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Free Radical Scavenging Activities of Chitosan Oligosaccharides Produced by Bioreactor

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Introduction

Chitosan, a deacetylated derivative of chitin, is one of the abundant resources, and its biological properties such as antibactierial activity, hypocholesterolemic activity, and hypertensive action are remarked. However, increasing attention has recently been given to converting chitosan to its oligosaccharides because they 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 (Jeon et al., 2001). However, little information on the free radical scavenging activity of chitosan oligosaccharides (COS) is available until now.

In recent years, the role of active oxygen and free radicals in tissue damage in various diseases such as cancer, gastric ulcer, and other diseases is becoming increasingly recognized. Especially, hydrogen peroxide promotes tumors in mouse skin, and several tumor promoters like 12-O-tetradecanoylphorbol 13-acetate, 12-O-retinoyl-phorbol 13-acetate and mezerein induced formation of hydrogen peroxide by human polymorphonuclear leukocytes and caused DNA damage. (Cunningham et al., 1987) demonstrated that superoxide produced single-strand breaks in DNA in Chinese hamster ovary cells in a dose-dependent manner. The number of breaks was decreased on the prior addition of a metal chelator, indicating that some breaks may have been caused by peroxide or hydroxyl radical.

In the present study, we investigated the scavenging effect of chitosan oligosaccharides on free radical scavenging effects by a stable free radical, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and hydroxyl radical scavenging activities by electron spin resonance (ESR) spectrometer.

Materials and Methods

Materials, Chitosan (degree of deacetylation : 89 %, average molecular weight : 685,000) was donated by Kitto Life Co. (Pyangtaek, Korea). Chitosanase (694 units (U)/g protein) for the preparation of chitooligosaccharides was from *Bacillus pumilus* BN-262, and purchased from Wako Chemical Industries, Ltd (Tyoko, Japan). Ultrafiltration membrane reactor (Millipore MinitanTM) system for production of chitooligosaccharides was from

Millipore Co. (Bedford, MA). 5,5-Dimethyl-pyrronine N-oxide (DMPO), and DPPH were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of the highest grade available.

Scavenging effect on DPPH radical, The effect of COS on DPPH radical was estimated according to the method of Hatano et al. (1988). The COS in 3.5 ml of distilled water were added to a methanolic solution of 1.5×10-4 DPPH (1 ml). The mixture was shaken and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

Hydroxyl radical scavenging assay, The hydroxyl radical reacts rapidly with DMPO; the resultant DMPO-OH is detectable with an electron spin resonance (ESR) spectrometer. The spectrum was recorded 2.5 min after chitosan oligosaccharide (COS-Ⅲ, 0.2 ml) were mixed with DMPO (0.3 M, 0.2 ml), Fe^{2+} (10 mM, 0.2 ml), and H_2O_2 (10 mM, 0.2 ml) in a phosphate buffer solution (pH 7.2) using an ESR spectrometer set at the following conditions: receiver gain, 2×105; modulation amplitude, 1.0 G; scan time, 200 s; field, 3461.3 ± 50 G; time constant, 0.5 s.

Conclusions

The free radical scavenging activities of five kinds of chitosan oligosaccharides (COS) were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and a spin-trapping electron resonance method. Five kinds of COS with relatively very higher molecular weights (COS-Ⅰ: 30-10 kDa), higher molecular weights (COS-Ⅱ: 10-5 kDa), medium molecular weights (COS-Ⅲ: 5-3 kDa), lower molecular weights (COS Ⅳ: 3-1 kDa) and very lower molecular weights (COS Ⅴ: below 1 kDa) were prepared using ultrafiltration membrane in conjunction with an enzymatic bioreactor. The radical scavenging effects of COS on the DPPH radical were decreased in the order of COS-Ⅲ > COS-Ⅳ > COS-Ⅴ > COS-Ⅱ > COS-Ⅰ. In addition, COS-Ⅲ scavenged above 90 % hydroxyl radical at a dosage of 0.25 mg/ml with a reaction mixture of Fe2+ and H2O2 in the presence of spin trapping agent 5,5-dimethyl-pyrronine N-oxide (DMPO). Therefore, It seems that chitosan oligosaccharides have scavenging activities against DPPH and hydroxyl radical.

References

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