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Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan

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Abstract—N-substituted chitosan and quaternized chitosan were synthesized and their antioxidant activity against hydroxyl radicals was assessed, respectively. Compared with the antioxidant activity of chitosan, the results indicated that the two kinds of chitosan derivatives had different scavenging ability on hydroxyl radicals, which should be related to the form of amido in the two kinds of chitosan derivatives.

Chitosan, a natural cationic polymer prepared by *N*-deacetylation of chitin, is the second abundant natural resource next to cellulose. Because chitosan is biodegradable and non-toxic, it has attracted people's attention for its unique physicochemical characters and bioactivities.[1–4] With the development of the study of chitosan on biomedicine, the antioxidant activity of chitosan and its derivatives has been studied accordingly.[5–8] But, there are less researches which reported the relation between the antioxidant activity and the molecular structure. Generally, there are such forms of amido as primary amine (–NH_2, chitosan), imine (–C=N, Schiff bases of chitosan), secondary amine (–NHR′, N-substituted chitosan), and quaternary ammonium (–$N^+RR'R''$, quaternized chitosan). And the last one can be synthesized from chitosan step by step according to the above-mentioned order. For these four kinds of compounds, the hydroxyl radicals' scavenging activity of Schiff bases has been investigated earlier, and it is the only one which has been measured except for chitosan. Among various reactive oxygen species, hydroxyl radicals have the strongest chemical activity, which can damage a wide range of essential biomolecules such as amino acids, proteins, and DNA.[9] And then, in this paper, the two other kinds of derivatives—N-substituted chitosan and quaternized chitosan were synthesized and the antioxidant activity against hydroxyl radicals was assessed. And the possible reason was discussed.

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). The degree of deacetylation was 97% and the viscosity average-molecular weight was 7.6×10^3. N-substituted chitosan and quaternized chitosan derivatives were synthesized as follows:[9] Three grams of chitosan was dissolved into 100 ml H_2O at rt, and various aldehydes 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 precipitated in acetone and the precipitants were filterted. And then the N-substituted chitosan derivatives (a, b, and c) were obtained after drying at 60 °C for 24 h. One gram of N-substituted chitosan was dispersed into 50 ml *N*-methyl-2-pyrrolidone (NMP) for 12 h at rt. 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 20 h. The solution was precipitated by excess acetone and the precipitations were filtered. The quaternized chitosan derivatives (A, B, and C) were obtained by drying at 60 °C for 24 h (Scheme 1).

The elemental analysis results, yield, and degree of N-substitution chitosan or quaternization chitosan are shown in Table 1, and the IR spectrum data of the quaternized chitosan derivatives are shown in Figure 1. As shown in Figure 1, the IR spectrum of chitosan

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Keywords: N-substituted chitosan; Quaternary chitosan; Antioxidant activity.

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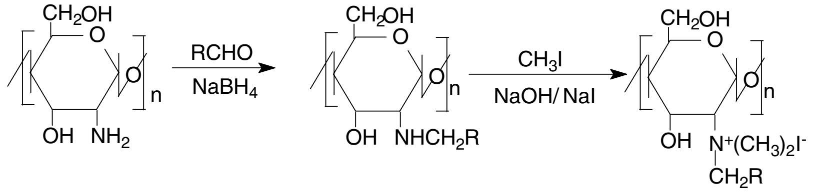

Scheme 1. Synthetic pathway of the derivatives of chitosan.

Table 1. The elemental analysis results, yield, and degree of N-substitution or quaternization of the chitosan derivatives

Compound	Yield (%)	Elemental analysis (%)			Degree of N-substitution or quaternization (%)
		C	N	H	
a	65.3[a]	60.88	5.71	6.78	90.8
b	68.5[a]	57.33	5.49	6.40	87.5
c	58.7[a]	54.33	5.89	6.14	93.4
A	55.3[b]	44.88	3.71	5.46	86.3
B	59.2[b]	43.55	3.65	5.36	80.5
C	53.4[b]	39.67	3.72	5.12	90.7

[a] Yield is the ratio of N-substituted chitosan and added chitosan.

[b] Yield is the ratio of quaternized chitosan and added N-substituted chitosan.

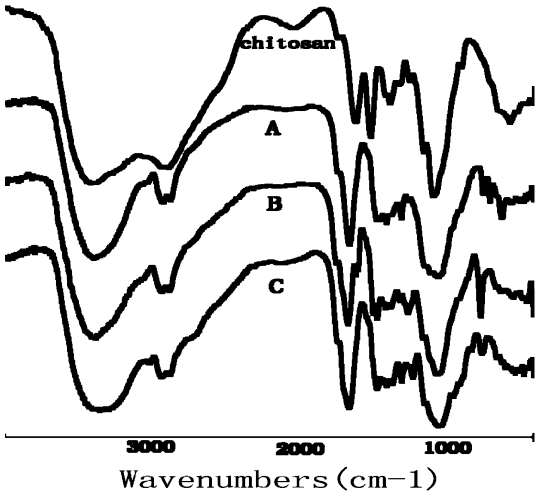

Figure 1. The IR spectra data of quaternized chitosan.

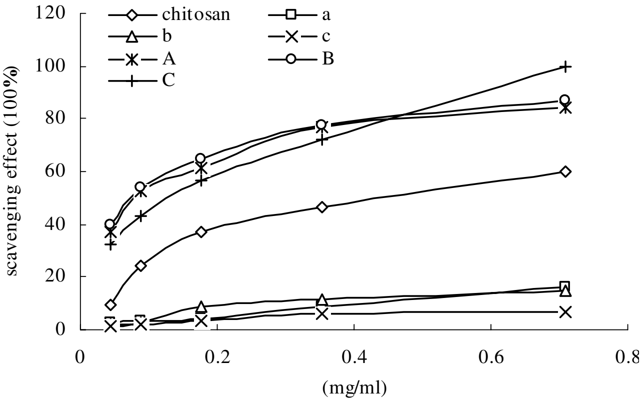

Figure 2. Scavenging effect of the different derivatives of chitosan on hydroxyl radicals.

shows peaks assigned to the saccharine structure at 895 and 1155 cm^{-1}. Characteristic peaks of amine (N–H) vibration deformation appeared at 1618 cm^{-1} for chitosan. After quaternized, new peaks appear at about 1662 cm^{-1}, which were assigned to the quaternary ammonium salt. There are peaks at about 1415–1430 cm^{-1}, which were assigned to the characteristic absorption of N–CH_3.[9] Moreover, (A) and (B) have peaks at about 1400, 1470, 1500, and 1580 cm^{-1} corresponding to the phenyl groups,[10] and (C) has peaks at 1472, 1404, and 758 cm^{-1} corresponding to the furfuryl group.[11] Above-mentioned results demonstrate that the quaternized chitosan derivatives were obtained.

The antioxidant activity was carried out according to Wang,[12] The reaction mixture, total volume 4.5 ml, containing the samples of chitosan derivatives (a, b, c, A, B, and C), 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 30 min 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 capability of scavenging hydroxyl radicals was calculated using the following equation:

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

where $A_{\text{blank 520nm}}$ was the absorbance of the blank (distilled water instead of the samples), $A_{\text{control 520nm}}$ 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 computer programme: Excel and Statistical software (1999).

The hydroxyl radicals, generated by the Fenton reaction in this system, were scavenged by the derivatives of chitosan. Figure 2 shows the % scavenging effect of chitosan and its derivatives. The scavenging effect on hydroxyl radicals of chitosan increases with the increase of the concentration, and the % scavenging effect was 60% at the concentration of about 0.7 mg/ml, and the IC_{50} is 0.48 mg/ml. For the N-substituted chitosan derivatives, the antioxidant activity against hydroxyl radicals was very soft and the scavenging effect was no more than 20% at the concentration from 0.04 to 0.7 mg/ml. On the other hand, the antioxidant activity of all the quaternized chitosan derivatives was better than that of chitosan and N-substituted chitosan derivatives, and the % scavenging effect were 84.6, 87.3, and 100% for A, B, and C, respectively.

Polysaccharides with scavenging effect on hydroxyl radicals have the same structural feature that all have one or more alcohol or phenolic hydroxyl groups, and the scavenging ability was related to these groups. For chitosan, there two kinds of hydroxyl groups at C3 and C6, and part of the relationship between the groups and the antioxidant activity had been studied.[8] There are not only hydroxyl groups but also amido groups, and the antioxidant activity of chitosan and its derivatives is affected by the amido groups. Furthermore, the antioxidant activity is affected by the form of amido groups of chitosan. For imine ($-C{=}N$, Schiff bases of chitosan), primary amine ($-NH_2$, chitosan), secondary amine ($-NHR'$, N-substituted chitosan), and quaternary ammonium ($-NRR'R''$, quaternized chitosan), the quaternized chitosan derivatives have the best scavenging effect against hydroxyl radicals. The Schiff bases and N-substituted chitosan derivatives have soft ability. The difference between quaternized chitosan and the other chitosan derivatives above mentioned is that quaternized chitosan has high positive charge density, and the best antioxidant activity against hydroxyl radicals of quaternized chitosan may have been caused by this positive charge. However, there is no report about the antioxidant activity of positive charge. And so, the potential mechanism of these results needs to be further researched.

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References and notes

1. Lim, S.-H.; Hudson, S. M. *Carbohydr. Res.* **2004**, *339*, 313.
2. Lin, H.-Y.; Chou, C.-C. *Food Res. Int.* **2004**, *37*, 8839.
3. No, H. K.; Park, N. Y.; Lee, S. H.; Meyers, S. P. *Int. J. Food. Microbiol.* **2002**, *74*, 65.
4. Nishimura, K.; Nishimura, N.; Nishi, N.; Saiki, I.; Tokura, S.; Azuma, I. *Vaccine* **1984**, *2*, 93.
5. Kim, K. W.; Thomas, R. L. *Food Chem.* **2007**, *101*, 308.
6. Sun, T.; Xie, W. M.; Xu, P. X. *Carbohydr. Polym.* **2004**, *58*, 379.
7. Xie, W. M.; Xu, P. X.; Liu, Q. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1699.
8. Guo, Z. Y.; Xing, R. E.; Liu, S.; Yu, H. H.; Wang, P. B.; Li, C. P.; Li, P. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4600.
9. Halliwell, B.; Gutteridge, J. M. C. *Methods Enzymol.* **1990**, *86*, 1.
10. Jia, Z. S.; Shen, D. F.; Xu, P. X. *Carbohydr. Res.* **2001**, *333*, 1.
11. Matsumoto, M.; Shimizu, T.; Kondo, K. *Sep. Purif. Technol.* **2002**, *29*, 229.
12. Wang, J. C.; Xing, G. S.; Hu, W. D.; Zhu, T. L.; Wang, Q.; Zhao, H. *Chin. Pharm. J.* **1994**, *29*, 23.