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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