Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 58 (2004) 379–382
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Superoxide anion scavenging activity of graft chitosan derivatives

Tao Sun[a,*], Wenming Xie[b], Peixin Xu[b]

[a]College of Food Science, Shanghai Fisheries University, Shanghai 200090, China
[b]Department of Chemistry, Yuquan Campus, Zhejiang University, Hangzhou 310027, China

Received 13 May 2004; accepted 25 June 2004
Available online 28 October 2004

Abstract

Two kinds of graft chitosan derivatives (CMCTS-*g*-MAS and HPCTS-*g*-MAS) were prepared by the graft copolymerization of maleic acid sodium onto etherified chitosans-carboxymethyl chitosan (CMCTS) and hydroxypropyl chitosan (HPCTS), respectively. Superoxide anion scavenging activity of the derivatives was evaluated in a luminal-enhanced autoxidaton of pyrogallol by chemiluminescence techniques. Compared with chitosan, the graft chitosan derivatives have much improved scavenging ability against superoxide anion. They have similar 50% inhibition concentrations (IC_{50}s) as ascorbic acid and superoxide dismutase (SOD). Graft chitosan derivatives with hydroxypropyl groups have relatively higher superoxide anion scavenging ability owing to the incorporation of hydroxyl groups. The graft chitosan derivatives (HPCTS-*g*-MAS 1, 2, and 3) with different grafting percentages exhibit IC_{50}s values ranging from 243 to 308 μg/mL, which could be related to the contents of active hydroxyl and amino groups in the polymer chains.

Keywords: Graft chitosan derivatives; Antioxidant; Superoxide anion

1. Introduction

Chitosan, an important naturally occurring polymer, is a marine polysaccharide with unique bioactivities. With the development of chitosan science and technology, chitosan and its derivatives have a prospective application in many fields especially in biomedicine. Recently, the research on antioxidant activity of chitosan and its derivatives has attracted much attention (Alexandrova, Obukhova, Domnina, & Topchiev, 1999; Esumi, Takei, & Yoshimura, 2003; Jeon et al., 2003; Kamil, Jeon, & Shahidi, 2002; Li, Jiang, Xue, & Chen, 2002; Matsugo et al., 1998; Park, Je, & Kim, 2004; Terada et al., 1999; Xue, Yu, Hirata, Terao, & Lin, 1998). Xue et al. reported that antioxidant activity of oligosaccharide chitosan, N, O-carboxymethyl chitosan and hydroxyprolated chitosan (Xue et al.). Chitosan derivatives partially quarternized with gallic acid had much improved antioxidant activity than chitosan and low molecular gallic acid (Alexandrova et al.). Acrylated or oxidized chitosan derivatives inhibited lipid peroxidations and had great effects on canine polymorphonuclear cells (Matsugo et al.; Terada et al.). Considering multifunction and low toxicity of chitosan, researches on antioxidant activity of chitosan derivatives will be helpful to expand their applications in biomedicine.

The superoxide anion, $O_2^{\cdot -}$, is formed in almost all aerobic cells and is a major agent in the mechanism of oxygen toxicity (Fridovich, 1978; Sawyer & Valentine, 1981). It related closely to the biological course of apolexis, tumor, and inflammation etc. Compared with other oxygen radicals, superoxide anion has a longer lifetime, can move to an aim at a longer distance, and thus has more dangerous. Therefore, it is very important to study the scavenging of superoxide anion.

Recent researches showed that after primary derivation then followed by graft modification, the products-graft chitosan derivatives would obtain multifunctions such as antibacterial and antioxidant activities (Sun, Xie, & Xu, 2003; Xie, Xu, Wang, & Liu, 2002a, 2002b). In this paper, two kinds of graft chitosan derivatives

* Corresponding author. Tel.: +86-216-5710-032; fax: +86-216-5684-287.
E-mail address: taosun@shfu.edu.cn (T. Sun).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.06.042

(CMCTS-*g*-MAS and HPCTS-*g*-MAS) were prepared and their superoxide anion scavenging ability was evaluated by chemiluminescence techniques.

2. Methods

Carboxymethyl chitosan (CMCTS) and hydroxypropyl chitosan (HPCTS) were synthesized from the etherification of alkali chitosan using chloroacetic acid and propylene epoxide as etherifying agents, respectively.

Graft chitosan derivatives (CMCTS-*g*-MAS and HPCTS-*g*-MAS) were prepared according to the previous method by the graft copolymerization of maleic acid sodium onto CMCTS and HPCTS using ammonium persulfate (APS) as an initiator (Xie et al., 2002a; Xie, Xu, Liu, & Xue, 2002; Sun, Xu, Liu, Xue, & Xie, 2003). The weight grafting percentage (G) was calculated as: $G=[(W_2-W_1)/W_1]\times 100$, where W_1 and W_2 represent the weights of CMCTS (HPCTS) and graft chitosan derivatives, respectively.

CMCTS was confirmed by absorption bands (FTIR) in the 1410 [γ_{sym} (CO_2)] and 1596 cm^{-1} [γ_{as} (CO_2)], and proton absorption (^{1}H NMR) at $\delta=3.39$ ppm. Elemental analysis results show that CMCTS has a substituting degree of 0.45. HPCTS (degree of substitution is 0.93 calculated from elemental analysis data) was characterized by the new adsorption peak at 2970 cm^{-1} (FTIR) and the protons peaks of the hydroxypropyl moiety successively absorbing at $\delta=3.10$, 5.05 and 3.80 ppm (^{1}H NMR). The grafted chitosan derivatives all showed characteristic absorption at 1700 cm^{-1}, and characteristic broad bands of carboxylate at 1560–1520 cm^{-1}.

Superoxide anion was generated by a luminol-enhanced autoxidaton of pyrogallol. 0.8 mL luminol (1×10^{-3} mol/L) and 0.2 mL Na_2CO_3/$NaHCO_3$ buffer solution (0.05 mol/L, pH = 10.3) were mixed vigorously, and the background were measured on a bio-chemical luminescence-measuring instrument (SGH-1, made in Shanghai). Then 0.1 mL pyrogallol (6.25×10^{-4} mol/L) was added to initiate the luminescence reaction. The luminescence kinetics curve (chemiluminescence (CL)–t) was measured with an integral time of 6 s, and accumulated continuously for 150 s (Guo & Wang, 1989). The relative content of superoxide anion radical $O_2^{\cdot-}$ in the system was calculated as the abstraction value of the peak value of curve CL–t and the background. Chitosan derivatives were added into the above system, and at the same time the volume of the buffer solution was reduced to keep the volume constant. The scavenging rate for superoxide anion $O_2^{\cdot-}$ was calculated as: $SR/\% = (CL_0 - CL_1)/CL_0$, where CL_0 and CL_1 represent peak values in the CL–*t* curves of the control group and test group, respectively. Every data point was obtained from three parallel determinations. The tolerance was no more than 3%.

The free radical produced in the system was proved to be superoxide anion tested by superoxide dismutase (SOD), catalase, and mannitol. Thiourea and ascorbic acid were used as a control.

3. Results and discussion

The antioxidant activity of chitosan derivatives was evaluated as superoxide anion scavengers by chemiluminescence technology. Fig. 1 showed superoxide anion scavenging activity of CMCTS and HPCTS. The scavenging rate increases with the increase of CMCTS (HPCTS) concentration. At the maximum concentration of about 1200 μg/mL, they have scavenging rates of 13.4 and 25%, respectively.

Compared with CMCTS or HPCTS, graft chitosan derivatives (CMCTS-*g*-MAS and HPCTS-*g*-MAS) show relatively strong scavenging activities against superoxide anion (shown in Fig. 2). Four graft chitosan derivatives have obvious scavenging ability against superoxide anion. And the scavenging rate gradually increases with the increase of the concentration of graft chitosan derivatives.

CMCTS-*g*-MAS and HPCTS-*g*-MAS have different superoxide anion scavenging effects. At an applied CMCTS-*g*-MAS concentration of 344 μg/mL in the final solution, a superoxide anion scavenging rate of approximately 50% is achieved, that is, the 50% inhibition concentration (IC_{50}) is 344 μg/mL. The maximum scavenging rate of CMCTS-*g*-MAS is 69.1% when its concentration reaches 680 μg/mL. However, HPCTS-*g*-MAS 1, 2, and 3 have IC_{50}s of 243, 275, and 308 μg/mL, respectively. And in the concentration range examined, the maximal scavenging rates of HPCTS-*g*-MAS (1, 2, and 3) are 71.7, 80.2, and 82.1%, respectively.

Polysaccharides with scavenging effect on superoxide anion have the same structural feature that they all have one or more alcohol or phenolic hydroxyl groups. And the scavenging effect was related to the number of active hydroxyl groups in the molecules (Li et al., 2002). Chitosan has two hydroxyl groups and one amino group in its construction unit. The researches of antioxidant activity of chitosan and its derivatives indicated that the hydroxyl groups were involved in the reaction with free radicals

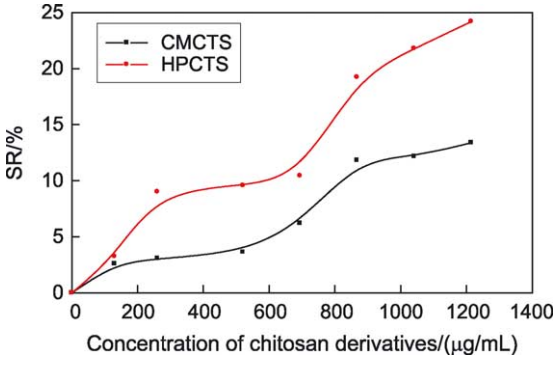

Fig. 1. Superoxide anion scavenging activity of CMCTS and HPCTS.

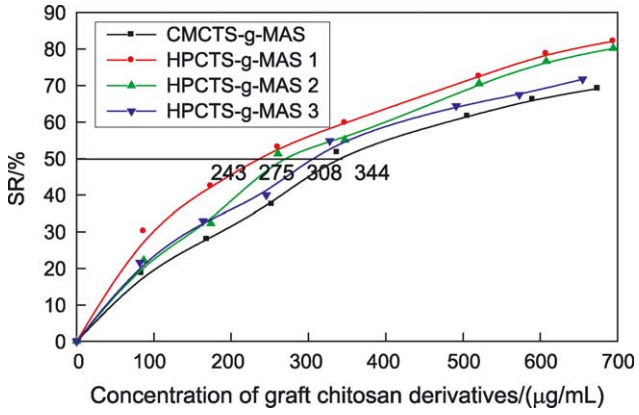

Fig. 2. Superoxide anion scavenging activity of CMCTS-*g*-MAS and HPCTS-*g*-MAS.

(Li et al., 2002; Xue et al., 1998). The scavenging effect of graft chitosan derivatives on superoxide anion may also be related to the active hydroxyl groups in the polymer chains. On the other hand, according to the free radical theory the amino groups in chitosan can react with free radicals to form most stable macroradicals (Sun et al., 2003; Xie et al., 2002; Yazdani, Lagos, Campos, & Retuert, 1992). According to the research of Park et al., the free radical scavenging activities of hetero-chitosans depend on their degree of deacetylation, this is, the content of amino group. The results indicated that the amino group may take part in the scavenging of free radicals. Therefore, the active hydroxyl and amino groups in the polymer chains are the origin of the scavenging ability of chitosan derivatives.

Compared with chitosan derivatives, chitosan itself has the highest content of hydroxyl and amino group and should have the strongest scavenging ability. But according to the report of Alexandrova et al., the antioxidant activity of chitosan was equal to zero (Alexandrova et al., 1999). And chitosan with different acrylation degrees did not show any scavenging activity toward superoxide anion (Matsugo et al., 1998). Li reported the superoxide anion scavenging ability of chitosan, but it has a high IC_{50} of 1.12×10^{6} μg/mL calculated from electron spin resonance (ESR) results (Li et al., 2002). This should be related to the formation of strong intermolecular and intramolecular hydrogen bonds that inhibited the reactivity of hydroxyl and amino groups in the polymer chains. After etherification, a longer hydroxyl group (HPCTS) or a substituting carboxylic group with strong electron-withstanding ability (CMCTS) were introduced into the chitosan chains. Both two substituting groups are very helpful to enhance the antioxidant activity of chitosan derivatives. Compared with the nearly zero scavenging ability of chitosan in our experiments, the scavenging activities of CMCTS and HPCTS against superoxide anion were moderately improved but not so much. The reason may be that the molecular structure of chitosan has not been so much changed.

HPCTS-*g*-MAS 1, 2 and 3 with different grafting percentages have different IC_{50}s. As shown in Table 1,

Table 1
Graft copolymerization of MAS onto CMCTS or HPCTS at 70 °C[a]

Copolymers	Concentration (mol/L)	*G%*	IC_{50} (μg/mL)
CMCTS-*g*-MAS	0.6	586	344
HPCTS-*g*-MAS1	0.6	560	243
HPCTS-*g*-MAS2	1.2	1633	275
HPCTS-*g*-MAS3	1.8	1720	308

[a] Reaction conditions: CMCTS = 0.20 g; HPCTS = 0.20 g; APS = 0.1 mmol; 2 h.

the IC_{50} is high when the grafting percentage is high, which indicates that the copolymer with high *G%* has low scavenging ability. With high grafting percentage, the copolymer has relatively low content of hydroxyl and amino groups, and thus low scavenging ability. Compared with CMCTS and HPCTS, the inner structure of chitosan was severely disrupted by the introduction of grafted polymer chains after graft modification. The ability to form hydrogen bond declines sharply, this is, the hydroxyl and amino groups are activated, and this is helpful to the reaction with superoxide anion.

Though have similar grafting percentages, CMCTS-*g*-MAS has higher IC_{50} and lower scavenging ability than HPCTS-*g*-MAS 1, which may be due to the incorporation of hydroxyl group. The results are different from their scavenging effects on hydroxyl radical (Xie et al., 2001), which perhaps is related to the different reaction mechanisms of graft chitosan derivatives with hydroxyl radical and superoxide anion. As known, the reaction of hydroxyl groups with O_2^- presumably occurs by the same initial proton-induced dismutation step (Dietz, Forno, Larcombe, & Peover, 1970), while the hydroxyl radical main reacts by H-abstraction reaction.

The scavenging ability of graft chitosan derivatives can be compared with the control samples. Thiuorea, ascorbic acid and SOD have IC_{50}s of 300, 250, and 220 μg/mL, respectively. Considering the good water-solubility and excellent antibacterial activities (Xie et al., 2002a, 2002b), graft chitosan derivatives can be developed into new kinds of marine polysaccharide drugs with several types of bioactivities such as antioxidant and antibacterial activities.

4. Conclusions

Antioxidant activity of chitosan and its derivatives mainly attributes to the active hydroxyl and amino groups. The introduction of substituting groups will destroy the structure of chitosan, decrease the intermolecular hydrogen bond, and thus will be helpful to increase the antioxidant activity of chitosan derivatives. The difference of

antioxidant activity of graft chitosan derivatives could be attributed to their different contents of active hydroxyl and amino groups in their polymer chains.

Acknowledgements

This project was supported by the President's Special Foundation of Shanghai Fisheries University (SFU200305).

References

Alexandrova, V. A., Obukhova, G. V., Domnina, N. S., & Topchiev, D. A. (1999). *Macromolecules Symposium*, *144*, 413.

Dietz, R., Forno, A. E. J., Larcombe, B. E., & Peover, M. D. (1970). *Journal of Chemical Society B* , 816.

Esumi, K., Takei, N., & Yoshimura, T. (2003). *Colloids and Surfaces B: Biointerfaces*, *2*, 117.

Fridovich, I. (1978). *Science*, *201*, 875.

Guo, A. G., & Wang, Z. Y. (1989). *Plant Physio Communication*, *3*, 54.

Jeon, T. I., Hwang, S. G., Park, N. G., Jung, Y. R., Shin, S. I., Choi, S. D., & Park, D. K. (2003). *Toxicology*, *187*, 67.

Kamil, J. Y. V. A., Jeon, Y. J., & Shahidi, F. (2002). *Food Chemistry*, *1*, 69.

Li, W. J., Jiang, X., Xue, P. H., & Chen, S. M. (2002). *Chinese Scientific Bulletin*, *11*, 887.

Matsugo, S., Mizuie, M., Matsugo, M., Ohwa, R., Kitano, H., & Konishi, T. (1998). *Biochemistry Molecular Biology International*, *5*, 939.

Park, P. J., Je, J. Y., & Kim, S. K. (2004). *Carbohydrate Polymer*, *1*, 17.

Sawyer, D. T., & Valentine, J. S. (1981). *Accounts Chemical Research*, *14*, 393.

Sun, T., Xie, W. M., & Xu, P. X. (2003). *Macromolecular Bioscience*, *6*, 320.

Sun, T., Xu, P. X., Liu, Q., Xue, J., & Xie, W. M. (2003). *European Polymer Journal*, *39*, 189.

Terada, N., Morimoto, M., Saimoto, H., Okamoto, Y., Minami, S., & Shigemasa, Y. (1999). *Chemistry Letters*, *12*, 1285.

Xie, W. M., Xu, P. X., & Liu, Q. (2001). *Bioorganic and Medicinal Chemistry Letters*, *11*, 1699.

Xie, W. M., Xu, P. X., Wang, W., & Liu, Q. (2002b). *Journal of Applied Polymer Science*, *85*, 1357.

Xie, W. M., Xu, P. X., Wang, W., & Liu, Q. (2002a). *Carbohydrate Polymer*, *50*, 35.

Xie, W. M., Xu, P. X., Liu, Q., & Xue, J. (2002). *Polymer Bulletin*, *49*, 47.

Xue, C. H., Yu, G. L., Hirata, T., Terao, J., & Lin, H. (1998). *Bioscience Biotechnology Biochemistry*, *2*, 206.

Yazdani, P. M., Lagos, A., Campos, N., & Retuert, J. (1992). *Journal of Polymeric Materials*, *18*, 25.