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The effect of the chitosan membrane properties on the enzyme adsorption and performance for the construction of horseradish peroxidase biosensors

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ABSTRACT

The molecular weight, degree of deacetylation and the acetic acid concentration of chitosan solutions were modulated to change the structures and properties of the chitosan membranes, including chemical structures, ionic conductivity and hydrophobicity, which were analyzed by FTIR-ATR, electrochemical impedance and water contact angle measurement, respectively. Consequently, the adsorption of horseradish peroxidase (HRP, as a model) was controlled by the structural and natural changes of chitosan membranes, exhibiting different adsorbed amount and activity. A HRP-based electrode coated with the chitosan membrane was further constructed to investigate the influence of the chitosan membrane properties on the electrochemical performance. Therefore, this work offered a fundamental understanding of the control of the enzyme adsorption and performance through changing the chitosan membrane structures and properties for the more extensive applications, especially in the construction of the highly efficient bioelectrodes.

1. Introduction

Chitosan membranes have been applied extensively in the wound dressing materials, protein adsorption and separation, enzyme immobilization and drug delivery (Khor, 2002; Krajewska, 2004; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Orrego, Salgado, Valencia, Giraldo, Giraldo, & Cardona, 2010; Rinaudo, 2006). Especially, chitosan membranes are applied in the construction of bioelectrodes and biosenors as an effective support to adsorb enzymes with the unique polycation property and excellent film-forming ability (Tan et al., 2010; Zhang & Ji, 2010). Thus the adsorption of enzyme on the chitosan membrane is an important process and the control of the process has also become interesting for the high efficiency in applications.

For the control of enzyme adsorption, compared with the optimization of the reaction conditions in the adsorption, such as the pH and ionic strength (Krajewska, 2000; Ye, Jiang, & Xu, 2007), the change of the chitosan membrane structures and properties can be considered as a key to offer a thorough change and control of the enzyme (or protein) adsorption. Some other chemicals have introduced to the chitosan membrane surface and network to change the structures and properties through a few physical and chemical methods, such as the surface modification and hybrid/blending techniques (Chao, 2008; Hoven, Tangpasuthadol, Angkitpaiboon, Vallapa, & Kiatkamjornwong, 2007; Liu, Li, Zhao, Yao, & Liu, 2002; Zhang, Li, Gong, Zhao, & Zhang, 2002). For example, the surface charge of the chitosan membrane can also be changed through a modification with the *N*-sulfofurfuryl groups. As a result, the negatively charged chitosan membrane can perform a selectively adsorption to the positively charged proteins rather than the negatively charged proteins (Hoven et al., 2007). These methods have revealed some potential factors influencing the enzyme adsorption, such as hydrophobicity and network structures. However, with the introduction of other chemicals, it may hardly confirm that the effects on the enzyme adsorption are indeed ascribed to either the changes of chitosan membrane structures and properties or the chemicals on the chitosan membrane surface and in the network, or a combination of both contributions.

For the chitosan membrane without the introduction of other substances, the changes of structures and properties, such as the permeability, ionic conductivity, swelling capability and mechanical property, can also be caused by two intrinsic and structural parameters of chitosan, molecular weight (MW) and degree of deacetylation (DDA), and the acid concentration of chitosan solutions in the membrane formation (Chen, Zheng, Wang, Lee, & Park, 2002; Ren, Yi, Wang, & Ma, 2005; Santos, Seabra, Veleirinho, Delgadillo, & Lopes da Silva, 2006; Takahashi, Imai, & Suzuki, 2007; Wan, Creber, Peppley, & Bui, 2003). These parameters are related to the chitosan chain conformation in aqueous solutions and influence the solubilization of chitosan, the protonation degree of chitosan amino groups, and the membrane formation (Berth & Dautzenberg, 2002; Brugnerotto, Desbrières, Heux, et al.,

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Table 1
The parameters of chitosan samples and membranes.[a]

Chitosan membranes	Chitosan samples	MW[b,c] (kDa)	DDA[c] (%)	AAC (%)
CM-48k	CS-48	48	92	10.0
CM-98k	CS-98	98	92	10.0
CM-180k	CS-180	180	92	10.0
CM-480k	CS-480	480	92	10.0
CM-LDA	CS-LDA	1000	86.5	10.0
CM-MDA	CS-MDA	1000	92.1	10.0
CM-HDA	CS-HDA	1000	97.5	10.0
CM-10	CS-Com[d]	1000	90	10.0
CM-7	CS-Com	1000	90	7.0
CM-5	CS-Com	1000	90	5.0
CM-4	CS-Com	1000	90	4.0
CM-1	CS-Com	1000	90	1.0

[a] The chitosan membrane, CM-48, was prepared with the chitosan sample, CS-48, dissolved in 10% acetic acid solution. Other samples were signed similarly.
[b] The molecular weight is the viscosity-average molecular weight.
[c] Data were supplied by the manufacturer.
[d] CS-Com is a commercial chitosan sample without controlling the MW and DDA precisely.

2001; Brugnerotto, Desbrières, Roberts, & Rinaudo, 2001; Rinaudo, Pavlov, & Desbrières, 1999).

Although these parameters play an important role in the changes of chitosan membrane structures and properties, less attention has yet been paid to the role of these parameters in the control of the enzyme adsorption on the chitosan membranes. Moreover, in recent years, chitosan membranes have also been prepared as a support for enzyme adsorption and immobilization extensively in the literature, but these researches often focus on the enzyme rather than the chitosan membranes (Krajewska, 2004). Therefore, it is required to further understand the influence of the structural and natural changes of chitosan membranes caused by these parameters on the enzyme adsorption. The control of the adsorption without the introduction of other substances may reveal the interactions between the enzyme and the membranes and the factors influencing the performance of the adsorbed enzyme.

In this work, our investigation on the structural and natural changes of the chitosan membrane dependent on the structural parameters and the relationship between the changes and the enzyme adsorption may allow us to control the enzyme adsorption on the chitosan membranes, and then offer the fundamental understandings for the applications of the chitosan membrane in the construction of highly efficient bioelectrodes and biosensors. The chitosan membranes with different MW and DDA were prepared. The acetic acid concentration (AAC) of chitosan solutions was also modulated in the membrane formation. The chemical structures, conductivity and hydrophobicity of different chitosan membranes were analyzed by attenuated total reflectance Fourier-transform infrared spectroscopy (FTIR-ATR), electrochemical impedance and water contact angle measurement, respectively. As a model, horseradish peroxidase (HRP) was chosen to be adsorbed on chitosan membranes. The amount and activity of adsorbed HRP were determined and related to the changes of the chitosan membranes. Finally, the different chitosan membrane adsorbed HRP were used to construct the bioelectrodes and the influence of the chitosan membrane properties on the electrode sensitivity was investigated.

2. Materials and methods

2.1. Materials

Chitosan samples were all donated by Golden-shell Biochemical Co., Ltd. (Zhejiang Province, China). The molecular weight (MW) and the degree of deacetylation (DDA) of chitosan samples were listed in Table 1. Data were supplied by the manufacturer. Horseradish peroxidase (HRP) (>250 U/mg) was purchased from Dongfeng Biotechnology Co. Ltd., Shanghai, China. Lysozyme (10,000 U/mg) was obtained from Zeheng Biotechnology Co., Ltd., Shanghai, China. Trypsin (>1000 U/mg) was offered by Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China. All other chemicals and reagents were of analytical grade.

2.2. Preparation and analysis of chitosan membranes

Chitosan was dissolved in 10% (v/v) aqueous acetic acid solution. The concentration of chitosan was 10.0 mg mL^{-1}. The solution was filtered to remove possible undissolved materials. Then 4.0 mL filtrated solution was poured into a polyethylene terephthalate (PET) dish (3.0 cm × 3.0 cm × 2.0 cm) and dried at 60 °C for 15 h. The obtained different chitosan membranes (Table 1) (about 3.0 cm × 3.0 cm × 0.035 cm) were analyzed by attenuated total reflectance Fourier-transform infrared spectroscopy (FTIR-ATR) (Nicolet Nexus FTIR 670 spectrophotometer, USA). For the comparison, the membranes were immersed into phosphate buffer solutions (PBS, pH 7.0, 0.1 mol L^{-1}) for 1 h to remove the residual acetic acid in the membranes and then dried at 60 °C for 15 h. The rinsed chitosan membranes were also analyzed by FTIR-ATR and their water contact angles (WCA) (placed on a flat glass) were also measured (Dataphysics OCA20, Germany).

2.3. The ionic conductivity of chitosan membranes

A bare gold electrode was used here and pretreated according to our previous work (Liu, Jin, Yang, Chen, & Lin, 2007; Yang, Li, Jiang, Chen, & Lin, 2006). 40 μL prepared chitosan solution was cast onto the surface of gold electrode and dried at 60 °C for 15 h. The ionic conductivity of chitosan membranes was measured by electrochemical impedance spectroscopy (EIS) method. EIS was performed on a CHI650C instrument (CH Instrument, Inc., China) with a conventional three-electrode cell. The gold electrode coated with the chitosan membrane was applied as the working electrode. A platinum foil and a saturated calomel electrode (SCE) were used as the counter electrode and the reference electrode, respectively. A 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution (PBS, pH 7.0, 0.1 mol L^{-1}) was used as the redox probe and the perturbation signal was 10 mV. EIS was recorded with the frequency range of 0.01–100,000 Hz (vs. SCE).

2.4. Enzyme adsorption

Three enzyme (HRP, lysozyme and trypsin) solutions were prepared using PBS (0.1 mol L^{-1}) with different pH equaling to the respective isoelectric point (pI) of the dissolved enzyme (pH = 7.2, 11.1, and 6.0 for HRP, lysozyme, and trypsin, respectively). The concentration of enzyme was 10.0 μmol L^{-1}. A 2.0 cm × 1.0 cm prepared chitosan membrane (without neutralized) was immersed into 4.0 mL enzyme solution for 1 h and then the chitosan membrane with HRP was rinsed thrice by deionized water. The chitosan membrane was immersed into 4.0 mL PBS (enzyme pI, 0.1 mol L^{-1}) without enzyme as the control experiment. The protein concentration of the enzyme solution was assayed by the Bradford's method (Bradford, 1976). Every assay was performed triplicately.

2.5. Determination of HRP activity

The Worthington method was used to determine the activity of HRP as described previously with some modifications (Jiang, Chen, Yang, Lin, & Lin, 2004; Maehly & Chance, 1954). The chitosan membrane with HRP was immersed into a 5.0 mL color reagent consisting of 1% (v/v) fresh H_2O_2 solution, 0.50 g L^{-1} 4-amino antipyrine and 16.0 g L^{-1} phenol in PBS (pH 7.0, 0.1 mol L^{-1}). The mixture was shaken at 37.0 °C and a red compound could be

produced. After 15 min, the absorbance was determined at 510 nm spectrophotometrically (Shimadzu UV-2550 spectrophotometer, Japan). The activity was directly proportional to the absorbance. Thus the unit (U g^{-1} protein) of enzyme activity was defined as 1 mg HRP producing the 1 absorbance value of the red compound from the mixture per minute. Every assay was performed triplicately.

2.6. Construction of the HRP-based electrodes and the electrochemical measurements

The gold electrode coated with a chitosan membrane was prepared as mentioned in Section 2.3. Then the modified gold electrode was immersed into 1.0 g L^{-1} HRP solutions (PBS, pH 7.0, 0.1 mol L^{-1}) for 1 h and rinsed thrice by deionized water. The electrochemical measurements were performed on the same instrument mentioned above. An Ag/AgCl (saturated with KCl) was the reference electrode here. Then the modified electrode was fixed in the electrochemical cell containing 200 μmol L^{-1} H_2O_2 in 25 mL PBS (pH 7.0, 0.1 mol L^{-1}) which was mechanically stirred at a constant rate at room temperature. The current was recorded at −0.15 V (vs. Ag/AgCl), allowing the steady-state current to be reached. Next, 50 μL 0.02 mol L^{-1} hydroquinone solution (PBS, pH 7.0, 0.1 mol L^{-1}) was added periodically and the changes of the current were recorded.

3. Results and discussion

3.1. The preparation and analysis of chitosan membranes

Chitosan membranes could be successfully prepared by a solvent evaporation method. FTIR-ATR spectra of the chitosan membrane surface reflected the change of the structures (shown in Fig. 1). The bands of 1540–1550 cm^{-1}, 1400–1410 cm^{-1} and 1100–1000 cm^{-1} were assigned to $–COO^-$ of the residual acetic acids, $–NH_4^+$ of the ionized chitosan molecules and C–O of the chitosan, respectively. A high intensity of the bands at 1540–1550 cm^{-1} and 1400–1410 cm^{-1} of CM-10 could be observed because a high AAC of the chitosan solution was used (Fig. 1A). The amount of $–COO^-$ and $–NH_4^+$ in the membrane increased with increasing chitosan MW (Fig. 1B). The higher MW chitosan and ACC solution were used, the more acetic acid could be retained. 25 kDa MW chitosan was also used to prepare membrane for the analysis, but the membrane was very friable and even spited during drying process. The phenomenon was also observed by Park's group, and the low chitosan MW could decrease the membrane mechanical properties, such as yield and tensile strength, which was ascribed to a loss of the membrane rigidity (Chen et al., 2002; Santos et al., 2006). When the AAC of the chitosan solution was lower than 1% (v/v), the phenomenon was similar to that of 25 kDa MW chitosan. It could imply that the $–COO^-$ in the membrane could have a strong interaction with $–NH_4^+$ of chitosan to form a stable matrix owing to hydrogen and ionic bonds, and then the rigidity of chitosan membrane could be enhanced.

The amount of residual acetic acid increased with the increasing DDA due to the more amino groups of the higher DDA chitosan (Fig. 1C). The membranes were immersed in PBS (pH 7.0, 0.1 mol L^{-1}) to remove the residual acetic acid. Two characteristic bands assigned to $–COO^-$ and $–NH_4^+$ in FTIR-ATR spectra both remarkably decreased (Fig. 1C). Most of residual acetic acid in all prepared chitosan membranes could be eliminated after 1 h in PBS. When these chitosan membranes were dried, all membranes became friable, similarly to the 25 kDa MW chitosan membrane. It also implied that, in the presence of acetic acid, the strong ionic interaction between $–COO^-$ and $–NH_4^+$ can enhance the rigidity of the chitosan matrix.

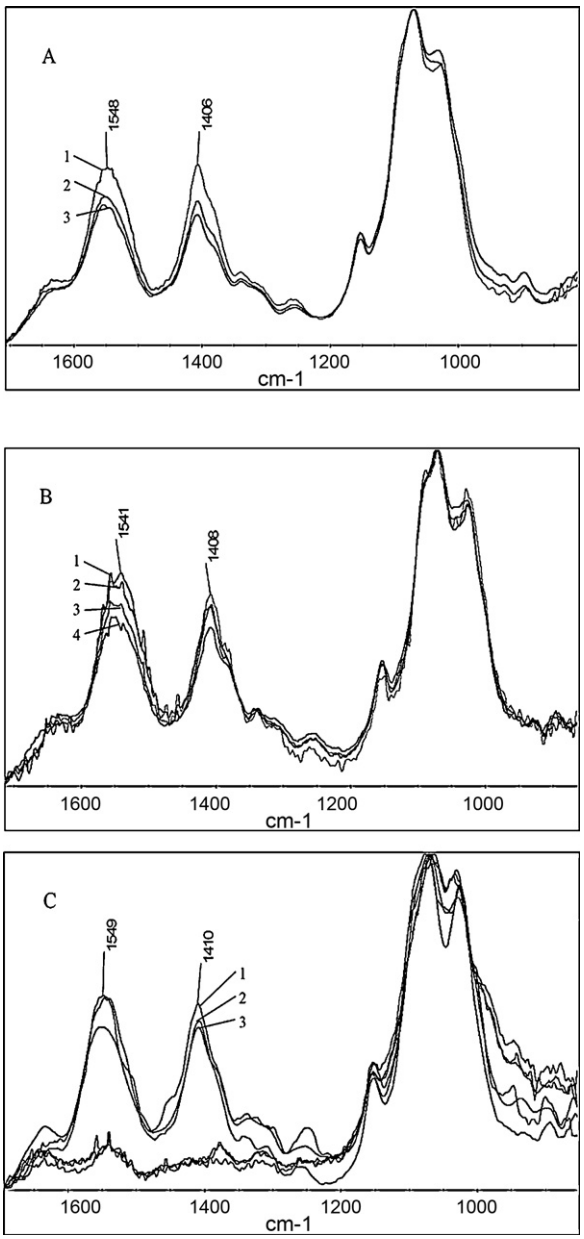

Fig. 1. FTIR-ATR spectra of the chitosan membranes. (A) 1: CM-10; 2: CM-5; 3: CM-1. (B) 1: CM-480k; 2: CM-180k; 3: CM-98k; 4: CM-48k. (C) 1: CM-HDA; 2: CM-MDA; 3: CM-LDA; the bottom three spectra are of the three chitosan membranes immersed in PBS (pH 7.0, 0.1 mol/L) for 1 h and dried.

EIS is a powerful tool for studying conductivity of membranes. The EIS results of different chitosan membranes were shown in Fig. 2, presented as Nyquist plots (Z_{re} versus Z_{im}). Z_{re} and Z_{im} were the real variable and the negative value of the imaginary variable of impedance, respectively. EIS consists of a semicircle portion observed at higher frequency range corresponding to the electron-transfer-limited process and a linear part at lower frequency representing the diffusion-limited process. The more difficultly the electron transferred through the membrane to the electrode surface, the more diameter of the semicircle should be shown in EIS. The membrane could limit the electron transfer and the limitation increased with decreasing chitosan MW (Fig. 2A) and DDA (Fig. 2B). The low AAC of the chitosan solution could also increase the limitation of chitosan membranes (Fig. 2C). The con-

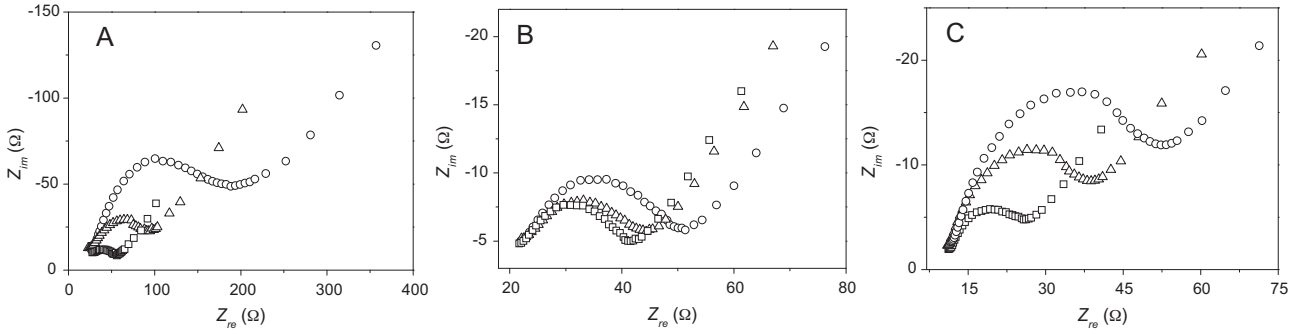

Fig. 2. Electrochemical impedance spectra for different chitosan membranes with different (A) MW, (B) DDA and (C) AAC of chitosan solutions in a 5.0 mmol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution (PBS, pH 7.0, 0.1 mol/L). AC mode; 10 mV perturbation signal; frequency range: 0.01–100,000 Hz. (A): (□) CM-480k; (△) CM-180k; (○) CM-98k. (B): (□) CM-HDA; (△) CM-MDA; (○) CM-LDA. (C): (□) CM-10; (△) CM-5; (○) CM-1.

ductivity of the chitosan membrane might be related to the amount of residual acetic acid. The ionization of chitosan molecules could be favorable to decrease the electron transfer limitation and further increase the conductivity.

The WCA of the membranes (placed on a flat glass and rinsed in PBS) was measured and the results were shown in Fig. 3. The high MW and DDA could lead to a high WCA of the chitosan membrane, while the WCA could not be affected by the AAC of chitosan solutions. The hydrophilicity of different MW chitosan molecules should be similar, but the low MW chitosan membrane, CM-48k, exhibited the highest "hydrophilicity". On the other hand, the membrane should become more hydrophilic with the DDA increasing, because the increase of amino groups could enhance the hydrophilicity of chitosan molecules, but the membrane "hydrophilicity" was decreased slightly with the DDA increasing. According to some results of other previous researches, decreasing the chitosan MW could increase the molecular permeability of chitosan membrane, but the swelling property and water adsorption could be decreased; the lower the chitosan DDA was, the higher water adsorption of chitosan membrane could be offered. It could be deduced that a loose network could be formed in the chitosan membranes with low MW and DDA, leading to a high molecular permeability and water adsorption (Chen et al., 2002; Ren et al., 2005; Santos et al., 2006; Takahashi et al., 2007). Therefore, the low WCA of low MW and DDA chitosan membrane was not ascribed to the membrane hydrophilicity, but to the high water permeability into the matrix of the chitosan membrane, and partial water adsorption simultaneously. Then the chitosan membrane prepared by different AAC chitosan solutions could only show the similar WCA, due to the similar molecule and matrix structures after the residual acetic acid was removed.

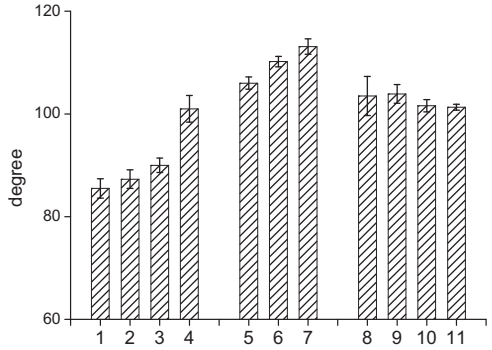

Fig. 3. The water contact degree of different chitosan membranes. 1: CM-48k; 2: CM-98k; 3: CM-180k; 4: CM-480k; 5: CM-LDA; 6:CM-MDA; 7: CM-HDA; 8: CM-10; 9: CM-7; 10: CM-4; 11: CM-1.

3.2. The enzyme adsorption

The chitosan membrane is an important carrier for the enzyme adsorption and immobilization. The adsorption occurred through some interactions between the enzyme and membranes, such as the hydrophobic forces, hydrogen bonds, ionic interactions and affinity. All the structural and natural changes related to these interactions could influence the enzyme adsorption. Here, HRP, as a model enzyme in many literature, were also adsorbed on the different prepared chitosan membranes in this work, and the results were shown in Table 2.

The amount of adsorbed HRP increased with decreasing the MW and DDA and increasing the AAC of chitosan solutions. The low MW and low DDA chitosan could form a loose structure of the membranes according to the results of the membrane WCA. HRP could be adsorbed into the loose network and a high amount of the adsorbed HRP could be obtained. When the AAC of chitosan solutions was modulated, the chitosan membranes had similar networks. The amount of adsorbed HRP was influenced by the residual acetic acid in the chitosan membranes which controlled the ionization of chitosan membrane and influenced the ionic interaction between HRP molecules and chitosan membranes.

Compared with some literature, hydrophobic forces and ionic interactions played the important roles in the protein adsorption, and the membrane swelling property also influenced it (Hoven et al., 2007; Liu et al., 2002). Especially, for polymer hydrogels, the adsorption of proteins could occur by an in-diffusion process into the matrix (Hoven et al., 2007; McArthur et al., 2000). Thus, according to the results of our work, the loose network could influence the adsorption of enzyme as a dominating factor. In

Table 2
HRP adsorption on different chitosan membranes.

Entry	Chitosan membranes	Adsorbed enzyme (mg cm^{-2})	Specific activity (U g^{-1} protein)
1	CM-48k	0.221	4.28
2	CM-98k	0.143	6.82
3	CM-180k	0.129	7.88
4	CM-480k	0.120	11.6
5	CM-LDA	0.101	7.60
6	CM-MDA	0.089	10.5
7	CM-HDA	0.056	25.8
8	CM-10	0.156	10.4
9	CM-7	0.127	13.9
10	CM-4	0.108	18.1
11	CM-1	0.097	22.3

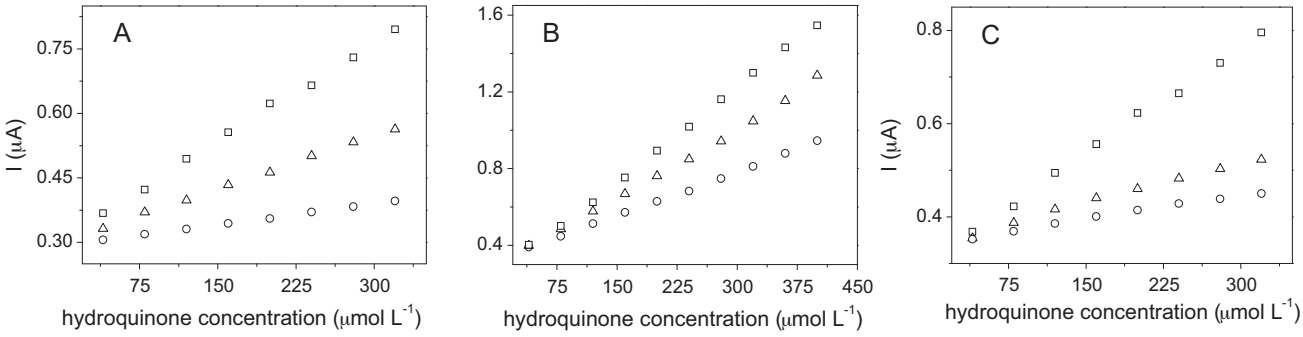

Fig. 4. The response of electrodes coated with HRP/chitosan membrane to hydroquinone was investigated by a steady-state current method. 50 μL 0.02 mol L^{-1} hydroquinone solution (PBS, pH 7.0, 0.1 mol L^{-1}) was added periodically into the electrochemical cell containing 200 μmol L^{-1} H_2O_2 in 25 mL PBS (pH 7.0, 0.1 mol L^{-1}), and the changes of the current were recorded. The influences of the chitosan MW (A), DDA (B), and the AAC of chitosan solutions (C) on the sensitivities of the electrodes: (A): (□) CM-480k; (△) CM-180k; (○) CM-98k. (B): (□) CM-HDA; (△) CM-MDA; (○) CM-LDA. (C): (□) CM-10; (△) CM-5; (○) CM-1.

addition, the strong ionic interaction was also favorable for the adsorption.

For a further investigation, three enzymes, lysozyme, trypsin and HRP, were adsorbed on CM-10 (Table 3) as a comparison. The molar amount of the adsorbed lysozyme was the highest (7.79 mmol cm^{-1}). It could be deduced that enzyme was adsorbed on chitosan membranes as a multilayer by an approximate calculation using the data of enzyme size. For example, according to the size of HRP (4.0 nm × 6.7 nm × 11.8 nm), there may be 10–100 layers of HRP molecules on the chitosan membrane surface. Thus, it might also imply that enzyme could diffuse into the membrane surface. The low MW proteins (<66.2 kDa) could even permeate through chitosan membranes (Chen et al., 2002). Therefore, the high molar amount of the adsorbed lysozyme with the lowest MW was measured due to an easy diffusion into the network of chitosan membranes. Consequently, it might also prove that the loose network could predominantly influence the enzyme adsorption.

The apparent specific activity of HRP adsorbed on the chitosan membrane was then investigated (Table 2). The activity increased with increasing MW and DDA of chitosan and decreasing AAC of chitosan solutions. The apparent specific activity was related to the velocity of enzymatic reaction catalyzed by HRP. The substrate phenol, the electron mediator H_2O_2 must diffuse into chitosan membranes and be combined with the enzyme in the network of the chitosan membrane. Then the product diffused into the solution. However, the diffusional limitation can lead to a low reaction rate and a low efficiency of the catalytic reaction, which was often accompanied by some activity loss (Tischer & Wedekind, 1999). The more easily enzyme molecules adsorbed into the deep network of chitosan membrane, the more difficultly the enzyme combined with the substrates. Thus the low MW and low DDA chitosan membranes could have a high diffusional limitation to decrease the apparent specific activity of HRP. In addition, the chitosan membrane prepared using a high AAC of chitosan solution could also have a strong ionic interaction with the substrate and product molecules, limit their diffusion in the membrane and decrease the apparent specific activity.

Table 3
Adsorption of three enzymes on CM-10.

Enzyme	MW of enzyme (kDa)	Isoelectric point (pH units)	Adsorbed enzyme (mg cm^{-2})	Adsorbed enzyme (nmol cm^{-2})
Lysozyme	14.3	11.1	0.111	7.79
Trypsin	23.3	6.0	0.146	6.27
HRP	40.0	7.2	0.151	3.76

3.3. Response of electrodes coated with HRP/chitosan membrane to hydroquinone

The amperometric response of the electrode coated with HRP/chitosan membrane was investigated by successively adding the hydroquinone solution into the electrochemical cell. The results were shown in Fig. 4. The slope of the electrode response versus the hydroquinone concentration was the sensitivity of the electrode. In Fig. 4A and B, the sensitivity of the electrode increased with the chitosan MW and DDA increasing. According to the previous results, the high MW and DDA chitosan membrane had a high conductivity and HRP adsorbed on it also had a high apparent specific activity. Therefore, the HRP electrode with the high MW and DDA chitosan membrane had a high sensitivity. In Fig. 4C, a high sensitivity of the HRP electrode could be obtained trough using chitosan solution with a high AAC. Although the apparent specific activity of adsorbed HRP on CM-1 was high, the high electrochemical impedance (low conductivity) could increase the electron transfer resistance and result in a decreased sensitivity. It might imply that the conductivity was a more important factor for the sensitivity than the specific activity of adsorbed HRP. The HRP-based electrode was also prepared by 10 μL CS-480 solution and compared with the 40 μL CS-480 solution casting HRP-based electrode. The sensitivity was increased about 4-fold from 1.5 to 6.0 mA L mol^{-1}. As mentioned in some other researches (Du, Liu, Wu, & Cai, 2007; Liu et al., 2007), the electron transfer could be obstructed by the thick and compact membrane/films on the electrode surface and the sensitivity was then decreased. It might also prove that lowering the electron transfer limitation was more efficient to improve the bioelectrode sensitivity than increasing the adsorbed enzyme activity.

4. Conclusions

Our investigation, where HRP were adsorbed on chitosan membranes prepared with different MW and DDA of chitosan and the AAC of chitosan solutions, revealed the relationship between the enzyme adsorption and the structural and natural changes of the chitosan membrane dependent on the structural parameters. The low MW and DDA chitosan membranes could form a looser network structure to adsorb more HRP molecules than the high MW and DDA chitosan membranes. The chitosan membrane prepared by a high AAC of the chitosan solution could lead to a high amount of adsorbed HRP due to the enhancement of the amino group protonation and the ionic interactions. The apparent specific activity of adsorbed HRP could be increased due to a low diffusional limitation of the small substrate and product molecules in the chitosan

membranes prepared with the high MW and DDA chitosan and the low AAC of chitosan solution. Finally, the sensitivity of the HRP-based electrode for the hydroquinone measurement could be increased with increasing the chitosan MW and DDA and the chitosan solution AAC predominantly due to a decrease of the electron transfer resistance. Therefore, these fundamental understandings could offer a potential approach to the control of enzyme adsorption and performance through changing chitosan membrane properties, which could extend the applications of chitosan membranes in the enzyme (or protein) adsorption and immobilization, especially in the construction of the highly efficient biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.03.048.

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