⁻¹ of Mb solution was stored at 4°C as stock solution. H₂O₂ (30% w/v solution) was purchased from Shanghai Jinlu Chemical Engineering Ltd. Co (China). Other reagents were of analytical reagent grade. 0.1 M phosphate buffer solutions (PBS) with different pH values were prepared by mixing the stock standard solutions of Na₂HPO₄ and NaH₂PO₄ and adjusting the pH with 0.1 M H₃PO₄ or NaOH. All solutions were prepared with twice-distilled water.

Grafted collagen was prepared according to the literatures (Cao et al. 2004). High-purity MWNTs were obtained through the catalytic chemical vapor deposition, and subsequent thermal heat treatment at 2800°C for 30 min in an argon atmosphere. 20 mg as-synthesized MWNTs were immersed into 1 M hydrochloric acid for 3 h and 2.6 M nitric acid for 4 h, respectively. The obtained products were dispersed in ethanol for further experiments. MWNTs enhanced grafted collagen matrix was prepared by mixing functionalized MWNTs and the grafted collagen in ethanol with vigorously stirring for 4 hours and ultrasonic vibration for 4–6 hours. After filtrated with the vacuumized filtration the final product was washed thoroughly with absolute ethanol and evaporated in room temperature to remove impurities and solvents.

Amperometric Biosensor for Hydrogen Peroxide**1559****Apparatus and Measurements**

UV-Vis absorbance spectroscopy was performed using a UV-Vis-3100-Nir Recording Spectrophotometer (Shimadzu, Japan). Fourier transform infrared (FT-IR) spectra were recorded on a Vector 22 FT-IR spectrometer (Bruker). For morphological analysis, the sample films were prepared in the same way as those for voltammetric measurements on different slides cleaned with nitric acid and the mixture of H₂SO₄:H₂O₂ (1:1). After coated with Au film to improve the conductivity, these films were examined under a scanning electron microscope (SEM, LEO 1530 VP, Germany) at 5.00 kV.

Electrochemical measurements were performed on a CHI 730 A electrochemical analyzer (CHI Co., China) at $(20 \pm 2)^\circ\text{C}$ with a conventional three-electrode system with the modified graphite electrode (GE) as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference against which all potentials were measured. The amperometric experiments were carried out by applying a potential of -400 mV on a stirred cell. The biosensor response was measured as the difference between total and residual currents. All experimental solutions were deoxygenated by bubbling highly pure nitrogen for 15 min and maintained under nitrogen atmosphere during measurements.

Preparation of Modified Electrodes

The substrate graphite electrodes (GE, 5.6 mm in diameter) were polished before each experiment with 1.0, 0.3, and 0.05 μm α -alumina slurry (Beuhler) respectively, rinsed thoroughly with doubly distilled water between each polishing step, then sonicated in 1:1 nitric acid, acetone, and doubly distilled water successively and allowed to dry at room temperature. To improve the dispersion of MWNTs enhanced grafted collagen, dimethyl sulfoxide (DMSO) was used to prepare MWNTs-grafted collagen suspension (4.0 mg in 1.0 ml DMSO). 10 μl 2.0 mg ml^{-1} of Mb solution and 5 μl of MWNTs-grafted collagen suspension were cast on GE surface to obtain the Mb/MWNTs-grafted collagen/DMSO modified electrode. Alternatively, only 10 μl Mb solution, 5 μl MWNTs-grafted collagen suspension, 10 μl Mb and 5 μl aqueous suspension of MWNTs enhanced grafted collagen or 10 μl Mb and 5 μl DMSO solution was cast on the graphite electrode to form Mb/GE, MWNTs-grafted collagen/DMSO/GE, Mb/MWNTs-grafted collagen/GE or Mb/DMSO/GE, respectively. A small bottle was fit tightly over the electrode for 2 h to ensure the slow evaporation of water and the formation of more uniform film. The film was then dried and aged overnight in a sealed flask at room temperature. Prior to electrochemical experiments, the electrode was rinsed thoroughly with doubly distilled water and kept in 0.1 M pH 7.0 PBS at 4°C in a refrigerator when not in use.

RESULTS AND DISCUSSION

Spectroscopic Analyses of Mb/MWNTs-Grafted Collagen/DMSO Film

The UV-Vis spectra of all systems containing Mb displayed a maximum absorption at about 408 nm (curves a, b, c, and d in Fig. 1), while no absorption of MWNTs-grafted collagen/DMSO and DMSO was observed in the studied wavelength range (curves e and f). Obviously, the absorption peak was attributed to the Soret band of Mb. No shift of the Soret band upon mixing of Mb with MWNTs enhanced grafted collagen or DMSO was observable. Thus MWNTs enhanced grafted collagen did not change the fundamental microenvironment of Mb, and the Mb mixed in these systems retained its natural secondary structure.

The shapes of the infrared absorption bands of amide I at 1700–1600 cm^{-1} and amide II around 1620–1500 cm^{-1} can provide detailed information on the secondary structure of polypeptide chain (Kauppinen et al. 1981). As shown in Fig. 2, the FT-IR spectra showed the interaction between MWNTs-grafted collagen and Mb. The FT-IR spectrum of MWNTs enhanced grafted collagen showed the amide I and amide II infrared absorbance of collagen at 1652 and 1544 cm^{-1} (curve a), while they located at 1652, 1540 cm^{-1} in Mb/MWNTs-grafted collagen film (curve b), which were close to 1654 and 1542 cm^{-1} obtained for Mb itself (curve e). The slight shift resulted from the interaction between Mb and MWNTs enhanced grafted collagen. The presence of DMSO (curve c) resulted in greater shifts in the peak positions of amide I (1649 cm^{-1}) and amide II (1534 cm^{-1}) infrared absorbance of Mb. But the shifts became smaller in presence of MWNTs enhanced grafted

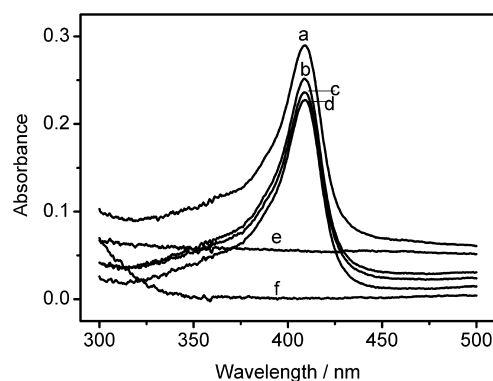


Figure 1. UV-vis spectra of Mb/MWNTs enhanced grafted collagen in DMSO (a), Mb/MWNTs enhanced grafted collagen in water (b), Mb in DMSO (c), Mb in water (d), MWNTs enhanced grafted collagen in DMSO (e), and DMSO (f).

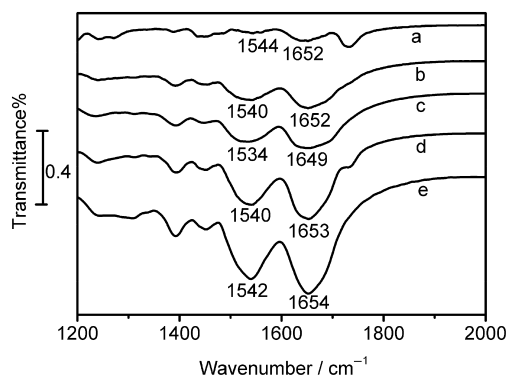
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Figure 2. FT-IR spectra of MWNTs-grafted collagen (a), Mb/MWNTs enhanced grafted collagen (b), Mb/DMSO (c), Mb/MWNTs-grafted collagen/DMSO (d), and Mb (e) films.

collagen hybrid nanocomposite (curve d) with the peak positions of 1653 and 1540 cm^{-1} for amide I and amide II of Mb, indicating the nanocomposite improved greatly the microenvironment for retaining the natural structure of the immobilized Mb.

SEM Characterization

The Mb molecules dispersed on a glass slice aggregated together in the absence of DMSO and MWNTs enhanced grafted collagen (Fig. 3a). The SEM micrograph of MWNTs enhanced grafted collagen film displayed that the grafted collagen molecules could strongly adsorb on MWNTs surface through covalent or hydrogen bond to form a porous three-dimensional structure. The average diameter of the MWNTs doped in the grafted collagen matrix ranged between 30 and 60 nm (Fig. 3b).

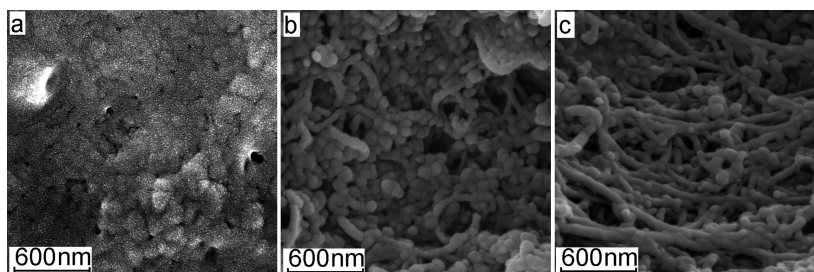


Figure 3. Scanning electron micrographs of Mb (a), MWNTs-grafted collagen/DMSO (b), and Mb/MWNTs-grafted collagen/DMSO (c) films on glass slices.

After mixing Mb with MWNTs enhanced grafted collagen/DMSO, a well-distributed film could be formed (Fig. 3c). In this case, the MWNTs enhanced grafted collagen was surrounded by Mb molecules to form robust, stereo, network, and porous structure. Therefore, MWNTs enhanced grafted collagen provides advantages in microscopic electrochemical reactions.

Direct Electrochemistry of the Immobilized Mb

No detectable response was observed on the cyclic voltammograms of GE and MWNTs-grafted collagen/DMSO/GE (curves a and b in Fig. 4), while MWNTs-grafted collagen/DMSO/GE showed a couple of stable and well-defined redox peaks at -330 and -386 mV at 100 mV s $^{-1}$ (curve f in Fig. 4). Thus, these peaks were attributed to the redox reaction of the electroactive center of Mb. Although Mb/GE and Mb/MWNTs-grafted collagen/GE and Mb/DMSO/GE also displayed a couple of redox peaks of Mb (curves c, d, and e in Fig. 4), not only the redox peak currents were much smaller but also the peak-to-peak separations were greater than those of Mb/MWNTs-grafted collagen/DMSO/GE, indicating a faster electron transfer rate at the latter. MWNTs enhanced grafted collagen improved the direct electrochemistry of immobilized Mb due to its three-dimensional stage and the presence of MWNTs.

From the integration of reduction peaks of Mb/MWNTs-grafted collagen/DMSO/GE at different scan rates, an average surface coverage of Mb was calculated to be $(4.0 \pm 0.2) \times 10^{-10}$ mol cm $^{-2}$, which was much larger than 1.4×10^{-10} mol cm $^{-2}$ at Mb-CNT (Zhao et al. 2006),

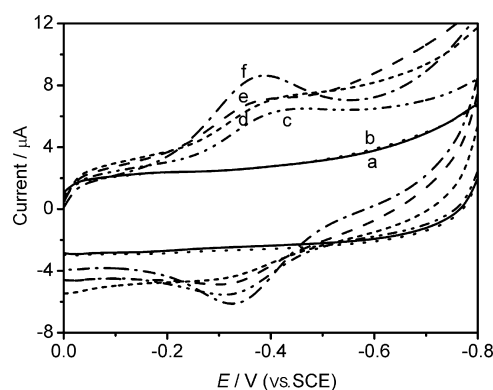


Figure 4. Cyclic voltammograms of GE (a), MWNTs-grafted collagen/DMSO/GE (b), Mb/GE (c), Mb/MWNTs-grafted collagen/GE (d), Mb/DMSO/GE (e), and Mb/MWNTs-grafted collagen/DMSO/GE (f) in 0.1 M pH 7.0 PBS at 100 mV s $^{-1}$.

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$5.18 \times 10^{-11} \text{ mol cm}^{-2}$ at Mb-agarose hydrogel electrode (Liu et al. 2004a), $8.85 \times 10^{-11} \text{ mol cm}^{-2}$ at $\{\{\text{SiO}_2\text{-(Mb/PSS)}_m\}/\text{PEI}\}_n$ (Liu et al. 2004b) and was similar to $2.785 \times 10^{-10} \text{ mol cm}^{-2}$ at Mb-Hcy/Au electrode (Zhang and Li 2000). The large surface coverage of Mb proved high loading of the Mb in MWNTs enhanced grafted collagen and its stability.

The formal potential of the heme $\text{Fe}^{\text{III/II}}$ couple in Mb/MWNTs-grafted collagen/DMSO/GE, estimated as the midpoint of reduction and oxidation potentials, was $-(358 \pm 3) \text{ mV}$ (vs. SCE) in 0.1 M pH 7.0 PBS. This value was similar to those of -350 mV at Mb-CNT (Zhao et al. 2006) and -342 mV at Mb-colloidal gold (Liu and Ju 2003), suggesting that most molecules preserved their native structure after being entrapped in the MWNTs enhanced grafted collagen.

The cyclic voltammogram of Mb/MWNTs-grafted collagen/DMSO/GE showed a couple of nearly symmetrical redox peaks proportional to scan rate (Fig. 5), indicating a surface-controlled electrode process. The peak-to-peak separations of the cyclic voltammograms at scan rates from 30 to 1000 mV s^{-1} were from 52 to 71 mV. Considering the α value between 0.3 and 0.7 and the peak-to-peak separation less than 200 mV, the electron transfer rate constant k_s was estimated to be $(9.7 \pm 0.2) \text{ s}^{-1}$ according to the model of Laviron (Laviron 1979) with $k_s = mnFv/RT$, where m is a parameter related to the peak-to-peak separation, F is Faraday constant, R is the gas constant, T is the temperature, and n is the number of electron transfer. The k_s value was much larger than those of 0.93 s^{-1} for Mb immobilized in DL-homocysteine self-assembled gold electrode (Zhang and Li 2000), 1.34 s^{-1} for Mb immobilized in silk fibroin film (Wu et al. 2006), 1.2 s^{-1} for Mb entrapped in agarose hydrogel films in room-temperature ionic liquids (Wang et al. 2005), suggesting a reasonably fast electron transfer between

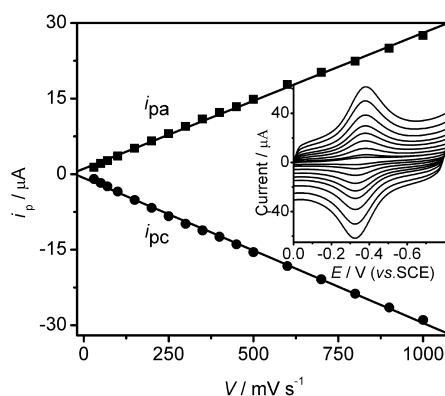


Figure 5. Plot of peak current vs. scan rate. Inset: cyclic voltammograms of Mb/MWNTs-grafted collagen/DMSO/GE in 0.1 M pH 7.0 PBS at 30, 70, 150, 250, 350, 450, 600, 800, and 1000 mV s^{-1} (from lowest to highest peak current).

the immobilized Mb and the electrode due to the presence of MWNTs enhanced grafted collagen.

With the increasing of solution pH from 5.0 to 10.0, the redox peaks shifted negatively. All changes in the peak potentials and currents with solution pH were reversible in the pH range from 5.0 to 10.0. The plot of formal potential versus pH showed a slope of -51 mV pH^{-1} ($R = 0.997$), which was close to -59 mV pH^{-1} expected for a reversible, one-electron coupled one-proton reaction process at 25°C .

Electrocatalytic Response of Mb/MWNTs-Grafted Collagen/DMSO/GE to Hydrogen Peroxide

Upon addition of H_2O_2 to the solution, the shape of cyclic voltammogram for direct electron transfer of immobilized Mb in Mb/MWNTs-grafted collagen/DMSO/GE changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 6A), while the change at bare or MWNTs-grafted collagen/DMSO modified GE was very small (not shown), displaying an obvious electrocatalytic behavior of the Mb to the reduction of H_2O_2 . At an applied potential of -400 mV the amperometric response of the Mb/MWNTs-grafted collagen/DMSO/GE to H_2O_2 was shown in Fig. 6B. Upon addition of an aliquot of H_2O_2 to the buffer solution, the reduction current increased steeply to reach a stable value. The modified electrode achieved 95% of the maximum steady-state current to H_2O_2 in less than 5 s. In the same (Zhao et al. 2006) or longer (Zhao et al. 2004) time Mb-MWNT modified electrode only achieved 90%. This demonstrated

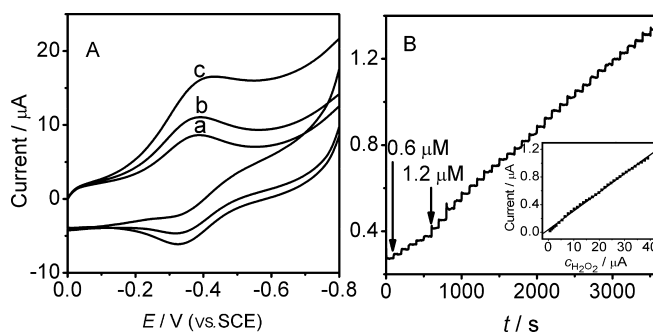


Figure 6. Cyclic voltammograms of Mb/MWNTs-grafted collagen/DMSO/GE in 0.1 M pH 7.0 PBS containing 0 (a) 30 (b) and 90 (c) μM H_2O_2 at 100 mV s^{-1} (A) and amperometric response of the sensor at -400 mV in 0.1 M pH 7.0 PBS upon successive additions of 0.6 and 1.2 μM H_2O_2 (B). Inset: plot of steady-state current vs. H_2O_2 concentration.

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that the electrocatalytic response was very fast. It could be used as an efficient biosensor for H_2O_2 detection. Although the current steps for the catalyzed signal displayed a decreasing current over time, we did not observe the difference among the signals determined for several times at the same concentration after H_2O_2 was added for 25 s. The catalytic current was stable and reproducible after 25 s. The decrease was due to the uneven concentration of H_2O_2 on the electrode surface that resulted from the addition of new H_2O_2 solution.

Analytical Performance of the Amperometric Biosensors

The calibration plot of the sensor was shown as inset in Fig. 6B. The linear response range of the biosensor to H_2O_2 was from 0.6 to 39.0 μM with a correlation coefficient of 0.9982 ($n = 19$). From the slope of 0.027 $\mu\text{A}/\mu\text{M}$ a limit of detection of 0.16 μM was obtained at a signal to noise ratio of 3, which was lower than 4.2 μM for a Mb-MWCNT modified electrode (Zhao et al. 2004) and 8.0 μM for a Mb-hydroxyethylcellulose modified electrode (Liu et al. 2006). The low limit of detection was due to the large specific surface area and high enzyme loading. The sensitivity of the Mb/MWNTs-grafted collagen/DMSO/GE to H_2O_2 was 110 $\text{mA M}^{-1} \text{cm}^{-2}$, which was much higher than those of 0.8125 $\text{mA M}^{-1} \text{cm}^{-2}$ for Mb in MWNT film (Zhao et al. 2006), and 1.7 $\text{mA M}^{-1} \text{cm}^{-2}$ for HRP in porous gold nanoparticle- CaCO_3 hybrid material immobilized with silica sol-gel (Cai et al. 2006).

An enzymatic saturation response was observed when the concentration of H_2O_2 was higher than 39.0 μM , showing a characteristic of the Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant (K_M^{app}) for H_2O_2 was obtained to be 0.106 mM from the electrochemical version of the Linweaver–Burk equation (Kamin and Wilson 1980). The K_M^{app} value for H_2O_2 was much smaller than that of 1.303 mM for Mb immobilized silver nanoparticles- modified electrode (Gan et al. 2004), 1.53 mM for Mb/ ZrO_2 /chitosan modified GCE (Zhao et al. 2005), and 0.65 mM for Mb immobilized colloidal gold nanoparticles-modified electrode (Liu and Ju 2003). Thus, the presence of MWNTs enhanced grafted collagen resulted in a high affinity to H_2O_2 due to the porous network structure of MWNTs enhanced grafted collagen and the good biocompatibility of grafted collagen.

The sensor could retain the direct electrochemistry of the immobilized Mb at constant current value in 0.1 M pH 7.0 PBS upon the continuous cyclic voltammetric sweep over the potential range from -0.8 to $+0.0$ V at 100 mV s^{-1} . After cyclically swept at 100 mV s^{-1} for 50 times the immobilized Mb lost only 4.2% of its initial activity. When the sensor was not in use, it was stored in 0.1 M pH 7.0 PBS at 4°C . A storage period of a week almost did not change the currents of the direct electron transfer and the response to H_2O_2 . The sensor could retain 93% of its initial response to H_2O_2 after a month. The stability was better than those of biosensors for

hydrogen peroxide reported previously (Zhao et al. 2005, Liu et al. 2006). Thus, the presence of grated collagen enhanced the stability of the biosensor and was very efficient for retaining the bioactivity of immobilized Mb and preventing it from leaking out of the biosensor. The fabrication of five electrodes, made independently, showed an acceptable reproducibility with the RSD of 1.4% for the current determination of 20 μM H_2O_2 .

Conclusions

Myoglobin can be effectively immobilized in a MWNTs enhanced grafted collagen matrix. The Mb/MWNTs-grafted collagen/DMSO modified electrode shows a fast direct electron transfer between the Mb and electrode. The biosensor for H_2O_2 exhibits fast response, high sensitivity, low limit of detection, good affinity, operational convenience, good storage stability, and acceptable reproducibility due to the presence of MWNTs enhanced grafted collagen with the uniform porous network structure. The MWNTs enhanced grafted collagen hybrid nanocomposite provides an efficient strategy and a new promising platform for the development of biosensors.

REFERENCES

- Britto, P.J., Santhanam, K.S.V., and Ajayan, P.M. 1996. Carbon nanotube electrode for oxidation of dopamine. *Bioelectrochem. Bioenerg.*, 41: 121–125.
- Cai, W.Y., Xu, Q., Zhao, X.N., Zhu, J.J., and Chen, H.Y. 2006. Porous gold-nanoparticle- CaCO_3 hybrid material: preparation, characterization and application for horseradish peroxidase assembly and direct electrochemistry. *Chem. Mater.*, 18: 279–284.
- Cao, Y., Zhou, Y.M., Shan, Y., Ju, H.X., Xue, X.J., and Wu, Z.H. 2004. $(\text{Ti},\text{Sn})\text{O}_2$ solid solution self-aligned into “sandwich” array on grafted modification collagen matrix. *Adv. Mater.*, 16: 1189–1192.
- Fan, C.H., Suzuki, I., Chen, Q., Li, G.X., and Anzai, J. 2000. An unmediated hydrogen peroxide sensor based on a hemoglobin-SDS film modified electrode. *Anal. Lett.*, 33: 2631–2644.
- Florescu, M. and Brett, C.M.A. 2004. Development and characterization of cobalt hexacyanoferrate modified carbon electrodes for electrochemical enzyme biosensors. *Anal. Lett.*, 37: 871–886.
- Gan, X., Liu, T., Zhong, J., Liu, X.J., and Li, G.X. 2004. Effect of on the electron transfer reactivity and the catalytic activity of myoglobin. *Chem. Bio. Chem.*, 5: 1686–1691.
- Garguilo, M.G., Huynh, N., Proctor, A., and Michael, A.C. 1993. Amperometric sensors for peroxide, choline, and acetylcholine based on electron transfer between horseradish peroxidase and a redox polymer. *Anal. Chem.*, 65: 523–528.
- Huang, W.M., Yang, X.R., and Wang, E.K. 2005. Mimetic membrane for biosensors. *Anal. Lett.*, 38: 3–18.

Amperometric Biosensor for Hydrogen Peroxide**1567**

- Iijima, S. 1991. Helical microtubules of graphitic carbon. *Nature*, 354: 56–58.
- Kamin, R.A. and Wilson, G.S. 1980. Rotating ring-disk enzyme electrode for biocatalysis kinetic studies and characterization of the immobilized enzyme layer. *Anal. Chem.*, 52: 1198–1205.
- Kauppinen, J.K., Moffatt, D.J., Mantsch, H.H., and Cameron, D.G. 1981. Fourier self-deconvolution: a method for resolving intrinsically overlapped bands. *Appl. Spectrosc.*, 35: 271–276.
- Koshio, A., Yudasaka, M., Zhang, M., and Iijima, S. 2001. A simple way to chemically react single-wall carbon nanotubes with organic materials using ultrasonication. *Nano. Lett.*, 1: 361–363.
- Kurusu, F., Koide, S., Karube, I., and Gotoh, M. 2006. Electrocatalytic activity of bamboo-structured carbon nanotubes paste electrode toward hydrogen peroxide. *Anal. Lett.*, 39: 903–911.
- Laviron, E. 1979. General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *J. Electroanal. Chem.*, 101: 19–28.
- Liao, X., Lu, Z., Du, X., Liu, X., and Shi, B. 2004. Collagen fiber immobilized myrica rubra tannin and its adsorption to UO_2^{2+} . *Environ. Sci. Technol.*, 38: 324–328.
- Li, J., Lu, Y.J., Ye, Q., Cinke, M., Han, J., and Meyyappan, M. 2003. Carbon nanotube sensors for gas and organic vapor detection. *Nano. Lett.*, 3: 929–933.
- Liu, H.H., Tian, Z.Q., Lu, Z.X., Zhang, Z.L., Zhang, M., and Pang, D.W. 2004a. Direct electrochemistry and electrocatalysis of heme-proteins entrapped in agarose hydrogel films. *Biosens. Bioelectron.*, 20: 294–304.
- Liu, H.Y., Rusling, J.F., and Hu, N.F. 2004b. Electroactive core-shell nanocluster films of heme proteins, polyelectrolytes, and silica nanoparticles. *Langmuir*, 20: 10700–10705.
- Liu, S.Q. and Ju, H.X. 2003. Electrocatalysis via direct electrochemistry of myoglobin immobilized on colloidal gold nanoparticles. *Electroanalysis*, 15: 1488–1493.
- Liu, S.Q., Leech, D., and Ju, H.X. 2003. Application of colloidal gold in protein immobilization, electron transfer and biosensing. *Anal. Lett.*, 36: 1–19.
- Liu, X.J., Chen, T., Liu, L.F., and Li, G.X. 2006. Electrochemical characteristics of heme proteins in hydroxyethylcellulose film. *Sensor. Actuat. B-Chem.*, 113: 106–111.
- Mimica, D., Zagal, J.H., and Bedioui, F. 2001. Electroreduction of nitrite by hemin, myoglobin and hemoglobin in surfactant Wlms. *J. Electroanal. Chem.*, 497: 106–113.
- Razola, S.S., Blankert, B., Quarin, G., and Kauffmann, J.M. 2003. Phenothiazine drugs as redox mediators in horseradish peroxidase bioelectrocatalysis. *Anal. Lett.*, 36: 1819–1833.
- Ricci, F., Amine, A., Moscone, D., and Palleschi, G. 2003. Prussian blue modified carbon nanotube paste electrodes: A comparative study and a biochemical application. *Anal. Lett.*, 36: 1921–1938.
- Se, K. and Aoyama, K. 2004. Preparation and characterization of graft copolymers of methyl methacrylate and poly(n-hexyl isocyanate) macromonomers. *Polymer*, 45: 79–85.
- Shen, L., Huang, R., and Hu, N.F. 2002. Myoglobin in polyacrylamide hydrogel films: direct electrochemistry and electrochemical catalysis. *Talanta*, 56: 1131–1139.
- Wang, S.F., Chen, T., Zhang, Z.L., Shen, X.C., Lu, Z.X., Pang, D.W., and Wong, K.Y. 2005. Direct electrochemistry and electrocatalysis of heme proteins entrapped in agarose hydrogel films in room-temperature ionic liquids. *Langmuir*, 21: 9260–9266.

- Wu, F.H., Zhao, G.C., and Wei, X.W. 2002. Electrocatalytic oxidation of nitric oxide at multi-walled carbon nanotubes modified electrode. *Electrochem. Commun.*, 4: 690–694.
- Wu, Y.H., Shen, Q.C., and Hu, S.S. 2006. Direct electrochemistry and electrocatalysis of heme-proteins in regenerated silk fibroin film. *Anal. Chim. Acta*, 558: 179–186.
- Xiao, Y., Ju, H.X., and Chen, H.Y. 1999. A reagentless hydrogen peroxide sensor based on incorporation of horseradish peroxidase in poly(thionine) film on a monolayer modified electrode. *Anal. Chim. Acta*, 391: 299–306.
- Xu, S.Y., Tu, G.L., Peng, B., and Han, X.Z. 2006. Self-assembling gold nanoparticles on thiol-functionalized poly(styrene-co-acrylic acid) nanospheres for fabrication of a mediatorless biosensor. *Anal. Chim. Acta*, 570: 151–157.
- Zhang, H.M. and Li, N.Q. 2000. The direct electrochemistry of myoglobin at a DL-homocysteine self-assembled gold electrode. *Bioelectrochemistry*, 53: 97–101.
- Zhao, G.C., Zhang, L., and Wei, X.W. 2004. An unmediated H₂O₂ biosensor based on the enzyme-like activity of myoglobin on multi-walled carbon nanotubes. *Anal. Biochem.*, 329: 160–161.
- Zhao, G.C., Zhang, L., Wei, X.W., and Yang, Z.S. 2003. Myoglobin on multi-walled carbon nanotubes modified electrode: direct electrochemistry and electrocatalysis. *Electrochem. Commun.*, 5: 825–829.
- Zhao, G., Feng, J.J., Xu, J.J., and Chen, H.Y. 2005. Direct electrochemistry and electrocatalysis of heme proteins immobilized on self-assembled ZrO₂ film. *Electrochem. Commun.*, 7: 724–729.
- Zhao, L.Y., Liu, H.Y., and Hu, N.F. 2006. Electroactive films of heme protein-coated multiwalled carbon nanotubes. *J. Colloid. Interf. Sci.*, 296: 204–211.