In vitro Bacteriostatic Effects of Dietary Polysaccharides

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The antimicrobial action of dietary polysaccharides on eight food-borne pathogenic bacteria were examined. Among the polysaccharides, the carrageenans showed the most pronounced inhibitory effect, the growth of all the bacterial strains except Listeria monocytogenes being significantly inhibited by them, particularly by ı-carrageenan. A growth-inhibition experiment using Salmonella enteritidis showed that the inhibitory effect of the carrageenans was not bactericidal but bacteriostatic. The removal of sulfate residues eliminated the bacteriostatic effect of ı-carrageenan, suggesting that the sulfate residue(s) in carrageenan played an essential role in this effect. The results of the present study suggest that dietary polysaccharides, and particularly carrageenans, may act as effective preservatives in various types of processed food.

Keywords: carrageenan, pectin, sulfated polysaccharide, antimicrobial activity, Salmonella enteritidis

Dietary polysaccharides are used in many processed food products as modifiers of rheological properties or as stabilizers. In addition to those physical properties, the undigestible polysaccharides or dietary fibers such as pectins, gums, cellulose and alginate are known to provide a physiological benefit: they improve the bowel conditions after an oral administration (Schneeberger, 1994). Dietary fibers have therefore been given increased attention as ingredients of physiologically functional food (Prosky, 2000). Some other physiological and biochemical functions of polysaccharides have also been reported. For example, the suppression of intestinal glucose uptake by dietary fibers can be expected to reduce the blood glucose level after a meal (Nut-tall, 1993), while the lowering effect on serum cholesterol has also been reported (Brown et al., 1999).

El-Nakeeb and Yousef (1970a, b) have studied the antimicrobial activity of pectin because the “apple diet” and various pectin-containing preparations have long been used in the relief of diarrhea. They found that pectin was antimicrobial to various microorganisms. Recent studies by Tazawa et al. (1999) have also shown that apple pectin exerted a bacteriostatic action that may change the composition of the intestinal flora. However, the antimicrobial properties of other dietary polysaccharides remain to be elucidated. The present study was undertaken to identify the antimicrobial properties of such dietary polysaccharides as pectins, gums and carrageenans against various food-borne pathogenic bacteria.

Salmonella enteritidis (strain no. E930448, phage type 4), Salmonella typhimurium (1030–96), Vibrio mimicus (753), Aeromonas hydrophila (088), enterotoxigenic Escherichia coli (K88) and Listeria monocytogenes were the kind gift of Dr. Shizunobu Igimi of the National Institute of Infectious Diseases. Staphylococcus aureus strains (SA110, S-6 and SA106, 494) were the gift of Dr. S. Fukuda of Kikkoman Co (Noda). The polysaccharide samples were all purchased from Sigma Chemical Co. (St. Louis, MO). Bactopeptone and trypticase soy broth were obtained from Difco Laboratories (Detroit, MI) and trypticase soy broth was from Becton Dickinson (Cockeysville, MD).

The bacteria were grown overnight in trypticase soy broth at 37˚C. The bacterial cells were collected by centrifuging the culture medium that was taken periodically, the turbidity being measured at 550 nm. Data were subjected to an analysis of variance and means separation by Scheffe’s tests. Statistical studies were performed with Statigraphic software (Statistical Graphic Co. and Graphic Software Systems Inc., Rockville, MD).

Guar gum, gum arabic, apple pectin, citrus pectin, λ-carrageenan, ε-carrageenan and κ-carrageenan (Fig. 1) were all purchased from Sigma Chemical Co.. Tritium labeling of the carrageenans was performed with 3H-NaBH4 according to the method of Gahmberg and Hakomori (1973). ε-Carrageenan was desulfated according to the method described by Ishizaka et al. (1989). The sulfate in the carrageenans was determined by the method of Dey et al. (1992).

The bacterial growth was initially evaluated in this study by two methods, turbidimetry and colony counting. Since the results obtained by these two techniques were well correlated (data not shown), we used the turbidimetric method for subsequent experiments.
The effects of dietary polysaccharides on the growth of eight different bacteria were examined. Figure 2 shows the turbidity of the eight bacterial cultures after 6 h of incubation in the presence of 0.25% of the polysaccharides. Gum arabic showed no growth-inhibitory effect on any of the bacterial strains used in this experiment, while guar gum significantly inhibited the growth of *Salm. typhimurium*, *V. mimicus* and *Staph. aureus* SA106. The pectins, particularly those of apple origin, were also inhibitory against various bacteria, including *Salm. enteritidis*, *Salm. typhimurium*, *V. mimicus*, *Aer. hydrophila*, *E. coli* and the *Staph. aureus* strains. However, the most pronounced inhibitory effect was observed with the carrageenans. The growth of all the bacteria used in this experiment except *L. monocytogenes* was significantly inhibited by the carrageenans, the inhibitory activity of *κ*-carrageenan being particularly high among the three types tested.

The reason of the inhibitory activity was different among the various bacteria is not known, since the mechanism for this inhibition has not yet been elucidated. One possible mechanism could be binding of the polysaccharides with the bacterial surface. Since the surface molecules present in various strains of bacteria differ, the binding ability with polysaccharides of bacteria having different structural properties may be diverse. Our recent results have, however, suggested that the inhibition was not via binding of the polysaccharides to the bacterial cell surface, because the experiment with ³H-carrageenan showed no binding with the bacteria (for example, *Salm. enteritidis*; data not shown). Another possible mechanism may be trapping of such nutrients as cationic minerals in the culture medium by the polysaccharides, by which the bioavailability of the nutrients was
polysaccharides significantly lowered the growth rate of the bacteria in a concentration-dependent manner, although the effect was not bactericidal but bacteriostatic.

Carrageenans contain sulfated D-galactose and anhydro-D-galactose. Since the sulfate residue is known to be involved in various functional properties of sulfated polysaccharides (Nakashima et al., 1987; Thornton et al., 1999; Witvrouw & De Clercq, 1997), we examined the growth-inhibitory effect of κ-carrageenan after desulfation. Figure 4 shows that the removal of sulfate (about 90% of sulfate had been removed; data not shown) eliminated the bacteriostatic effect of κ-carrageenan, suggesting that the sulfate in carrageenan played an essential role in its bacteriostatic effect. The inhibitory activity, however, is not likely to have been dependent on the sulfate content alone, because the sulfate content of κ-carrageenan, which had the highest inhibitory activity among the three types of carrageenan, was not the highest of the three (λ, 32%; μ, 35%; κ, 25%). The mechanism for the bacteriostatic effect needs to be studied in more detail.

The results of the present study suggest that various polysaccharides, particularly carrageenans, may act as effective preservatives in processed food. Although their antimicrobial activity is not very high, their bacteriostatic property could be of help by enhancing the effect of other preservatives.

**References**


