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Superoxide Anion Radical Scavenging Ability of Quaternary Ammonium Salt of Chitosan

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Abstract: A series of N-alkyl or N-aryl chitosan quaternary ammonium salt were prepared using 96% deacetylated chitosan. Their scavenging activities against superoxide anion radical were investigated by chemiluminescence. The IC_{50} values of these compounds range from 280 to 880 μg/mL, which should be attributed to their different substitutes.

Keywords: Chitosan quaternary ammonium salts, antioxidant activity, chemiluminescence, superoxide anion radical.

The increasing evidence suggests that some age-related human diseases such as heart disease, cancer, inflammation, arthritis, immune system decxline, brain dysfunction and cataracts are the result of cellular damage by free radicals[1,2]. When antioxidant defense systems, which can scavenge and minimize the formation of free radicals are not effective, oxidative damage does occur *in vivo*. To minimize potential oxidative damage *in vivo*, antioxidants from various sources have been studied. Chitosan is a cationic polysaccharide made from alkaline N-deacetylation of chitin. Chitosan has found a wide variety of applications in both industrial and medical fields[3]. However, chitosan shows its biological activity only in acidic medium because of its poor solubility above pH 6.5. Thus, water soluble chitosan derivatives which are soluble in both acid and basic physiologic circumstances might have a wide-ranging application. Recently, the antioxidant activity of chitosan and its derivatives has attracted the most attention[4--7]. The scavenging activities against hydroxyl radical of some water-soluble chitosan derivatives were investigated[8]. In this communication, we demonstrate the utilization of water soluble quaternary ammonium salts of chitosan as a superoxide anion radical scavenger by chemiluminescence technique.

The water soluble quaternary ammonium salts of chitosan, such as N, N, N –trimethyl chitosan (TC), N-benzyl, N-dimethyl chitosan (BDC), N-phenylethyl-N-, N-dimethyl chitosan (PHDC), N-salicyl-N,N-dimethyl chitosan (SDC) and N-furfuryl-N, N-dimethyl chitosan (FDC) were prepared using 96% deacetylated chitosan according to the method that we reported previously[9]. The structure of quaternary ammonium salts of chitosan was confirmed by FT-IR and ^{1}H NMR spectra , which were compared with

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that of the corresponding chitosan and glucosamine. There are some characteristic peaks of it's derivatives in IR spectrum. The intensities of the peaks in range of 2889-2970 cm^{-1} and 1460 cm^{-1} were increased due to the presence of methyl or methylene groups. Formation of the N-aryl derivatives was also confirmed by the presence of characteristic peaks of phenyl or furfuryl group. Specially the peak around 1593 cm^{-1} (N-H) disappeared, it was confirmed that the N-alkyl (or N -aryl) chitosan derivatives were formed. Examination of ^1H NMR spectrum provided the evidence for the structure of the obtained derivatives. The proton signals were similar with the data of Xu[10].

The superoxide-scavenging activity was determined using chemiluminescence methods. The assay was carried out on a SHG-C luminemeter (Shanghai Shangli instrument factory). Superoxide anion were generated by pyrogallol autoxidantion[11]. 0.4 mL 1mmol/L luminol and 0.7 mL 0.05 mol/L $NaCO_3$-$NaHCO_3$ buffer solution (pH 10.2) were added in a 1.2 ×5 cm glass tube and mixed intensively. 0.1 mL 0.625mmol/L pyrogallol was added to initiate the luminescence reaction, and the chemiluminescent emission from the resulting mixture was counted at an interval of 6 s for 25 times as CL_0. Different volume of solution of chitosan derivatives was used instead of buffer solution and the other processes are the same as described above , and the chemiluminescent emission was recorded as CL_1. The amount of superoxide anion radical in the system was represented by the chemiluminescence intensity. The scavenging ability on superoxide anion radical was calculated according to the following formula:

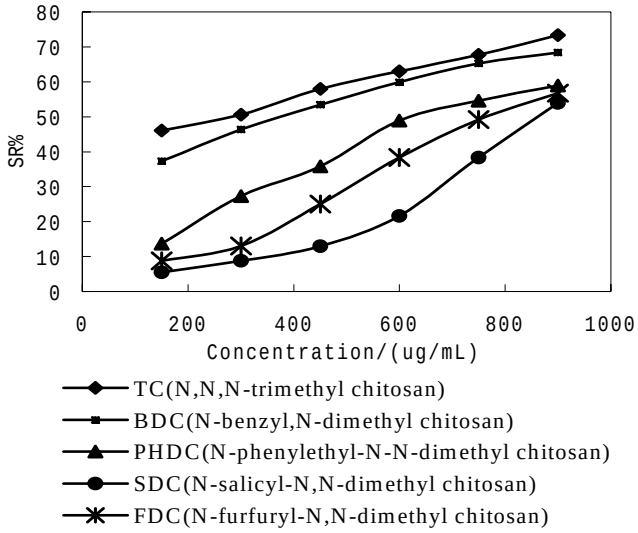
$$S=(CL_0-CL_1)/CL_0\times100\%$$

Where CL_0 , CL_1 were the chemiluminescence intensity in the system without and with scavenger, respectively. The free radical produced in this system was proved to be superoxide anion radical tested by superoxide dismutase (SOD), catalase and mannitol.

The scavenging ability of various concentrations of quaternary ammonium salts of chitosan on superoxide anion radical are shown in **Figure 1**. Five compounds had obvious scavenging activities. The scavenging rate increased with the increasing of concentration of chitosan derivatives.

The IC_{50} (The 50% inhibition concentration) of TC, BDC,PHDC, FDC and SDC were 280,380,650,770 and 880 μg/mL, respectively. The scavenging mechanism may be related to the fact that $O_2^{-\cdot}$ can react with active hydrogen atoms in quaternary ammonium salts of chitosan to from a most stable macromolecule radical. It is well known that the free radical's scavenging activities are closely related to bond dissociation energy of O-H and the stability of the formed radicals. Chitosan has strong intramolecular and intermolecular hydrogen bonds. The OH groups are difficult to dissociate and react with $O_2^{-\cdot}$. So chitosan has almost no antioxidant activity[4]. The different scavenging effects of chitosan derivatives on $O_2^{-\cdot}$ should be attributed to their different structures. Compared with other quaternary ammonium salts of chitosan, TC has three strong electron-withdrawing methyl groups. The electron-withdrawing groups improve the energy level of the highest occupied molecular orbital (HOMO) and deline the dissociation energy of O-H[12]. Therefore TC has the highest scavenging ability.

Figure 1 The dependence of scavenging effect on the concentrations of quaternary ammonium salts of chitosan



The SDC has lowest scavenging ability, for that it has a electron releasing phenolic group.

These scavenging ability can be compared with those of some commonly used superoxide anion scavengers, such as thiourea and ascorbic acid (IC_{50} is 250 μg/mL for thiourea and 280 μg/mL for ascorbic acid, respectively). The above results suggest the potential uses of quaternary ammonium salts of chitosan in the biochemical or pharmaceutically field.

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