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Hydroxyl Radical Scavenging Effect of Partially N-Acetylchitooligosaccharides by ESR Spectroscopy

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Introduction

Chitosan, a deacetylated derivative of chitin (a linear polysaccharide of -1,4-linked N-acetylglucosamine residues), is one of the abundant resources, and its biological properties such as antibactierial activity, hypocholesterolemic activity and antihypertensive action are remarked. However, increasing attention has recently been given to converting chitosan to its oligosaccharides. Even though chitosan has very strong functional properties in many areas, its high molecular weights and high viscosity may restrict the uses *in vivo*. Therefore, the actions of chitosan *in vivo* still remain ambiguous as the physiological functional properties because most animal intestine, especially the human gastrointestinal tract, do not possess enzyme such as chitosanase which directly degrades the β-glucosidic linkage in chitosan and consequently the unbroken polymers may be poorly absorbed into the human intestine. Additionally, chitosan oligosaccharides possess various functional properties like antitumor activity, immuno-enhancing effects, enhancing protective effects against infection with some pathogens in mice, antifungal activity, and antimicrobial activity. There, however, little information on the free radical scavenging activity of chitosan oligosaccharides.

In the present study, we investigated the scavenging effect of chitosan oligosaccharides on hydroxyl radical scavenging effects by electron spin resonance(ESR) spin-trapping technique.

Materials and Method

Preparation of Chitosan oligosaccharides The chitosan oligosaccharides (COS) was prepared from chitosan by an enzymatic reaction using the chitosanase in a bioreactor system according to the previously reported method (Jeon and Kim, 2000), and fractionated into three kinds of the COS with relatively higher molecular weights (90, 75 and 50-HMWCOS), medium molecular weights (90, 75 and 50-MMWCOS) and low molecular weights (90, 75 and 50-LMWCOS) were

prepared using ultrafiltration membrane in conjunction with an enzymatic bioreactor. The COS recovered was lyophilized on a freezing-drier for 5 days.

Hydroxyl radical scavenging assay The hydroxyl radicals were generated via iron-catalyzed Haber-Weiss reaction (Fenton driven Haber-Weiss reaction) and spin trapped with DMPO. The resultant DMPO-OH adduct was detected using an electron spin resonance (ESR) spectrometer (JEOL-JES-FA, JEOL Ltd., Tokyo, Japan). The COS were dissolved in 0.1 M phosphate buffer (pH 7.4) from 0.4mg/ml to 0.1mg/ml. The COS solution (200$\mu\ell$) were mixed with 200$\mu\ell$ of 0.3 M DMPO, 200$\mu\ell$ of 10 mM $FeSO_4$ and 200$\mu\ell$ of 10 mM hydrogen peroxide. All solutions were prepared in 0.1 M phosphate buffer (pH 7.4). After 3 min, 50$\mu\ell$ of the mixture were drawn into a syringe and transferred into a quartz capillary tube. The spectrum was recorded in the ESR spectrometer set at 2×105 receiver gain, 1.0 G modulation amplitude, 200s scan time, 3460 G center field, 100 G sweep width and 0.5 s time constant (Shi et al., 1991). Hydroxyl radical scavenging capacities of the additives were calculated using the following equation:

Hydroxyl radical scavenging capacity, % = 100 - (ESR signal intensity for medium containing the additives of concern/ESR signal intensity for the control medium)×100.

Results

Partially N-acetylated chitosans with different degrees of N-deacetylation were prepared by deacetylation of chitin. The degrees of deacetylation of the products were about 50%, 75% and 90%, respectively. Three partially deacetylated chitosans were hydrolyzed by various enzymes, and the N-deacetylchitooligosaccharides with relatively higher molecular weights (90, 75 and 50-HMWCOS), medium molecular weight (90, 75 and 50-MMWCOS) and lower molecular weight (90, 75 and 50-LMWCOS), respectively, was evaluated by using electron spin resonance (ESR) spectroscopy. All N-deacetylchitooligosaccharides used showed hydroxyl radical scavenging effect of above 50% by ESR spin-trapping technique. In addition, 50-MMWCOS showed the highest hydroxyl radical scavenging effect.

References

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