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Hydrogen peroxide biosensor based on hemoglobin modified zirconia nanoparticles-grafted collagen matrix

Shuizhen Zong [a,b], Yong Cao [c], Yuming Zhou [c], Huangxian Ju [a,*]

[a] MOE Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China
[b] Department of Chemistry, Changshu Institute of Technology, Changshu 215500, PR China
[c] School of Chemistry and Chemical Engineering, Southeast University, Nanjing 210096, PR China

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Abstract

A novel method for the immobilization of hemoglobin (Hb) and preparation of reagentless biosensor was proposed using a biocompatible non-toxic zirconia enhanced grafted collagen tri-helix scaffold. The formed membrane was characterized with UV–vis and FT-IR spectroscopy, scanning electron microscope and electrochemical methods. The Hb immobilized in the matrix showed excellent direct electrochemistry with an electron transfer rate constant of 6.46 s^{-1} and electrocatalytic activity to the reduction of hydrogen peroxide. The apparent Michaelis–Menten constant for H_2O_2 was 0.026 mM, showing good affinity. Based on the direct electrochemistry, a new biosensor for H_2O_2 ranging from 0.8 to 132 μM was constructed. Owing to the porous structure and high enzyme loading of the matrix the biosensor exhibited low limit of detection of 0.12 μM at 3σ, fast response less than 5 s and high sensitivity of 45.6 mA M^{-1} cm^{-2}. The biosensor exhibited acceptable stability and reproducibility. ZrO_2-grafted collagen provided a good matrix for protein immobilization and biosensing preparation. This method was useful for monitoring H_2O_2 in practical samples with the satisfactory results.

Keywords: Biosensor; Direct electrochemistry; Grafted collagen; Zirconia nanoparticles; Hemoglobin; Hydrogen peroxide

1. Introduction

The interaction and direct electron transfer between redox proteins and electrode surface are of great importance for not only studying the electron transfer between biomolecules in biological system, but also investigating the novel enzyme biosensors [1]. Hemoglobin (Hb) is an ideal model molecule for the study of electron transfer reactions of heme proteins, biosensing and electrocatalysis [2]. It has been incorporated in many organic membranes or biomembrane-like microenvironment such as polyacylamide hydrogel [3], SP Sephadex [4], DNA [5], Nafion [6], dimyristoyl phosphatidylcholine [7], poly(ester sulfonic acid) [8], and egg-phosphatidylcholine [9] films for this purpose. Recently, some oxide nanoparticles such as SiO_2 [10,11], TiO_2 [12–14], ZrO_2 [15,16], SnO_2 [17], and WO_3 [18] have also been used for immobilizing proteins and accelerating the electron transfer between the immobilized proteins and electrodes. These studies show the nano-sized materials possess good biocompatibility and high active surface areas for protein loading, regular structures and good mechanical, thermal and chemical stability. This work used a novel biocompatible hybrid material prepared with zirconia nanoparticles and grafted collagen to immobilize Hb on an electrode surface for studying the direct electron transfer of heme proteins and constructing new biosensors.

Advanced hybrid materials, especially nanoparticles enhanced hybrid materials, have been extensively synthesized. However, few researchers pay their attention to the biocompatibility and biosensing application of these materials. Collagen is one of the biopolymers most extensively used to construct functionalized hybrid structures. To strengthen its mechanical and thermal stability extensive efforts have been made to mimic [19] or stabilize [20] its soft conformation. Owing to the abundant oxygen and nitrogen atoms [21], collagen molecule shows good affinity to metal oxide for stabilizing metal oxide nanoparticles away from their aggregation [22]. The presence of nano-sized zirconia enhanced the tri-helix scaffold of collagen, increased the loading of Hb and accelerated the electron transfer of immobilized Hb, which were significant for

* Corresponding author. Fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

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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 [23–25]. Many techniques such as titrimetry [26], spectrometry [27], chemiluminescence [28] and electrochemistry [29–36] have been employed for hydrogen peroxide analysis. The analytical methods for hydrogen peroxide based on the first three techniques are relatively time-consuming, subject to interferences and need expensive reagents. Electrochemical methods overcome these drawbacks. Many electrochemical methods based on the electrocatalysis of immobilized enzymes and/or Hb to H_2O_2 reduction have been developed. Two electrochemical sensors for hydrogen peroxide, prepared with zirconia nanoparticles, have also been reported [15,16]. In this paper the Hb was immobilized in a new tri-helix scaffold of collagen matrix enhanced with nano-sized zirconia. In comparison with those reported previously [15,16], the proposed biosensor showed better analytical performance, such as faster response, wider detection range, lower detection limit, better affinity, higher loading, more simpler operation and better storage stability for electrochemical detection of hydrogen peroxide due to the introduction of the collagen to form well-distributed porous three-dimensional structure. Furthermore, the preparation of the present sensor was much simpler than that in Ref. [16]. The biosensor possessed preparation reproducibility, indicating that the metal oxide nanoparticles-grafted collagen hybrid materials were suitable for protein immobilization and preparation of the third generation biosensors.

2. Experimental

2.1. Reagents

Hb was obtained from Sigma and used without further purification. About 5.0 mg mL^{-1} of Hb solution was stored at 4 °C as stock solution. H_2O_2 (30%, w/v solution) was purchased from Shanghai Jinlu Chemical Engineering Ltd. Co (China). ZrO_2 nanoparticles and grafted collagen were prepared according to the literatures [37,38], respectively. Zirconia enhanced grafted collagen tri-helix scaffold was prepared by dispersing ZrO_2 nanoparticles and grafting collagen in alcohol which was stirred overnight and refluxed at 60 °C for 8 h. The mixture was refrigerated for 2 h to remove impurities, and then separated in room temperature to obtain the hybrid composite (ZrO_2-grafted collagen powder). Other reagents were of analytical reagent grade. About 0.1 M phosphate buffer solutions (PBS) with different pH values were prepared by mixing the stock standard solutions of Na_2HPO_4 and NaH_2PO_4 and adjusting the pH with 0.1 M H_3PO_4 or NaOH. All solutions were prepared with twice-distilled water.

2.2. Apparatus

Electrochemical measurements were performed on a CHI 730A electrochemical analyzer (CHI Co., China) at (18 ± 2) °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 −350 mV on a stirred cell. The sensor 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.

UV–vis absorbance spectroscopy was performed using a UV-Vis-3100-Nir Recording Spectrophotometer (Shimadzu, Japan). 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 H_2SO_4:H_2O_2 (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.

2.3. Preparation of the Hb/ZrO_2-grafted collagen/DMSO modified electrode

The substrate graphite electrodes (GE, geometric area: 28.3 mm^2) 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 ZrO_2-grafted collagen nanoparticles and Hb in the matrix dimethyl sulfoxide (DMSO) was used to prepare ZrO_2-grafted collagen suspension (4.0 mg in 1.0 mL DMSO). About 10 μL of Hb solution and 5 μL of ZrO_2-grafted collagen suspension were cast on GE surface to obtain the Hb/ZrO_2-grafted collagen/DMSO modified electrode. Alternatively, only 10 μL Hb solution, 5 μL ZrO_2-grafted collagen suspension, 10 μL Hb and 5 μL aqueous suspension of ZrO_2-grafted collagen or 10 μL Hb/DMSO solution were cast on the graphite electrode to form Hb/GE, ZrO_2-grafted collagen/DMSO/GE, Hb/ZrO_2-grafted collagen/GE or Hb/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.

3. Results and discussion

3.1. UV–vis and FT-IR spectroscopic analyses

The structure of the obtained hybrid composite (ZrO_2-grafted collagen powder) and the bonding properties of zirconia and collagen were similar to those of Al_2O_3–ZrO_2 co-nanoparticles enhanced tri-helix scaffolds reported in our previous report [39]. Fig. 1 shows the UV–vis spectra of different systems. All sys-

Table 1
Comparison among three Hb/nanoparticles films and relative sensors for H_2O_2

Performance	This work	Reported in Ref. [15]	Reported in Ref. [16]
Peak-to-peak separation at 100 mV s^{-1}	55 mV	33 mV	100 mV
Shift of λ_{max} of UV upon mixing (nm)	No shift	No shift	From 408 to 410
Formal potential (mV)	−328	−361	32
Coverage (10^{-10} mol cm^{-2})	8.48	–	3.3
Linear range	0.8–132 μM	1.5–30 μM	10–1500 μM
Limit of detection	0.12 μM	0.14 μM	4.0 μM
K_m (mM)	0.026	0.31	1.77
Response time (reach 95% maximum steady-state current)	5 s	–	10 s
Stability	94% after 2 months	91% after 2 months	80% after 1 month

tems containing Hb displayed a maximum absorption at about 405 nm (curves a–d). No absorption of DMSO and ZrO_2-grafted collagen/DMSO was observed (curves f and e). Obviously, the absorption peak was attributed to the Soret band of Hb. The presence of ZrO_2-grafted collagen resulted in increase of the absorption (curves a and b). No shift of the Soret band upon mixing of Hb with ZrO_2-grafted collagen or DMSO was observable, as control, the shift of 2 nm when Hb was immobilized in Hb/ZrO_2 self-assembled film was listed in Table 1 [16]. Previous studies have demonstrated that the absorption band would diminish upon the full protein denaturation [40]. Similar to the result reported in Ref. [15], ZrO_2-grafted collagen did not change the fundamental microenvironment of Hb. The Hb mixed in current system retained its natural secondary structure.

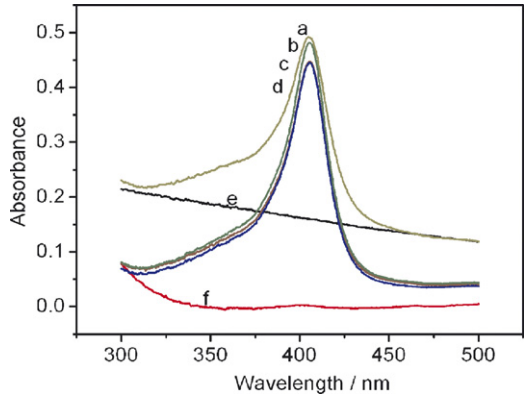

Fig. 1. UV–vis spectra of Hb/ZrO_2-grafted collagen in DMSO (a), Hb/ZrO_2-grafted collagen in water (b), Hb in water (c), Hb in DMSO (d), ZrO_2-grafted collagen in DMSO (e) and DMSO (f).

3.2. SEM characterization

The SEM micrograph of ZrO_2-grafted collagen film displays a chemically clean porous three-dimensional structure. This three-dimensional structure possessed a very narrow particle size distribution with the average diameter ranged between 30 and 40 nm (Fig. 2a). Dispersing of Hb onto a glass slice, the Hb molecules aggregated together in the absence of DMSO and ZrO_2-grafted collagen (Fig. 2b). After mixing Hb with ZrO_2-grafted collagen/DMSO, a well-distributed porous film could be formed (Fig. 2c). In this case, the ZrO_2-grafted collagen nanoparticles were surrounded by Hb molecules, leading to bigger size of nanoparticles. The uniform porous structure of the ZrO_2-grafted collagen film increased the homogeneous loading and affinity to the substrate of protein molecules, and provided good preparation reproducibility of the Hb modified electrodes.

3.3. Direct electrochemistry of Hb/ZrO_2-grafted collagen/DMSO modified electrode

The cyclic voltammogram of Hb/ZrO_2-grafted collagen/DMSO/GE displayed a couple of stable and well-defined redox peaks at −300 and −357 mV at 100 mV s^{-1}, while no obvious electrochemical response was observed at both GE and ZrO_2-grafted collagen/DMSO/GE (Fig. 3). Thus, these peaks were attributed to the redox reaction of the electroactive center of Hb. Hb/GE and Hb/ZrO_2-grafted collagen/GE exhibited only small reduction peaks. Although the Hb/DMSO/GE also displayed a couple of redox peaks of Hb (curve e in Fig. 3), these peak currents were much smaller than those at the Hb/ZrO_2-grafted collagen/DMSO/GE. The improvement in direct electrochemistry in presence of DMSO alone was due to the decrease of the dielectric constant of the microenvironment around Hb molecules, which decreased the reorganization

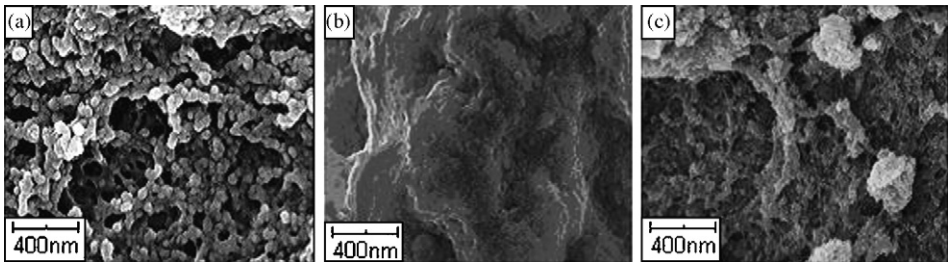

Fig. 2. Scanning electron micrographs of ZrO_2-grafted collagen/DMSO (a), Hb (b) and Hb/ZrO_2-grafted collagen/DMSO (c) films on glass slices.

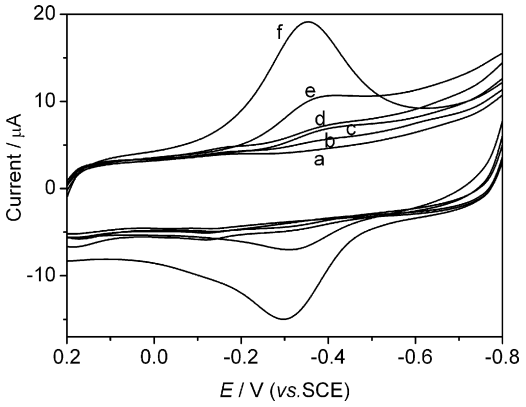

Fig. 3. Cyclic voltammograms of GE (a), ZrO_2-grafted collagen/DMSO/GE (b), Hb/GE (c), Hb/ZrO_2-grafted collagen/GE (d), Hb/DMSO/GE (e) and Hb/ZrO_2-grafted collagen/DMSO/GE (f) in 0.1 M pH 7.0 PBS at 100 mV s^{-1}.

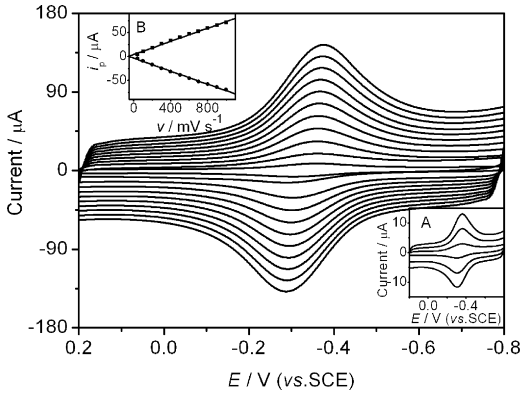

Fig. 4. Cyclic voltammograms of Hb/ZrO_2-grafted collagen/DMSO/GE in 0.1 M pH 7.0 PBS at 40, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV s^{-1} (from lowest to highest peak current). Insets: cyclic voltammograms of this system at 10, 40 and 70 mV s^{-1} (A) and plot of peak current *vs.* scan rate (B).

energy of biological electron transfer [41]. The further increase of the peak currents indicated ZrO_2-grafted collagen was very important for facilitating the electron exchange. From the integration of the reduction peak of Hb/ZrO_2-grafted collagen/DMSO/GE at different scan rates, an average surface coverage of Hb was calculated to be 8.48×10^{-10} mol cm^{-2}, which was larger than 5.60×10^{-11} mol cm^{-2} at $\{SWNT/Hb\}_8$ [42], 1.5×10^{-11} mol cm^{-2} at $\{TiO_2/Hb\}_n$ [34], 2.1×10^{-10} mol cm^{-2} at Hb-gold nanoparticles [43] and 2.93×10^{-11} mol cm^{-2} at Hb-agarose hydrogel [44], indicating a better loading of the Hb in the hybrid composite matrix.

The formal potential $E_{1/2}$ of the heme $Fe^{III/II}$ couple in Hb/ZrO_2-grafted collagen/DMSO/GE, estimated as the mid-point of reduction and oxidation potentials, was $-(328 \pm 2)$ mV (*versus* SCE) in 0.1 M pH 7.0 PBS. This value was similar to those of −348 mV at Hb-agarose hydrogel [44], −334 mV at Hb-silk fibroin [45], −343 mV at Hb-carbon nanotube (CNT) [46], suggesting that most molecules preserved their native structure after being entrapped in the ZrO_2-grafted collagen matrix. The cyclic voltammogram of the Hb/ZrO_2-grafted collagen/DMSO/GE showed a nearly equal height of reduction and oxidation peaks at the same scan rate (Fig. 4). With an increasing scan rate from 10 to 1000 mV s^{-1}, the anodic and cathodic peak potentials of the Hb showed shift in positive and negative directions, respectively, and the redox peak currents increased linearly (inset B in Fig. 4), indicating a surface-controlled electrode process. The peak-to-peak separations of the cyclic voltammograms at 40, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mV s^{-1} were 54, 55, 57, 61, 64, 69, 72, 75, 80, 84 and 88 mV, respectively. The separation of 55 mV at 100 mV s^{-1} indicated a much faster electron transfer rate than that of 100 mV for Hb immobilized in Hb/ZrO_2 self-assembled film [16]. Considering the α value between 0.3 and 0.7 and the peak-to-peak separation less than 200 mV, the average electron transfer rate constant k_s was estimated according to the formula $k_s = mnFv/RT$ [47] to be 6.46 s^{-1}, where m is a parameter related to the peak-to-peak separation. The k_s value was larger than those of 1.05 s^{-1} for Hb immobilized on gold nanoparticles [43], 0.8 s^{-1} for Hb immobilized in agarose hydrogel [44], 0.53 s^{-1} for Hb adsorbed on nanocrystalline tin oxide [17], and 0.062 s^{-1} for Hb entrapped in carbon nanotube [48], suggesting a reasonably fast electron transfer between the immobilized Hb and the electrode due to the presence of ZrO_2-grafted collagen.

3.4. Effect of solution pH on the direct electron transfer of immobilized Hb

Fig. 5 shows the effect of solution pH on the direct electrochemistry of the immobilized Hb. With the increasing of solution pH from 5.4 to 10.0, the negative shift of both reduction and oxidation peak potentials was observed. In general, all changes in the peak potentials and currents with solution pH were reversible in the pH range from 5.4 to 10.0, that was, the same cyclic voltammograms could be obtained if the electrode was transferred from a solution with a different pH value to its original solution. The plot of formal potential *versus* pH showed a slope of −44.7 mV pH^{-1} ($R = 0.996$) (inset in Fig. 5), indicating that one proton participated in the electron transfer process [3].

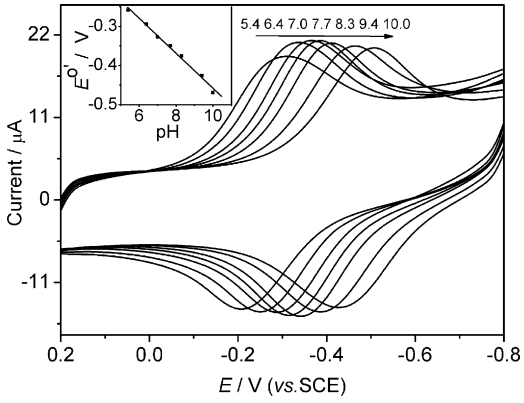

Fig. 5. Cyclic voltammograms of Hb/ZrO_2-grafted collagen/DMSO/GE in 0.1 M PBS with various pH values at 100 mV s^{-1}. Inset: plot of formal potential *vs.* pH.

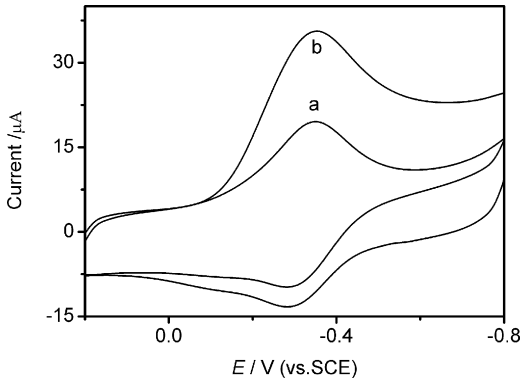

Fig. 6. Cyclic voltammograms of Hb/ZrO_2-grafted collagen/DMSO/GE in 0.1 M pH 7.0 PBS containing 0 μM H_2O_2 (a) and 100 μM H_2O_2 (b) at 100 mV s^{-1}.

3.5. Electrocatalysis of Hb/ZrO_2-grafted collagen/DMSO/GE to reduction of H_2O_2

Upon addition of H_2O_2 to 0.1 M pH 7.0 PBS, the shape of cyclic voltammogram for the direct electron transfer of immobilized Hb changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 6), while the change of cyclic voltammogram of bare or ZrO_2-grafted collagen/DMSO modified GE was very small (inset A in Fig. 7), displaying an obvious electrocatalytic behavior of the Hb to the reduction of H_2O_2. At an applied potential of −350 mV the amperometric response of the Hb/ZrO_2-grafted collagen/DMSO/GE to H_2O_2 was shown in Fig. 7. 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, which was shorter than 10 s at the absences of collagen (Table 1) [16]. This demonstrated clearly that the electrocatalytic response was very fast. 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.

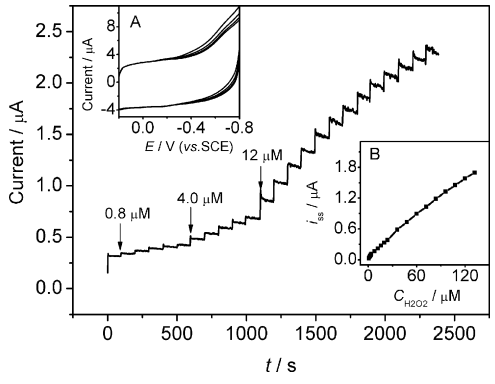

Fig. 7. Amperometric response of the sensor at −350 mV upon successive additions of 0.8, 4.0 and 12.0 μM H_2O_2 to 0.1 M pH 7.0 PBS. Inset: cyclic voltammograms of ZrO_2-grafted collagen/DMSO/GE in 0.1 M pH 7.0 PBS containing 0, 40, 80 and 100 μM H_2O_2 at 100 mV s^{-1} (from inner to outer) (A) and plot of steady-state current *vs.* H_2O_2 concentration (B).

The linear response range of the biosensor to H_2O_2 was 0.8–132 μM with a linear regression equation of I (μA) = 0.068 + 0.013c (μM) (R = 0.9982, n = 19). The linear range was much wider than that reported previously (Table 1) [15]. From the slope the limit of detection for H_2O_2 at a signal-to-noise ratio of 3σ was estimated to be 0.12 μM, which was lower than those listed in Table 1 [15,16], 1.2 μM at Hb/pluronic [35] and 1.0 μM at Hb entrapped clay nanoparticle [36]. The sensitivity of the Hb/ZrO_2-grafted collagen/DMSO/GE to H_2O_2 was 45.6 mA M^{-1} cm^{-2}, which was much higher than those of 2.85 mA M^{-1} cm^{-2} to H_2O_2 at $\{TiO_2/Hb\}_{15}$ [34] and 0.56 mA M^{-1} cm^{-2} to H_2O_2 at Hb-single-walled carbon nanotubes [42].

An enzymatic saturation response was observed when the concentration of H_2O_2 was higher than 132 μM, showing a characteristic of the Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant (K_M^{app}) was obtained to be 0.026 mM for H_2O_2 from the electrochemical version of the Linweaver–Burk equation [49]. The K_M^{app} value for H_2O_2 was much smaller than those listed in Table 1 [15,16], and 0.65 mM at Hb/zirconium phosphate/Au nanoparticles [50]. Thus, Hb had a high affinity to H_2O_2 due to the presence of collagen, which possessed good biocompatibility.

3.6. Interference and real sample analyses

The possible interference might occur in real samples was tested. The results were list in Table 2. No significant interference could be observed for matters such as SO_4^{2-}, CO_3^{2-}, ClO_3^-, Cl^-, Br^-, I^-, glycine and ascorbic acid at concentrations 10 times that of H_2O_2, indicating these matters coexisting in the sample matrix did not affect its determination, indicating that this method was useful for real sample.

To demonstrate the applicability of the proposed biosensor for real sample analysis, 60 and 100 μM H_2O_2 were added to rainwater samples, respectively. The average recovery was 98.4% and 103.5% (n = 5), respectively. The rainwater samples without adding H_2O_2 did not show any detectable signal. Obviously the method presented was simplicity, rapidness and convenience with satisfactory results.

Table 2
Interference of external matters to response of the Hb/ZrO_2-grafted collagen/DMSO/GE to 50.0 μM hydrogen peroxide in 0.1 M pH 7.0 PBS

External matters	Concentration spiked (μM)	Response change (%)
SO_4^{2-}	500	−1.9
CO_3^{2-}	500	−1.3
ClO_3^-	500	0.5
Cl^-	500	−4.9
Br^-	500	1.2
I^-	500	4.6
Glycine	500	6.1
Ascorbic acid	500	2.2

3.7. Stability and reproducibility of the H_2O_2 biosensor

The Hb/ZrO_2-grafted collagen/DMSO/GE could retain the direct electrochemistry of the immobilized Hb at constant current values in 0.1 M pH 7.0 PBS upon the continuous cyclic voltammetric sweep over the potential range from −0.8 to +0.2 V at 100 mV s^{-1}. After cyclically swept at 100 mV s^{-1} for 40 times the immobilized Hb lost only 5.6% 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 responses to H_2O_2. The sensor could retain 94% of its initial response to H_2O_2 after 2 months, which was better than those proposed in Refs. [15,16] (Table 1). Thus, the presence of collagen enhanced the stability of the biosensor and was very efficient for retaining the bioactivity of immobilized Hb and preventing it from leaking out of the biosensor. The fabrication of five electrodes, made independently, showed an acceptable reproducibility with the R.S.D. of 1.4% for the current determination of 50 μM H_2O_2.

4. Conclusions

Hemoglobin can be effectively immobilized in a ZrO_2-grafted collagen matrix. The Hb/ZrO_2-grafted collagen/DMSO modified electrode shows a fast direct electron transfer between Hb and electrode. The uniform porous structure of ZrO_2-grafted collagen provides a microenvironment around the proteins to retain their bioactivity, increases the loading of protein molecules and their affinity to substrates, gives a good reproducibility for preparation of the Hb/ZrO_2-grafted collagen/DMSO modified electrode or biosensor for H_2O_2. The biosensor exhibits good affinity, fast response, wide linear range, low limit of detection, high sensitivity, operational convenience, storage stability and acceptable reproducibility. The ZrO_2-grafted collagen hybrid composite provides an efficient strategy and a new promising platform for the further study on the direct electron transfer of proteins and the development of biosensors.

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References

[1] Z. Zhang, S. Chouchane, R.S. Magliozzo, J.F. Rusling, Anal. Chem. 74 (2002) 163.
[2] J. Ye, R.P. Baldwin, Anal. Chem. 60 (1988) 2263.
[3] H. Sun, N.F. Hu, H.Y. Ma, Electroanalysis 12 (2000) 1064.
[4] C.H. Fan, H.Y. Wang, S. Sun, D.X. Zhu, G. Wagner, G.X. Li, Anal. Chem. 73 (2001) 2850.
[5] A.-E.F. Nassar, J.F. Rusling, J. Am. Chem. Soc. 118 (1996) 3043.
[6] Q.D. Huang, Z.Q. Lu, J.F. Rusling, Langmuir 12 (1996) 5472.
[7] J. Yang, N. Hu, Bioelectrochem. Bioenerg. 48 (1999) 117.
[8] J. Yang, N. Hu, J.F. Rusling, J. Electroanal. Chem. 463 (1999) 53.
[9] X.J. Han, W.M. Huang, J.B. Jia, S.J. Dong, E.K. Wang, Biosens. Bioelectron. 17 (2002) 741.
[10] P.L. He, N.F. Hu, J.F. Rusling, Langmuir 20 (2004) 722.
[11] P.L. He, N.F. Hu, Electroanalysis 16 (2004) 1122.
[12] H. Zhou, X. Gan, J. Wang, X.L. Zhu, G.X. Li, Anal. Chem. 77 (2005) 6102.
[13] Q.W. Li, G.A. Luo, J. Feng, Electroanalysis 13 (2001) 359.
[14] C.A. Paddon, F. Marken, Electrochem. Commun. 6 (2004) 1249.
[15] S.Q. Liu, Z.H. Dai, H.Y. Chen, H.X. Ju, Biosens. Bioelectron. 19 (2004) 963.
[16] G. Zhao, J.J. Feng, J.J. Xu, H.Y. Chen, Electrochem. Commun. 7 (2005) 724.
[17] E. Topoglidis, Y. Astuti, F. Duriaux, M. Grätzel, J.R. Durrant, Langmuir 19 (2003) 6894.
[18] J.J. Feng, J.J. Xu, H.Y. Chen, Electrochem. Commun. 8 (2006) 77.
[19] G.A. Kinberger, W.B. Cai, M. Goodman, J. Am. Chem. Soc. 124 (2002) 15162.
[20] R. Berisio, V. Granata, L. Vitagliano, A. Zagari, J. Am. Chem. Soc. 126 (2004) 11402.
[21] S. Connolly, S.N. Rao, R. Rizza, N. Zaccheroni, D. Fitzmaurice, Coordin. Chem. Rev. 185 (1999) 277.
[22] T. Koide, M. Yuguchi, M. Kawakita, H. Konno, J. Am. Chem. Soc. 124 (2002) 9388.
[23] E.S. Forzani, G.A. Rivas, V.M. Solis, J. Electroanal. Chem. 382 (1995) 33.
[24] J. Kulys, L. Gorton, E. Domingues, J. Emneus, H. Jarskog, J. Electroanal. Chem. 372 (1994) 49.
[25] J. Kulys, L. Wang, A. Maksimoviene, Anal. Chim. Acta 274 (1993) 53.
[26] E.C. Hurdis, H. Romeyn Jr., Anal. Chem. 26 (1954) 320.
[27] C. Matsubara, N. Kawamoto, K. Takamura, Analyst 117 (1992) 1781.
[28] K. Nakashima, K. Maki, S. Kawaguchi, S. Akiyama, Y. Tsukamoto, I. Kazuhiro, Anal. Sci. 7 (1991) 709.
[29] J. Li, S.N. Tan, H. Ge, Anal. Chim. Acta 335 (1996) 137.
[30] M.G. Garguilo, N. Huynh, A. Proctor, A.C. Micheal, Anal. Chem. 65 (1993) 523.
[31] Y. Xiao, H.X. Ju, H.Y. Chen, Anal. Chim. Acta 391 (1999) 299.
[32] H.Y. Liu, N.F. Hu, Anal. Chim. Acta 481 (2003) 91.
[33] S.Y. Xu, G.L. Tu, B. Peng, X.Z. Han, Anal. Chim. Acta 570 (2006) 151.
[34] H.Y. Lu, J. Yang, J.F. Rusling, N.F. Hu, Electroanalysis 18 (2006) 379.
[35] W.J. Shan, P.L. He, N.F. Hu, Electrochim. Acta 51 (2005) 432.
[36] Y. Liu, H.Y. Liu, N.F. Hu, Biophys. Chem. 117 (2005) 27.
[37] A. Lecomte, F. Blanchard, A. Dauger, M.C. Silva, R. Guinebretière, J. Non-Cryst. Solids 225 (1998) 120.
[38] Y. Cao, Y.M. Zhou, Y. Shan, H.X. Ju, X.J. Xue, Z.H. Wu, Adv. Mater. 16 (2004) 1189.
[39] Y. Cao, Y.M. Zhou, Y. Shan, H.X. Ju, X.J. Xue, Adv. Mater. 18 (2006) 1838.
[40] A.-E.F. Nassar, W.S. Willis, J.F. Rusling, Anal. Chem. 67 (1995) 2386.
[41] H. Zhou, J. Am. Chem. Soc. 116 (1994) 10362.
[42] L.Y. Zhao, H.Y. Liu, N.F. Hu, Anal. Bioanal. Chem. 384 (2006) 414.
[43] L. Zhang, X.E. Jiang, E.K. Wang, S.J. Dong, Biosens. Bioelectron. 21 (2005) 337.
[44] S.F. Wang, T. Chen, Z.L. Zhang, X.C. Shen, Z.X. Lu, D.W. Pang, K.Y. Wong, Langmuir 21 (2005) 9260.
[45] Y.H. Wu, Q.C. Shen, S.S. Hu, Anal. Chim. Acta 558 (2006) 179.
[46] C.X. Cai, J. Chen, Anal. Biochem. 325 (2004) 285.
[47] E. Laviron, J. Electroanal. Chem. 101 (1979) 19.
[48] Y.D. Zhao, Y.H. Bi, W.D. Zhang, Q.M. Luo, Talanta 65 (2005) 489.
[49] R.A. Kamin, G.S. Wilson, Anal. Chem. 52 (1980) 1198.
[50] J.J. Feng, J.J. Xu, H.Y. Chen, J. Electroanal. Chem. 585 (2005) 44.