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ORIGINAL PAPER

A magnetic electrode modified with hemoglobin for determination of hydrogen peroxide: distinctly improved response by applying a magnetic field

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Abstract

A facile and highly sensitive biosensor was developed for the determination of hydrogen peroxide (H_2O_2) via electrochemical catalytic reduction of H_2O_2 by hemoglobin (Hb). Hb was enriched and immobilized simply in a chitosan (Chit) membrane on a magnetic electrode to construct an enzyme-like biosensor. The biosensor catalyzes the electrochemical reduction of H_2O_2 under an external magnetic field. The response improved roughly twice as Hb was adsorbed by Chit in an alkaline medium. The response of the biosensor under the magnetic field increased by 16% owing to the paramagnetism of Hb. The effect of pH values on Hb adsorption by Chit, as well as the effect of an external magnetic field on Hb configuration were investigated by UV-vis spectroscopy. The reduction peak current has linear and log-linear relationships with H_2O_2 concentration in the range of 5–250 μmol·L^{-1} and 0.01–1 μmol·L^{-1}, respectively. The detection limit was 0.003 μmol·L^{-1}, with a good sensitivity of 0.227 μA·μM^{-1}·cm^{-2}. The biosensor was successfully applied to the determination of H_2O_2 in milk samples and in disinfectant solutions. Recoveries ranged from 96.3 to 105.4%, and from 95.3 to 107.7%, respectively.

Keywords Hemoglobin · Chitosan · Hydrogen peroxide · Biosensor

Introduction

Hydrogen peroxide (H_2O_2) has significant effects on biological processes of living cells such as regulatory processes of plant hormone response, root growth and stomatal closure [1]. H_2O_2 is an important chemical with the function of bleaching, disinfecting and sterilizing in paper industry, textile industry and food hygiene industry [2]. H_2O_2 is also an enzymatic by-product of many enzymatic reactions. Alcohol oxidase (AOx) is used to oxidize ethanol, and ethanol can be indirectly determined by measuring the by-product H_2O_2 [3]. Glucose oxidase (GOx) is used to quantify glucose in real human perspiration samples by monitoring the reduction of H_2O_2 [4]. L-lactate also can be determined indirectly through H_2O_2, which is generated by the enzymatic reactions [5]. Therefore, it is necessary to sensitively and accurately determine H_2O_2 in many fields [6].

Many analytical methods such as spectrophotometry [7], chromatography [8], chemiluminiscence [9], fluorescence [10] and electrochemistry [11] have been applied for the determination of H_2O_2. In 1960s, residual H_2O_2 in milk was determined with spectrophotometry based on chromatic complex formation between H_2O_2 and titanium salts (titanium tetrachloride or titanium sulfate) [12] or enzymatic reaction between H_2O_2 and chromogenic o-dianisidine under catalysis of horseradish peroxidase [13]. The determination limit was 147 μmol·L^{-1} and 29.4 μmol·L^{-1}, respectively. The spectrophotometric method had some limitations such as corrosive titanium salts and poisonous chromogenic reagent, troublesome and complicated pretreatment, and relatively low sensitivity. Using acidified potassium iodide solution as the mobile phase and the reverse phase capillary column, H_2O_2 was determined with HPLC-DAD, and the limit of detection was 0.829 μmol·L^{-1} [14]. A luminescent $[EuW_{10}O_{36}]^{9-}$-Chitosan film (Eu-POM/Chit) has been synthesized for

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H_2O_2 determination with a detection limit of 0.11 μmol·L^{-1} [15]. The fluorescence-based strategy was inexpensive, sensitive and stabile, but it also has some drawbacks such as complicated construction of fluorescent Eu-POM/Chit films, which might lead to the poor reproducibility.

Because the electrochemical method is simple, sensitive and low-cost, it has attracted attentions of the researchers [16]. There are many materials such as nanomaterials [17], graphene [18], carbon-nanotube [19], and polymer substance [20] modified on the electrodes to achieve the non-enzymatic or enzymatic detection of H_2O_2. Furthermore, the human whole blood modified on graphitized mesoporous carbon electrode was constructed for electrochemical reduction of H_2O_2 with the current sensitivity of 0.048 μA·$μM^{-1}$ [21]. Hb had been immobilized by chitosan/gold nanoparticles/cysteine (Chit/Au NPs/Cys) on Au electrode to determine H_2O_2 with the detection limits of 6.4 μmol·L^{-1}. It showed that the film of Chit/Au NPs provided a friendly environment to Hb [22]. Among these experiments, it was shown that Hb would have an enzyme-like activity, because a heme group consists of an Fe(II) ion, an electron active center held in the porphyrin ring of Hb. A H_2O_2 biosensor was constructed by immobilizing Hb by magnetite-reduced graphene (Fe_3O_4-RGO). The response of H_2O_2 on the Fe_3O_4/RGO/Hb electrode had a significant improvement compared with that on RGO/Hb electrode [23]. Therefore, it was speculated that the tertiary structure of Hb might be unfold partially, and the active center was exposed under the external magnetic field. In the previous work, we proposed a H_2O_2 biosensor based on magnetic molecularly imprinted polymers nanoparticles (MMIPs NPs) modified on the electrode to immobilize Hb for the electrocatalysis of H_2O_2 reduction, and the detection limit was 3 μmol·L^{-1} [24]. Although the sensitivity was improved significantly, a complicated synthesis of MMIPs NPs was needed and the non-conductive MMIPs NPs might hinder the electron transfer.

In the present work, chitosan (Chit) membrane modified on a magnetic glassy carbon electrode (MGCE) was used to immobilize Hb. We found by chance that Hb adsorbed by Chit on MGCE under the alkaline condition had higher bioelectrocatalytic activity than under the neutral condition. The biosensor, Hb/Chit/MCGE, can electrochemically catalyze H_2O_2 reduction. Therefore, a simple, rapid and ultrasensitive biosensor for the determination of H_2O_2 was developed based on Hb enzyme-like activity.

Experimental section

Materials

Hemoglobin (Hb) was obtained from Baoman Biochemical Technology Co., Ltd. (http://baomanbio.bioon.com.cn/). Chitosan (Chit), H_2O_2, sodium dihydrogen phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4) were purchased from Sinopharm Chemical Reagent Co., Ltd. (https://www.reagent.com.cn/). The glassy carbon electrode (GCE) is 4 mm in diameter, and has a detachable core with a magnetic field strength of 0.4 T, which was obtained from Gaoshi Ruilian Technology Co., Ltd. (https://gaossunion.cn.made-in-china.com/).

Preparation of the modified biosensor

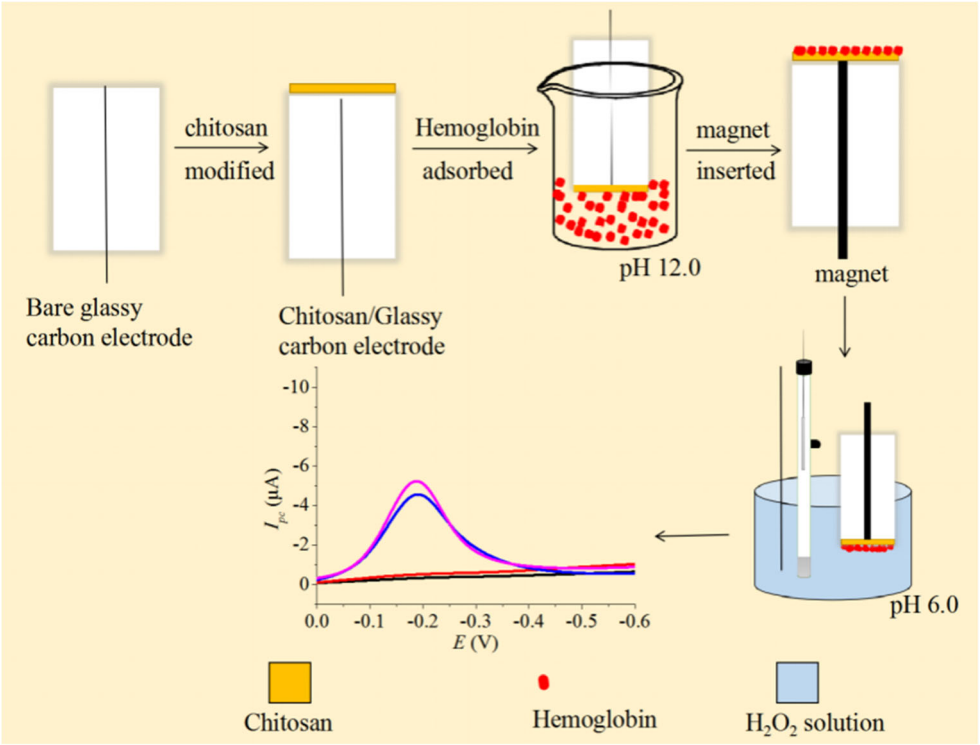
The GCE was burnished with 0.05 μm alumina slurries, and rinsed thoroughly with ultrapure water. 1% acetic acid was used to prepare 2 mg·mL^{-1} Chit aqueous solution. 10 μL Chit solution was cast onto the electrode surface. After completely drying at room temperature, the Chit/GCE was immersed into Hb solution under the alkaline condition, and adsorbed Hb with oscillation (BX2200H, Shanghai) for 20 min. After that, the magnetic core was inserted into Hb/Chit/GCE, and Hb/Chit/MGCE was achieved. Then, ultrapure water was used to wash the Hb/Chit/MGCE several times, Hb/Chit/MGCE biosensor was accomplished as the working electrode for the electrochemical detection. Scheme 1 shows the preparation of the biosensor and the electrochemical detection of H_2O_2.

Apparatus and measurements

The electrochemical measurements of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI660E electrochemical workstation (Chen Hua instruments Co., Shanghai, China). A three-electrode system was used to conduct CV and DPV, the working electrode was the Hb/Chit/MGCE, a platinum electrode was the counter electrode, and the saturated calomel electrode (SCE) was used as a reference electrode. CV conditions: the potential range was from 0.2 V to −0.8 V. DPV conditions: the potential range was from 0 V to −0.6 V, pulse amplitude was 0.15 V, pulse width was 0.04 s, sample width was 0.0167 s and pulse period was 0.08 s. Before the electrochemical experiment, phosphate buffer was purged with N_2 for 30 min to remove O_2 and maintained in the N_2 atmosphere during electrochemical measurements.

Preparation of the samples

3% H_2O_2 disinfectant solution and milk samples were bought from a local market. The preparation of the milk sample was referred to Ref. [25] by acidifying the milk samples, centrifuging and precipitating the proteins. 10 mL milk sample was mixed with 10 mL 0.1 mol·L^{-1} acetate buffer solution (pH 4.5). After cooling to 4 °C and centrifuging at 8000 rpm for 10 min, the supernatants were collected. 10 mL of the supernatant was diluted with 30 mL phosphate buffer



Scheme 1. Construction of the biosensor and principle of H_2O_2 determination based on Hb bioelectrocatalysis

(pH 6.0, 0.02 mol·L^{-1}) to obtain an 8-fold diluted test solution. 3% H_2O_2 disinfectant solution was diluted with an appropriate amount of the phosphate buffer, and was directly determined.

Results and discussion

Electrochemical behaviors

Differential pulse voltammetry (DPV) of H_2O_2 on the different electrodes

Figure 1 shows DPV curves of the background solution and 100 μmol·L^{-1} H_2O_2 on the different electrodes. There was no response on the Hb/Chit/MGCE in the background solution (curve a), which indicated an impossibility of directly electrochemical reduction of Hb, as well as a complete removal of O_2 in the electrolyte solution. Curve b showed that none of response occurred on bare MGCE as 100 μmol·L^{-1} H_2O_2 added in the electrolyte solution. It implied that it was difficult for electrochemical reduction of H_2O_2 on bare electrode without the bioelectrocatalysis of Hb. The reduction peak current (I_{pc}) of H_2O_2 at −0.188 V on the Hb/Chit/GCE was 4.08 μA in curve c. I_{pc} on Hb/Chit/MGCE was 4.74 μA, increased by 16.2%, shown in curve d. It revealed that an external magnetic field can induce the active center of Hb exposed and increase the catalytic activity of Hb on account of the paramagnetism of Hb,.

To verify whether the external magnetic field can influence the molecular conformation of Hb, UV-Vis spectroscopic (TU-1901, Beijing Pu Analysis General Instrument Co., Ltd.) characterization was done, shown in Fig. 2. The absorbance of 100 μg·mL^{-1} Hb in 0.02 mol·L^{-1} phosphate buffer (pH 6.0) was 0.311 at λmax of 405 nm. Under the magnetic field (0.3 T), the

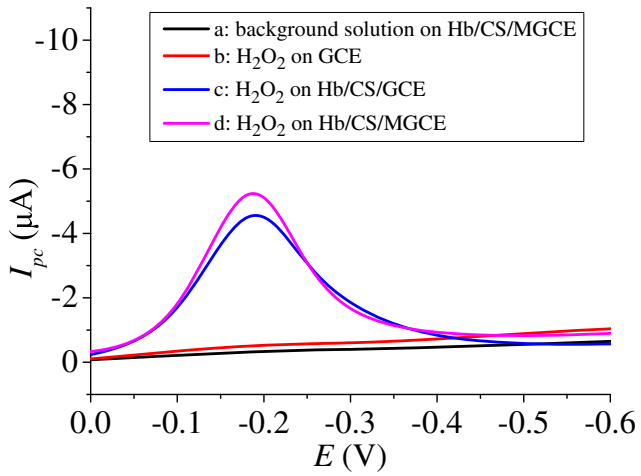


Fig. 1 DPVs of the background solution on Hb/Chit/MGCE (**a**), 100 μmol·L^{-1} H_2O_2 on GCE (**b**), 100 μmol·L^{-1} H_2O_2 on Hb/Chit/GCE (**c**) and 100 μmol·L^{-1} H_2O_2 on Hb/Chit/MGCE (**d**) in 0.02 mol·L^{-1} phosphate buffer (pH 6.0) in N_2 atmosphere. DPV conditions: scan range from 0 V to −0.6 V, pulse amplitude of 0.05 V, pulse width of 0.05 s and pulse period of 0.2 s

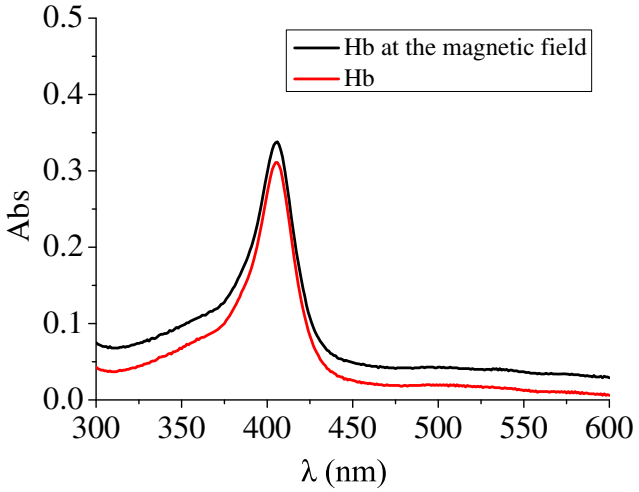


Fig. 2 UV-visible spectra of 100 μg·mL^{-1} Hb in 0.02 mol·L^{-1} phosphate buffer (pH 6.0) (red line) and that under the magnetic field (0.3 T) (black line)

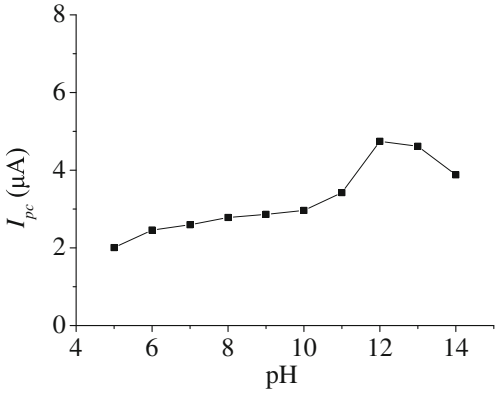


Fig. 3 Optimization of pH of Hb solution during the period of adsorbing Hb by Chit/MGCE. The adsorption condition H_2O_2 concentration: 100 μmol·L^{-1}, adsorption time: 20 min. The DPV conditions were the same as in Fig. 1

absorbance increased to 0.338, while λmax was still 405 nm. Zolghadri found if the peptide chains in Hb spread, the absorption of Sorbet band would increase, while the maximum absorption wavelength remain unchanged [26]. Accordingly, under the external magnetic field, the tertiary structure of Hb might unfold partially, and the active center was exposed. It made the biosensor more sensitive under an external magnetic field.

Effect of scan rates

The relationship between the peak current and scan rate was investigated, shown in Fig. S1. The Fig. S1A shows CV curves of 100 μmol·L^{-1} H_2O_2 in 0.02 mol·L^{-1} phosphate buffer (pH 6.0) changed with the scan rates. The linear relationship between the scan rate and the peak current was shown in Fig. S1B. The linear regression equation was represented as I_{pc} (μA) = 24.051 ν (V·s^{-1}) + 4.516, R^2 = 0.992, indicating it was an adsorption-controlled process.

Optimization of the experimental variables

Adsorption of hemoglobin (Hb)

Hb modified directly on the surface of GCE would make it denatured and inactivated. Chit is a kind of biocompatible polymer, which is a common used support as drug carrier and protein embedding to maintain biological activity. Furthermore, when pH is greater than the isoelectric point of Hb (Ip 6.8), there is an electrostatic interaction between the positively charged Chit and the negatively charged Hb. Therefore, Chit membrane was firstly modified on MGCE to immobilize Hb. In order to adsorb Hb molecules more and steadily, it is very important to optimize pH of Hb solution in the adsorption process by Chit on MGCE. As shown in Fig. 3, I_{pc} increased smoothly as pH ranged from 5.0 to 10.0. While pH increased from 10.0 to 12.0, I_{pc} increased sharply. When pH was larger than 12.0, I_{pc} decreased significantly. I_{pc} was 4.74 μA at pH 12.0, 1.83 times as high as I_{pc} (2.60 μA) at pH 7.0. The alkaline condition favors the dissociation of the carboxyl group in Hb, and Hb carries more negative charges. So, Hb can be enriched and immobilized by positively charged Chit on the electrode under the alkaline medium. However, as the alkalinity was further increased, Hb might denature and inactivate, which lowered down Hb enzyme-like activity. So the optimum pH during adsorbing Hb by Chit/MGCE was 12.0.

Adsorption time for Hb by the modified electrode was also a key factor affecting the analysis time as well as the sensitivity of the determination. The Fig. S2 shows the effect of adsorption time on I_{pc}. I_{pc} increased until the adsorption time was 20 min, after that, the adsorption of Hb reached equilibrium. Therefore, the optimum adsorption time for Hb on Chit / MGCE was 20 min.

UV-vis spectroscopic study on Hb-chit interaction at different pH values

The configuration and enzyme-like activity of Hb would be influenced by pH of Hb aqueous solution. UV-Vis absorption spectrum of Hb provides the information on the conformational integrity of the heme group region in heme protein. Therefore, UV-Vis absorption spectra of Hb and Hb-Chit solution at different pH were conducted, shown in Fig. 4. A Soret band, the intrinsic absorption peak of Hb, kept at 405 nm as pH 5.0–8.0, which indicated that Hb maintained its configuration at pH 5.0–8.0. However, as Hb dissolved in acid or alkaline medium (pH 4.0 or 12.0–14.0), the Soret band of Hb would be

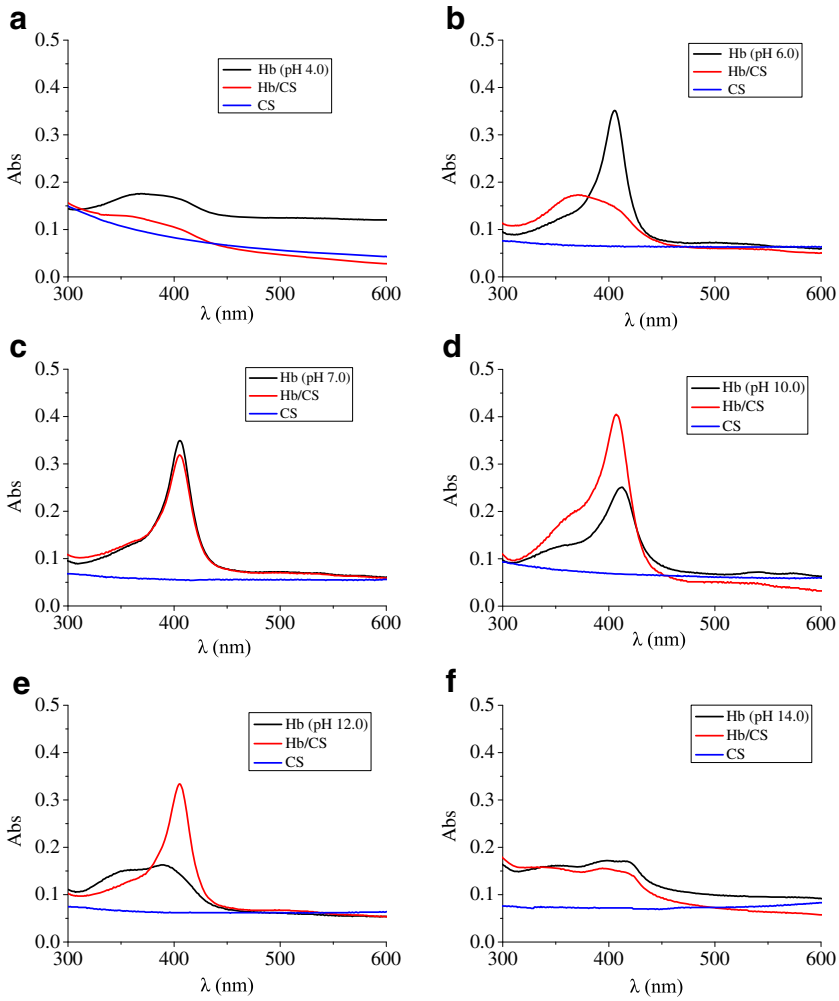


Fig. 4 UV-visible spectra of 100 μg·mL^{-1} Hb (black lines), Hb/Chit (100 μg·mL^{-1}/ 200 μg·mL^{-1}) (red lines) and 200 μg·mL^{-1} Chit (blue lines) at different pH (4.0, 6.0, 7.0, 10.0, 12.0, 14.0)

blue-shifted, and a new broad band appeared. Whereas, Hb in weak alkaline conditions (pH 9.0–11.0), the Soret band was red-shifted slightly, and a new broad band appeared too. It was speculated that the partial denaturation of Hb molecules occurred [27]. Fixed Hb at 100 μg·mL^{-1}, 200 μg·mL^{-1} Chit was added in different pH buffer solution. At pH 7.0, close to *Ip* 6.8, the UV-Vis absorption spectrum of Hb-Chit was the same as that of Hb. It implied the smallest interaction between Hb and Chit at pH 7.0. As pH at 10.0 and 12.0, the absorbance of Hb-Chit increased significantly. Unexpectedly, both Soret band of Hb in Hb-Chit mixed solution at 10.0 and 12.0 came back 405 nm, blue-shift from 412 nm at pH 10.0 and red-shift from 388 nm at pH 12.0. It was concluded that strong electrostatic interaction between Hb and Chit in the alkaline condition made Hb tertiary structure unfolded partially and electroactive center exposed. On the other hand, the Soret band returned to 405 nm revealed that Chit membrane maintained Hb biological activity at pH 12.0. Therefore, an alkaline condition makes more Hb molecules adsorbed by Chit, and it would not destroy Hb enzyme-like activity kept by Chit biocompatible environment. Thus, Hb adsorbed by Chit membrane on MGCE at pH 12.0 gave the highest sensitivity for the determination of H_2O_2 by Hb bioelectrocatalysis.

Electrochemical measurements

Hb is an enzyme-like catalyst, which should have the most suitable pH to catalyze H_2O_2 in the electrolyte solution. As shown in Fig. 4, the Soret band of Hb kept at 405 nm as pH 5.0–8.0. Therefore, at the pH range, Hb maintained the intrinsic configuration and enzyme-like activity. The assumption was accorded with the experimental results, shown in Fig. S3A. The peak current of H_2O_2 was high as pH between 5.0 and 8.0. Beyond the pH range, the current declined. On the other hand, pH of the phosphate buffer is an important parameter for the reduction reaction of H_2O_2. E_{pc} varies linearly with the pH of the electrolyte solution, shown in Fig. S3B. The

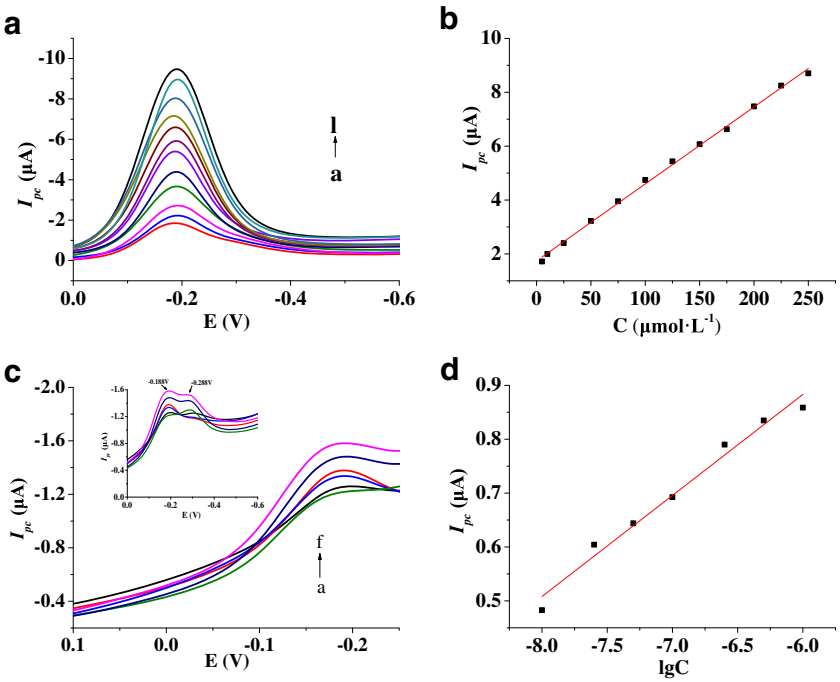


Fig. 5 DPVs of H_2O_2 in 0.02 mol·L^{-1} phosphate buffer (pH 6.0) on Hb/Chit/MGCE. Concentration of H_2O_2 (a-l): 5, 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 μmol·L^{-1}(**a**); the linear relationship between peak currents and the H_2O_2 concentration (**b**); DPVs of H_2O_2 in different concentration on Hb/Chit/MGCE. Concentration of H_2O_2 (a-f): $1.0'10^{-8}$, $2.5'10^{-8}$, $5.0'10^{-8}$, $1.0'10^{-7}$, $2.5'10^{-7}$, $5.0'10^{-7}$, $1.0'10^{-6}$ mol·L^{-1}(**c**); the linear relationship between peak currents and the logarithmic of H_2O_2 concentrations (**d**). The DPV conditions were the same as in Fig. 1

equation was $E_{pc} = -0.0604\ pH + 0.1576$, $R = 0.993$. The slope of −0.0604 V·pH $^{-1}$ was near to the theoretical value −0.0591 V·pH $^{-1}$, which proved an electron-proton reaction and verified the following mechanisms [28]:

$$HbFe(II) + H_2O_2 \rightarrow HbFe(IV) = O + H_2O$$
$$HbFe(IV) = O + H^+ + e^- \rightarrow HbFe(III)$$
$$HbFe(III) + H^+ + e^- \rightarrow HbFe(II)$$

Thus, a proton takes part in the electrochemical reaction. The acidic condition would be helpful for electrochemical reduction of H_2O_2. pH of the electrolyte solution at 6.0 was the reasonable compromise based on electrochemical reaction and Hb enzyme-like activity.

Response time was the important parameter to evaluate the performance of the biosensor. In order to quickly and accurately determine H_2O_2, the effect of accumulation time on the I_{pc} of H_2O_2 was studied, shown in Fig. S3C. Till the incubation time larger than 3 min, I_{pc} remained unchanged, and the response reached equilibrium. Thus, the greatest I_{pc} was at the incubation time of 3 min, which was chosen as the optimum response time for electrochemical reduction of H_2O_2 by Hb catalysis.

Analytical performance

Under the optimum conditions, a set of H_2O_2 standard solution with different concentrations were determined on Hb/Chit/MGCE in a N_2 atmosphere. I_{pc} increased with the increase of H_2O_2 concentration, shown in Fig. 5. I_{pc} and the concentration of H_2O_2 had a linear relationship between 5 and 250 μmol·L^{-1}, shown in Fig. 5a and b. The linear

Table 1 Comparison of different Hb modified electrodes for the determination of H_2O_2

Hb modified electrode	Linear range (μmol·L^{-1})	Detection limit (μmol·L^{-1})	References
Hb/Polyaniline/multiwall carbon nanotubes/ starch/CPE	100–5000	32	[30]
Hb/sodiumalginate MWCNTs/GCE	40–200	16.41	[28]
Hb/ triacetone triperoxide/CPE	19.58–117.45	5.53	[31]
Hb/Au–zirconium phosphate nanocomposite/ GCE	15–480	7.4	[32]
Hb/MMIPs NPs/MGCE	25–200	3	[24]
Electrospun-Hb-collagen/GCE	5–30	0.37	[33]
Hb-AgNPPdop@CNPs/GCE	1–147	0.3	[34]
Hb/Au/graphene-Chit/GCE	2–935	0.35	[35]
Hb/Chit/MGCE	5–250, 0.01–0.1	0.003	This work

Table 2 Results of the H_2O_2 detection in real samples

Samples	I_{pc} (μA)	Detected (μmol·L^{-1})	Average (μmol·L^{-1})	RSD ($n = 3$) %	Content in sample
milk	0.781	0.281	0.284 ±0.011	3.62	77.25 ± 2.99 μg·L^{-1}
	0.784	0.295			
	0.779	0.275			
disinfectant	3.129	48.71	51.04 ± 2.33	3.48	3.09 ± 0.12%
	3.203	51.34			
	3.253	53.09			

equations were I_{pc} (μA) = 0.0285 C_{H2O2} (μmol·L^{-1}) +1.74 (R = 0.998). As H_2O_2 concentration was lower than 1 μmol·L^{-1}, I_{pc} was linear with logarithmic concentration of H_2O_2 ranged from 0.010 μmol·L^{-1} to 1.0 μmol·L^{-1}. The semi-logarithmic equation was I_{pc} (μA) =0.187 lgC_{H2O2} (mol·L^{-1}) + 2.09 (R = 0.991), shown in Fig. 5c and d. Besides H_2O_2 reduction peak at E_{pc} = −0.188 V, the small peaks at E_{pc} = −0.288 V appeared, shown in the inset of Fig. 5c. These small peaks were ascribed to the reduction of oxygen, which might be brought in during injecting samples into the phosphate buffer. Such a small amount of oxygen could be detected, because the electrochemical reduction of O_2 was also catalyzed by Hb [29]. Luckily, the reduction peak of oxygen (E_{pc} = −0.288 V) was apart from the peak of hydrogen peroxide (E_{pc} = −0.188 V). Therefore, O_2 and H_2O_2 did not interfere with each other. Based on the signal of three-fold noise (standard deviation of the response in background solution), the detection limit for H_2O_2 was 0.003 μmol·L^{-1}, and the sensitivity was 0.227 μA·μM^{-1}·cm^{-2}. Table 1 compared the analytical performance for determination of H_2O_2 on Hb/Chit/MGCE with on the other Hb modified electrodes reported in the literature. Hb/Chit/MGCE made the sensitivity improved significantly and the determination range broaden.

Under the same optimum conditions, three Hb/Chit/MGCEs were used to determine 100 μmol·L^{-1} H_2O_2 for evaluation of the biosensor reproducibility. The RSD for the I_{pc} was 2.2%, implying the reproducibility of the biosensor was good. To evaluate the stability of the Hb/Chit/MGCEs, 100 μmol·L^{-1} H_2O_2 were determined by Hb/Chit/MGCEs after stored at 4 °C in a refrigerator for seven days under the optimum conditions. The peak current (4.55 μA) was about 96% of its initial response (4.74 μA). These results indicated that the stability of the Hb/Chit/MGCE could be acceptable.

The selectivity of the Hb/Chit/MGCE was studied by adding 5-fold concentration of the biological compounds, electrochemical active substance as well such as L-glutamic acid, L-cysteine, L-tyrosine, L-tryptophan, glutathione, ascorbic acid, dopamine and glucose into 100 μmol·L^{-1} H_2O_2 solution to evaluate the electrochemical response on the biosensor. All of the RSDs for I_{pc} were less than 5%. So these coexistent species did not interfere with the determination of H_2O_2. A large amount of O_2 would interfere the determination of H_2O_2, so O_2 should be removed during the electrochemical measurement. Luckily, O_2 at trace level did not influence H_2O_2 determination, since the peaks of them were separated completely.

Determination of H_2O_2 in the real samples

H_2O_2 is used as an antibacterial agent in the process of treating milk samples, and 0.05% was the maximum allowance treatment level in food [36]. However, a trace residue of H_2O_2 might still be in the milk samples [37]. Here, H_2O_2 content in milk sample was determined with the Hb/Chit/MGCEs. The results were shown in Table 2. H_2O_2 in the test solutions of milk samples were only 0.284 μmol·L^{-1}. Since the milk samples were diluted by 8 fold, the content of H_2O_2 in the

Table 3 Results of the recovery experiments (n = 3)

Samples	Added (μmol·L^{-1})	Found (μmol·L^{-1})	RSD (%)	Recovery (%)
milk	0	0.284 ± 0.011	3.62	/
	0.25	0.541 ± 0.025	4.33	102.8
	0.50	0.766 ± 0.026	3.10	96.3
	0.75	1.075 ± 0.036	3.15	105.4
disinfectant	0	51.04 ± 2.33	3.48	/
	25	77.42 ± 1.22	1.86	107.7
	50	94.98 ± 0.97	1.38	98.7
	75	122.52 ± 2.77	1.53	95.3

milk was 77.25 μg·L^{-1}. Such ppb level of H_2O_2 in milk samples could be detected, which testified the high sensitivity of the biosensor.

3% H_2O_2 disinfectant solution samples were diluted by 4412 times with 0.02 mol·L^{-1} phosphate buffer (pH 6.0), and measured directly under the optimal conditions. The average content of H_2O_2 in disinfectant solution was 3.09%, RSD = 3.48% (n = 3). The experimental results of the disinfectant solution content measured with the biosensor were agreed with the declared values.

In order to evaluate the accuracy and reliability of the biosensor further, the standard solution of H_2O_2 were spiked to the milk and disinfectant samples. The results of the recovery experiments were summarized in Table 3. The range of recoveries was 96.3%–105.4%, and 95.3%–107.7%, respectively.

Conclusions

A simple, robust and highly sensitive biosensor for determination of H_2O_2 was developed. Since Chit and Hb have a strong electrostatic interaction in an alkaline medium, Hb was enriched and immobilized by Chit on the electrode. Therefore, the electrochemical response increased roughly twice. Under an external magnetic field, I_{pc} of H_2O_2 increased by 16.2% owing to Hb paramagnetism. Without any nanomaterial modification, the biosensor was constructed through Hb simply adsorbed by Chit on a magnetic electrode. The developed biosensor can determine ppb-level H_2O_2 in milk samples successfully, which should be promising for the determination of H_2O_2 at trace level in practical applications.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interest.

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