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Influence of Hydroxyl Groups of Quaternized Chitosan Derivatives on Antioxidant Activity

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Four quaternized chitosan derivatives *i.e.*, N-phenmethyl-N,N-dimethyl chitosan, N-(2-hydroxyl-phenmethyl)-N,N-dimethyl chitosan, N-(4-hydroxyl-phenmethyl)-N,N-dimethyl chitosan and N-(3,4-dihydroxyl-phenmethyl)-N,N-dimethyl chitosan were synthesized and their antioxidant activity against hydroxyl radicals was assessed. The result indicated that all the quaternized chitosan derivatives have better hydroxyl radical scavenging activity than chitosan and the antioxidant activity of derivatives increases when more hydroxyl groups are grafted.

Keywords: Quaternized chitosan, Hydroxyl group, Antioxidant activity.

INTRODUCTION

As one of the most abundant natural biotic resources, chitosan has attracted people's attention for its unique physicochemical characteristics and bioactivities[1-3]. Among various bioactivities of chitosan, the antioxidant activity has received considerable attention in recent years[4-6]. In the course of the research and development of chitosan, various chitosan derivatives with better antioxidant activity have been synthesized such as carboxymethyl chitosan, quaternized chitosan, chitosan sulfates, *etc*[5,7,8]. We have reported that quaternized chitosan has better antioxidant activity than Schiff bases of chitosan and N-substituted chitosan[5]. The antioxidant activity is affected by the positive charge of amino group in the molecule of chitosan derivatives.

Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, *t*-butylhydroquinone and propyl gallate, play an important role in the elimination of free radical and protecting cells against toxic affects of free radical[9]. However, their use is increasingly restricted because of side effects on human body.

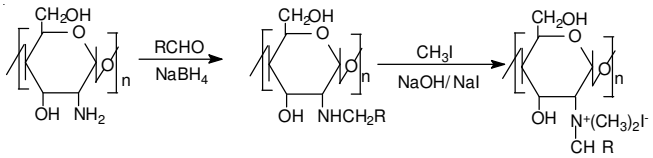
There are one or more hydroxyl groups in the molecules of above mentioned synthetic antioxidants, which have important influence on antioxidant activity. Therefore, in order to improve the antioxidant activity of chitosan derivatives and study the influence of hydroxyl group on antioxidant activity, N-phenmethyl-N,N-dimethyl chitosan (PDCS), N-(2-hydroxyl-phenmethyl)-N,N-dimethyl chitosan (2HPDCS), N-(4-hydroxyl-phenmethyl)-N,N-dimethyl chitosan (4HPDCS) and N-(3,4-dihydroxyl-phenmethyl)-N,N-dimethyl chitosan (3,4DHPDCS) were synthesized and their antioxidant activity against hydroxyl radicals was assessed in this paper. The possible mechanism was also discussed.

EXPERIMENTAL

Chitosan was purchased from Qingdao Yunzhou Biochemistry Co., Ltd. (China). The degree of deacetylation is 93 % and the viscosity average-molecular weight is 8.5×10^3D. The other reagents were all analytical grade and were used without further purification. The IR spectra were measured on a Jasco-4100 Fourier transform infrared (FTIR) spectrometer (Tokyo, Japan, provided by JASCO China (Shanghai), Co., Ltd. Shanghai, China) with KBr disks.

Synthesis of quaternized chitosan: Quaternized chitosan derivatives were synthesized as follows[5,10]: 1 g chitosan was dissolved into 30 mL H_2O at room temperature and benzaldehyde, 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde were added, respectively with stirring. After 2 h, 10 % $NaBH_4$ (1.5 fold excess to added aldehyde) was added and the reaction was carried out for 2 h. The solution was then precipitated in excess acetone and precipitants were filtrated. N-substituted chitosan derivatives were obtained after drying at 60 °C for 6 h. 1 g obtained N-substituted chitosan was dispersed into 50 mL N-methyl-2-pyrrolidone (NMP) for 12 h at room temperature. To this mixture, 0.12 mL NaOH (1 M), 1.5 g NaI and 4 mL CH_3I were added and each reaction was carried out with stirring at 50 °C for 24 h. After that, the solution was precipitated by excess

acetone and the precipitations were filtered. The quaternized chitosan derivatives were obtained by drying at 60 °C in vacuum for 6 h (Fig. 1).



PDCS, R = phenyl; 2HPDCS, R = 2-hydroxyl-phenyl; 4HPDCS, R = 4-hydroxyl-phenyl; 3,4DHPDCS, R = 3,4-dihydroyl-phenyl

Fig. 1. Synthetic pathway of quaternized chitosan

Hydroxyl radicals scavenging activity assay: The antioxidant activity was carried out according to Guo *et al.*[5] and Wang *et al.*[11]. The reaction mixture, with a total volume of 4.5 mL, containing the samples (chitosan, PDCS, 2HPDCS, 4HPDCS and 3,4DHPDCS), was incubated with EDTA-Fe^{2+} (220 μM), safranine O (0.23 μM) and H_2O_2 (60 μM) in potassium phosphate buffer (150 mM, PH 7.4) for 0.5 h at 37 °C. The absorbance of the mixture was measured at 520 nm. Hydroxyl radicals bleached the safranine O, so increased absorbance of the reaction mixture indicated decreased hydroxyl radicals scavenging ability and the scavenging effect of the samples was computed using the following equation:

$$\text{Scavenging effect}(\%) = \frac{(A_{\text{sample 520 nm}} - A_{\text{blank 520 nm}})}{(A_{\text{control 520 nm}} - A_{\text{blank 520 nm}})} \times 100$$

Where A blank 520 nm is the absorbance of the blank (distilled water instead of the samples) and A control 520 nm is the absorbance of the control (distilled water instead of H_2O_2).

All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$ and the means separated by Duncan's multiple range test. The results were processed by Excel and Statistical software (2003).

RESULTS AND DISCUSSION

Structure of quaternized chitosan: The IR spectra of chitosan and quaternized chitosan derivatives are shown in Fig. 2. The IR spectrum of chitosan shows peaks assigned to the saccharine structure at 898 and 1150 cm^{-1}. Characteristic peaks of amine (N-H) vibration deformation appeared at 1600 cm^{-1} for chitosan. After quaternization, new peaks appear at about 1650 cm^{-1}, which corresponds to quaternary ammonium salts. There are peaks in 1430-1415 cm^{-1} region, which are related to the characteristic absorption of N-CH_3[10]. Moreover, PDCS, 2-HPDCS, 4-HPDCS and 3,4-DHPDCS have peaks at about 701, 763, 821, 1470 and 1550 cm^{-1} corresponding to phenyl groups[5]. These results demonstrated that the quaternized chitosan derivatives were obtained.

Antioxidant activity: The hydroxyl radicals, generated by the Fenton reaction in this measuring system, were scavenged by the added samples. Fig. 3 shows the scavenging effect of chitosan and quaternized derivatives. The scavenging effect of chitosan on hydroxyl radicals increases with the increase of concentration and the scavenging effect is 40.5 % at 0.8 mg/mL. The quaternized chitosan derivatives have better

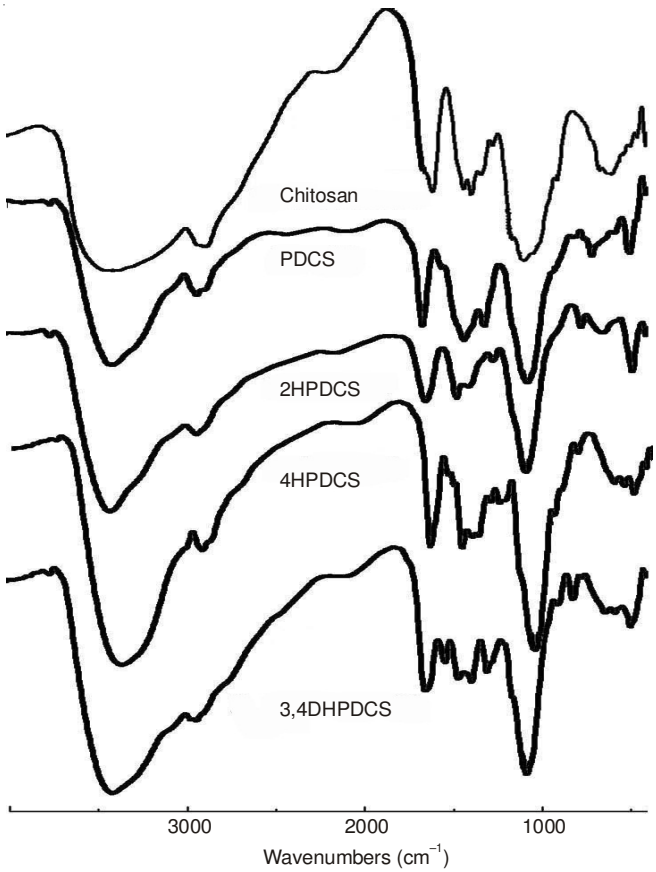


Fig. 2. IR spectra data of chitosan and quaternized chitosan derivatives

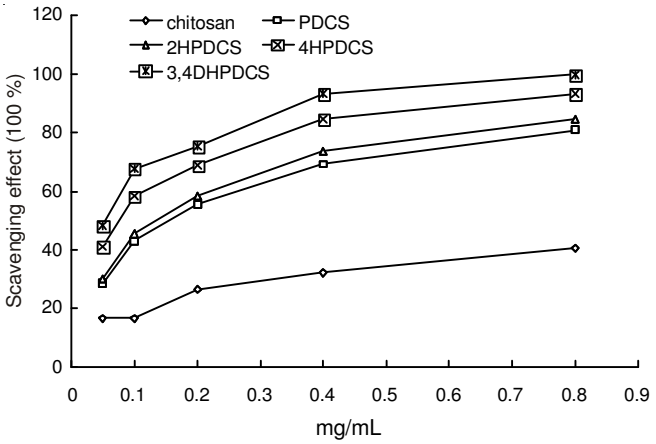


Fig. 3. Scavenging effect of chitosan and quaternized chitosan derivatives on hydroxyl radicals

scavenging effect on hydroxyl radicals than chitosan because of the positive charge[5]. For the synthesized quaternized chitosan in this paper, scavenging ability on hydroxyl radicals is found to be in the order of 3,4DHPDCS > 4HPDCS > 2HPDCS > PDCS. There are hydroxyl groups in the molecule of 3,4DHPDCS, 4HPDCS and 2HPDCS, which should be the reason for the increase of scavenging ability on hydroxyl radicals. And 3,4DHPDCS has the best scavenging effect among all the measured samples just because of the two hydroxyl groups grafted to 3,4DHPDCS. One hydroxyl group is grafted to the molecule of 2HPDCS and 4HPDCS, respectively, but 4HPDCS has better scavenging ability on hydroxyl radicals than 2HPDCS. The possible reason should be the

grafted hydroxyl group in the molecule of 2HPDCS is difficult to approach the hydroxyl radicals easily owing to steric hindrance.

Conclusion

Polysaccharides with scavenging effect on hydroxyl radicals have the similar structural feature that all have one or more alcohol or phenolic hydroxyl groups and the scavenging ability is related to these groups. For chitosan, with two kinds of hydroxyl groups at C3 and C6 and part of the relationship between the groups and the antioxidant activity had been studied. When the active hydroxyl groups were substituted, the antioxidant activity reduced[8]. The importance of hydroxyl groups on scavenging effect against hydroxyl radicals was confirmed in this study. More hydroxyl especially phenolic hydroxyl groups grafted to chitosan, more increase of scavenging effect against hydroxyl radicals. And so, a feasible method may be provided to get possible antioxidants derived from chitosan.

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