

The hydroxyl radical scavenging activity of chitosan, hyaluronan, starch and their O-carboxymethylated derivatives

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ABSTRACT

In order to study the effect of active hydroxyl and amino groups on scavenging activity against hydroxyl radicals of polysaccharides, three kinds of carboxymethylated polysaccharides (carboxymethyl chitosan (O-CM-chitosan), carboxymethyl hyaluronan (CMHA), and carboxymethyl starch (CMS)) were prepared and their antioxidant activities against hydroxyl radicals were assessed, respectively. Results showed that O-CM-chitosan had lower scavenging ability on hydroxyl radicals than chitosan. CMHA and CMS had the same tendency. For the three kinds of polysaccharides, scavenging ability on hydroxyl radicals was found to be in the order of chitosan > HA > starch. The scavenging ability of carboxymethylated polysaccharides had the same order as related to its corresponding polysaccharides at higher concentrations (≥ 0.8 mg/ml). There were not only hydroxyl groups but also amino or acetamino ($\text{CH}_3\text{CONH}-$) groups in the molecules of chitosan and HA, but only hydroxyl group for starch. It was suggested that the sequence influence the scavenging activity against hydroxyl radicals might be amino group > acetamide group > hydroxyl group.

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1. Introduction

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 (Halliwell & Gutteridge, 1990). Chitosan, one of the most important polysaccharide, has stronger scavenging activity on hydroxyl radicals than Vitamin C (Vc), and the IC_{50} is about 0.48 mg/mL (Guo, Liu, Chen, Ji, & Li, 2006). Earlier, we reported that the hydroxyl radical scavenging activity was related to the number of active hydroxyl and amino groups ($-\text{NH}_2$) in the molecule of chitosan (Guo et al., 2005). Parts of these active groups were substituted in the molecule of carboxymethylated chitosan, and it caused the decrease of the scavenging activity against hydroxyl radicals (Guo et al., 2005). In order to further study the effect of active hydroxyl or amino groups on scavenging activity against hydroxyl radicals of polysaccharides, we chose chitosan, hyaluronan (HA) and starch as the object. The corresponding carboxymethylated polysaccharides (carboxymethyl chitosan (O-CM-chitosan), carboxymethyl starch (CMS) and carboxymethyl HA (CMHA)) were synthesized and their scavenging activities against hydroxyl radi-

cals were assessed in this paper. The possible reason was discussed, too.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Qingdao Baicheng Biochemical Corp (China). Its degree of deacetylation was 96%. The viscosity-average molecular weight was 2.0×10^5 . HA was purchased from Shandong Freda Biochem. Co., Ltd (China), and the molecular weight was 2.5×10^5 . Starch and the other reagents were all of analytical grade and supplied by Shanghai Chemicals Co. (Shanghai, China), and the molecular weight of starch was 1.8×10^5 . Double distilled water was used to prepare solutions for antioxidant test.

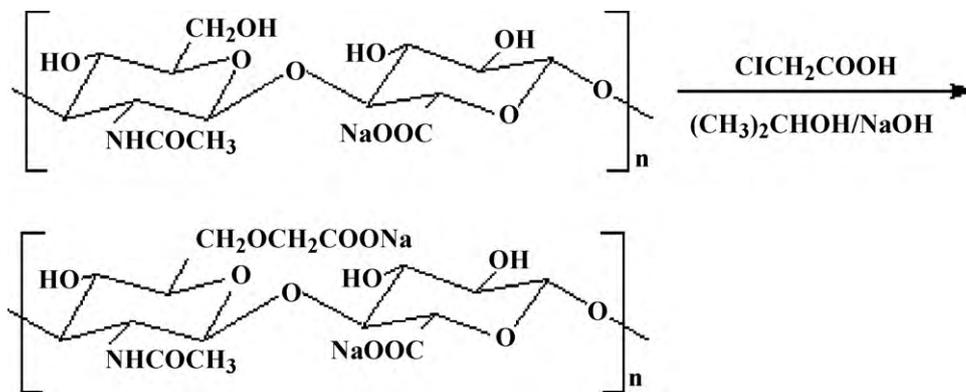
2.2. Synthesis of carboxymethylated polysaccharides

O-CM-chitosan was prepared according to Chen (Chen, Du, & Liu, 2000). CMS was prepared according to Zhang (Zhang, Wang, & Gao, 2003). CMHA was prepared as followed: 1 g HA was dispersed into 10 mL of 2-propanol at room temperature, and 2.52 mL NaOH (10 mol/L) was added with stirring. After 1 h, 1.20 g chloroacetic acid was added, and the reaction was carried out with stirring at 40 °C for 3 h. The product was filtered and washed by MeOH for three times. After vacuum dry for 12 h at room temperature, CMHA was prepared (synthetic pathway was shown in Scheme 1).

Abbreviations: O-CM-chitosan, carboxymethyl chitosan; CMHA, carboxymethyl hyaluronan; CMS, carboxymethyl starch; HA, hyaluronan.

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Scheme 1. Synthetic pathway of CMHA.

2.3. Characterization of CMHA

The IR spectra were measured on a Nicolet Magne-Avatar 360 instrument using KBr disks. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Apx 500 (500 MHz) NMR spectrometer in D_2O solvent.

2.4. Evaluation of hydroxyl radical scavenging activity

The antioxidant activity was carried out according to the method described previously (Wang et al., 1994). Samples were prepared at the original concentration of 10 mg/ml in distilled water. The reaction mixture, total volume 4.5 ml, was incubated with EDTA-Fe^{2+} (220 μM), safranin O (0.23 μM), and H_2O_2 (60 μM) in potassium phosphate buffer (150 mM, pH7.4) for 30 min at 37 °C. The absorbance of the mixture was measured at 520 nm. Hydroxyl radical bleached the safranin O, so decreased absorbance of the reaction mixture indicated decreased hydroxyl radical scavenging ability and the capability of scavenging hydroxyl radicals was calculated using the following equation:

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

where $A_{\text{blank } 520 \text{ nm}}$ was the absorbance of the blank (distilled water instead of the samples), $A_{\text{control } 520 \text{ nm}}$ was the absorbance of the control (distilled water instead of H_2O_2).

All data were expressed as means \pm 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.

3. Results and discussion

The IR spectra of HA and CMHA were shown in Fig. 1. The ^1H NMR spectrum and the ^{13}C NMR spectrum of CMHA were shown in Figs. 2 and 3. As shown in Fig. 1, the IR spectrum of CMHA showed new peak at 1741 and 1600 cm^{-1} , which assigned to $-\text{COOH}$ and $-\text{COONa}$ groups. In Fig. 3, there were four peaks at 172.24, 174.42, 177.94, and 180.00, which assigned to the $-\text{COOH}$ ($-\text{COONa}$) group, and $\text{CH}_3\text{CONH}-$ group. The above-mentioned results demonstrated that CMHA was successfully obtained. Yield of CMHA was 76.5%. CMS was synthesized from starch and chloroacetic acid in the presence of sodium hydroxide. Determined according to an earlier method (Zhang, Wang, & Gao, 2003), the degree of substitution (DS) was 0.337. O-CM-chitosan was synthesized from the etherification of alkali chitosan using chloroacetic acid as etherifying agent, and it had a substituting degree of 0.48. Yields of the CMS and O-CM-chitosan were 77% and 84.5%, respectively.

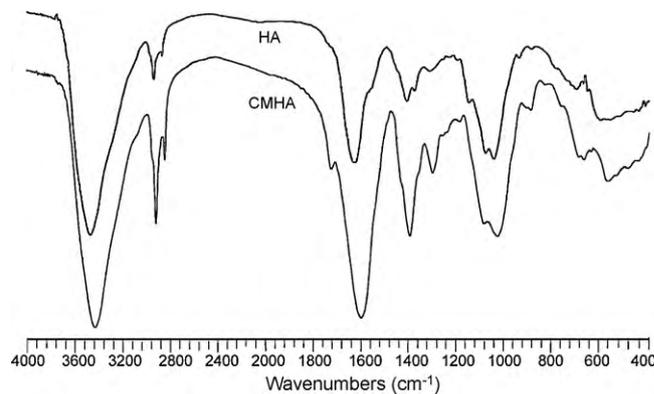
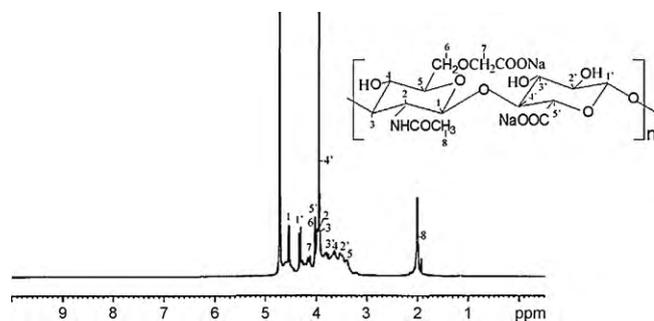
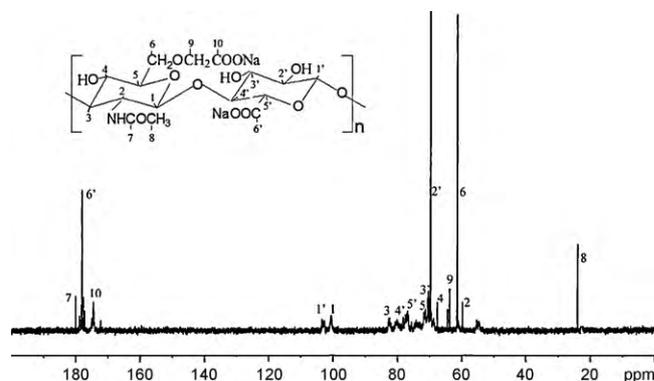


Fig. 1. IR spectra of HA and CMHA.

Fig. 2. The ^1H NMR spectrum of CMHA.Fig. 3. The ^{13}C NMR spectrum of CMHA.

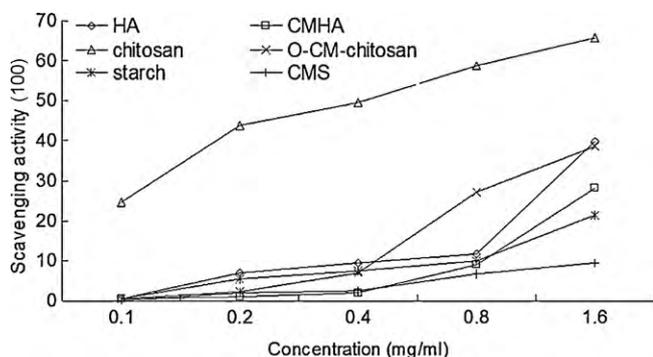


Fig. 4. Scavenging ability of hydroxyl radicals by three kinds of polysaccharides and carboxymethylated polysaccharides.

Fig. 4 shows the % scavenging effect of HA, chitosan, starch and their carboxymethyl derivatives. The scavenging ability on hydroxyl radicals increased with the increase of the concentration in each of the tested group. At a concentration of 0.1–1.6 mg, the percentage scavenging effect was 24.7–65.9% for chitosan, and 0.4–38.6% for O-CM-chitosan, respectively. The scavenging ability of chitosan was better than that of O-CM-chitosan at each concentration point tested, which was consistent with our earlier report (Guo et al., 2005). HA had the same tendency compared with CMHA. The scavenging index of HA and CMHA were 0.5–39.9% and 0.4–28.3% at a concentration of 0.1–1.6 mg/mL, respectively. For starch and CMS, at a concentration of 0.1–1.6 mg/mL, the percentage scavenging effect was comparatively soft and it was no more than 22%. And similarly, starch had better scavenging ability than that of CMS.

Polysaccharides with scavenging effect on hydroxyl radicals had the same structure feature in that all of them had one or more alcohol or hydroxyl groups, and the scavenging ability was related to the number of active hydroxyl groups in the molecules (Li, Jiang, Xue, & Chen, 2002). Decreased scavenging ability of the carboxymethylated polysaccharides could be rationalized by the fact that part of the hydroxyl groups were substituted by carboxyl groups in the molecules of CMHA, O-CM-chitosan, and CMS. For the three kinds of polysaccharides, scavenging ability on hydroxyl radicals

was found to be in the order of chitosan > HA > starch. The scavenging ability of carboxymethylated polysaccharides had the same order as related to its corresponding polysaccharides at higher concentrations, which was O-CM-chitosan > CMHA > CMS. There were not only hydroxyl groups but also amino or acetamino ($\text{CH}_3\text{CONH}-$) groups in the molecule of chitosan and HA. Chitosan had the best scavenging ability among the samples measured because of its active amino and hydroxyl groups. The molecule of HA had acetamide and hydroxyl groups, and then the scavenging ability decreased. Starch had hydroxyl group, which was the solo origin of its scavenging ability on hydroxyl radicals, and it had the least scavenging ability than that of chitosan and HA. It was suggested that both hydroxyl and amino groups affect the scavenging ability, and the sequence influence the scavenging activity against hydroxyl radicals might be amino group > acetamide group > hydroxyl group.

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References

- Chen, L., Du, Y., & Liu, Y. (2000). Structure–antimicrobial ability relationship of carboxymethyl chitosan. *Journal of Wuhan University*, 46, 191–194.
- Guo, Z., Liu, H., Chen, X., Ji, X., & Li, P. (2006). Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan. *Bioorganic & Medicinal Chemistry Letters*, 16, 6348–6350.
- Guo, Z., Xing, R., Liu, S., Yu, H., Wang, P., Li, C., & Li, P. (2005). The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. *Bioorganic & Medicinal Chemistry Letters*, 15, 4600–4603.
- Halliwell, B., & Gutteridge, J. M. (1990). Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymology*, 186, 1–85.
- Li, W., Jiang, X., Xue, P., & Chen, S. (2002). Inhibitory effects of chitosan on superoxide anion radicals and lipid free radicals. *Chinese Science Bulletin*, 47, 887–889.
- Wang, J., Xing, G., Hu, W., Zhu, T., Wang, Q., & Zhao, H. (1994). Effects of Ge-132 on oxygen free radicals and the lipid peroxidation induced by hydroxyl free radical *in vitro*. *Chinese Pharmaceutical Journal*, 29, 23–25.
- Zhang, L., Wang, W., & Gao, J. (2003). Synthesis and properties of medical carboxymethyl starch. *Journal of Hebei University*, 23, 48–54.