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Preparation of high antimicrobial activity thiourea chitosan–Ag^+ complex

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Abstract

Thiourea chitosan was prepared by the reaction of chitosan with ammonium thiocyanate in ethanol. Thiourea chitosan was characterized by FT-IR, ^{13}C NMR, and elemental analysis; XPS confirmed that in thiourea chitosan–Ag^+ complex, S atoms coordinated to silver ions and were the major electron donors; O atoms also coordinated to silver ions and they were another electron donors next to S atoms. N atoms did not take part in coordination. Thiourea chitosan–Ag^+ complex overcomes the instability of Ag^+. Antimicrobial activities of the complex was evaluated against six species of bacteria and molds. The complex showed a wide spectrum of antimicrobial activities, whose MIC values against bacteria were 20 times lower than those of chitosan, 100 times lower than those of sodium diacetate and 200 times lower than those of sodium benzoate, respectively; the complex has a better antibacterial activity than antifungal activity.

Keywords: Chitosan; Thiourea chitosan; Antimicrobial activity; Silver iron; Complex

1. Introduction

Chitosan is a natural nontoxic biopolymer derived by the deacetylation of chitin. Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activities (Jung et al., 1999). The antimicrobial activity of chitosan depend on several factors such as the kind of chitosan (deacetylation degree, molecular weight) used, the pH of the medium, the temperature, etc. The mechanism of the antimicrobial activity has not been fully elucidated yet, but several hypotheses have been postulated. The most feasible hypothesis is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Chen, Liau, & Tsai, 1998; Feng et al., 2000; Papineau, Hoover, Knorr, & Farkas, 1991; Sudarshan, Hoover, & Knorr, 1992; Young & Kohle, 1982). Other mechanisms are the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis (Hadwiger et al., 1986) and the chelation of metals, spore elements and essential nutrients (Cuero, Osuji, & Washington, 1991).

Silver or silver ions have long been known to have powerful antibacterial activity. Silver is widely applied in some medical fields for its high antimicrobial activity and low concentration (Kang, Jung, & Jeong, 2000). The complex of chitosan with Ag^+ exhibits antibacterial activity (Zhan, Xiong, Liu, & Xie, 2002). However, Ag^+ including the adsorptive Ag(I) by chitosan is instable, and can be easily reduced as Ag, which limits its medical application.

According to hard and soft acid theory, thiourea compounds are very efficient at chelating noble metals and widely used for extracting noble metals including silver, gold, etc. (Chanda & Rempel, 1990). In order to introduce thiourea group into chitosan main chain, thiourea grafting chitosan using glutaraldehyde

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as cross-linking agent was prepared (Guibal, Vincent, & Navarro Mendoza, 2000a; Guibal et al., 2001). However, the obtained thiourea grafting chitosan can not dissolve into any solvent, which restricts its use as antimicrobial. A kind of soluble chitosan modified by thiourea is desired.

In this paper, thiourea chitosan (TU-CTS) was prepared by the reaction of citosan with ammonium thiocyanate, which was easy to be dissolved into 1% acetic acid solution. Thiourea chitosan and Ag^+ formed a stable thiourea chitosan—Ag^+(TU-CTS-Ag^+) complex, which remarkably enhanced the antimicrobial activity of chitosan as well as the stability of Ag^+.

2. Experimental

2.1. Materials

Chitosan with deacetylation degree of 90% was purchased from Yuhuan Organisms Co. Ltd. (Zhejiang Province, China). Beef extract, potato starch, glucose and peptone were purchased from Shanghai Chemical Agent Co. (Shanghai, China).

The microorganisms tested were provided by Xiangtan University in China.

The other reagents were of analytical grade and used without further purification.

2.2. Characterizations of thiourea chitosan and thiourea chitosan–Ag^+

^{13}C NMR spectra were performed on a Bruker (AVACE) AV-500 spectrometer in D_2O/HCl and D_2O/CF_3COOD, respectively; IR spectra were recorded on Nicolet-170 SX FT-IR spectrophotometer in KBr discs; S% was measured in a SC-132 sulfur meter (LECO), while C%, N% and H% were measured by Elemental Analyzer-MOD 1106 (Carlo Erba Strumentazione); Ag^+ content was measured by a Hitachi 180-80 atomic absorption spectrometry. X-ray photoelectron and X-ray excited Auger spectra were obtained with a Physical Electronics PHI 5700 spectrometer with using non-monochromated Mg Kα radiation ($h\nu = 1253.6$ eV) and operating at 15 kV and 20 mA; the vacuum in the analysis chamber was better than 10^{-9} mbar. Binding energies were corrected using the binding energy values for C 1 s of adventitious carbon fixed at 284.8 eV.

2.3. Preparation of thiourea chitosan and thiourea chitosan–Ag^+

A mixture of 16.1 g (0.1 mol) chitosan powder, 15.2 g (0.2 mol) ammonium thiocyanate and 150 ml ethanol in a three-neck flask with amagenetic stirring was refluxed for 12 h. After cooling down to room temperature, the precipitate was collected by filtration and repeatedly washed with ethanol, then was dissolved in 500 ml of 1% (v/v) acetic acid solution. By adding 10% (w/v) NaOH solution into the solution and filtrating, the precipitate was collected and successfully washed with water and finally dried to give 16.5 g of thiourea chitosan.

0.3 g of thiourea chitosan was dissolved in 30 ml of acetic acid (1%, v/v), followed by addition of 25 ml of $AgNO_3$ solution (0.01 g $AgNO_3$) into the solution by stirring. After the mixture was stirred for 3 h at room temperature, the precipitate obtained by adding 200 ml acetone into the mixture was collected by filtration, successively washed with 95% ethanol, and dried to give thiourea chitosan–Ag^+ complex. The sorption amount of Ag^+ in thiourea chitosan–Ag^+ complex was 1.44 mg/g as determined by atomic absorption spectrometry.

2.4. Evaluation of antimicrobial activity in vitro

The agar plate method was used to determine the minimum inhibition concentration (MIC) of chitosan, sodium diacetate, sodium benzoate and thiourea chitosan–Ag^+ complexe. The 1% (w/v) solutions of chitosan, sodium diacetate, sodium benzoate and thiourea chitosan–Ag^+ were prepared in 1% (v/v) acetic acid. Duplicate two-fold serial dilutions of each sample were added to nutrient broth (beef extract 5 g, peptone10 g to 1000 ml distilled water, pH 7.0) for the final concentration of 0.1, 0.05, 0.025, 0.0025, 0.00125, and 0.00025%; 0.1%(v/v) of acetic acid was used as a control instead of samples. Then they were autoclaved at 121 °C for 25 min. The culture of each bacterium was diluted by sterile distilled water to 10^5–10^6 CFU/ml. A loop of each suspension was inoculated on nutrient medium with the sample or the control added. After inoculation, the plates were incubated at 37 °C for 72 h, and the colonies were counted and the MIC values were obtained. The MIC was considered to be the lowest concentration that completely inhibits against bacteria comparing with the control, disregarding a single colony or a faint haze caused by the inoculum (Speciale, et al., 2002).

Following the procedure described above, slight modifications were made in order to obtain the MIC values of chitosan, sodium diacetate, sodium benzoate and thiourea chitosan–Ag^+ against molds. Potato starch glucose media (potato starch 10 g, glucose 20, agar 20 g and pH value in nature) was used for culturing molds. The culture of each mold spores was diluted by sterile distilled water to 10^5 CFU/ml. A loop of each suspension was inoculated on nutrient medium with the sample or the control added. The inoculated plates were incubated at 30 °C for 10 days, and the colonies were counted and the MIC values were obtained.