Antimicrobial Activity of Medium Molecular Weight Chitosan against 
Streptococcus mutans

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ABSTRACT

The purpose of this study was to evaluate the antimicrobial activity of medium molecular weight chitosans (MMWCs; 3,500~7,800 Da) and high molecular weight chitosan (HMWC, 200 kDa) with a deacetylation degree of 95% against representative oral pathogen, Streptococcus mutans, which is a principal etiological agent of dental caries in humans. Among bioactive substances, HMWC shows significant inhibitory effect on S. mutans and chitosans exerted molecular weight dependent growth inhibition of 80% on growth rate of S. mutans. Furthermore, synthesis of exopolysaccharide (EPS) and acidogenic properties of S. mutans were also inhibited by MMWCs, showing that MMWC1 having 7,800 Da among the MMWCs exhibited the strongest antibacterial efficacy against S. mutans. Consequently, it was found that MMWC1 is of a potent natural material available in antibacterial component useful for oral health.

Keywords: Chitosan, Molecular weight, Streptococcus mutans, Antimicrobial activity

INTRODUCTION

Chitosan, known as a linear polysaccharide of β-1, 4 linkage and partially or fully deacetylated form of chitin, has been reported to show potent inhibitory effect on bacteria growth (1). There are several mechanism proposed for the antimicrobial activity of chitosan and its hydrolysates. One of the mechanisms proposed is the reduction of bacterial metabolism by stacking of chitosan molecules to cell wall of bacteria (2). Another mechanism proposed is the inhibition of description to RNA from DNA by adsorption of chitosan directly to DNA of bacteria (1). Subsequently, these mechanisms could be fully influenced by number of factors including the type of chitosan, degree of depolymerization, degree of deacetylation, chemical and physiological features (3-5). Owing to these features concerted with its biodegradability and biocompatibility, chitosan has been being paid high attention as an interesting biopolymer to be used in pharmaceutical (6,7) and cosmetical components (8), food (9), as well as in many other research fields of biomedicine (10). However, among these factors suggested above, poor water-solubility of high molecular weight chitosan (HMWC) is limited factor for the application in various fields. Therefore, many studies have focused on conversion of HMWC to chitosan oligosaccharide since depolymerized chitosan is readily soluble in water due to their shorter chain (11). Furthermore, such chitooligosaccharides are potentially more advantageous than high molecular weight chitin and chitosan as nutraceutical food additives because chitooligosaccharides are easily degraded in the human intestine (12).

Among many biological activities of HMWC and its hydrolysates, we aimed to evaluate the antibacterial activity toward Streptococcus mutans, which is a principal etiological agent of dental caries in humans (13). As long as we know, S. mutans produce water insoluble and soluble polysaccharide from dietary sugar by the action of enzyme identified as glycosyltransferase and fructosyltransferase for adsorbing to enamel surface. Also, it adheres tenaciously to glucan coated surfaces and is acidogenic and acid tolerant by producing lactic acid as end-product (14,15). Consequently, dental caries occurred by biofilm produced in biological function described above of S. mutans. Although chitosan is well known to have considerable antimicrobial activity, there was little work reported on its inhibitory activity against periodontal pathogens except in the case of reports of Choi et al. (16) and Tarsi et al. (17). Therefore this study was employed to estimate the biological activity of MMWCs derived from the HMWC against the representative periodontal pathogen, S. mutans. For this purpose, we have evaluated the inhibitory growth of bacteria by bioactive substances including monosaccharides, MMWCs and HMWC was compared in terms of antibacterial efficacy as biological functions including (i) growth rate, (ii) synthesis of EPS and (iii) acidification of S. mutans.

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MATERIALS AND METHODS

Materials and chemicals
A high molecular weight chitosan (HMWC, 95% degree of deacetylation, DD) of 200 kDa was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used for the preparation of medium molecular weight chitosans (MMWCs) with different molecular weights ranging from 3,500 to 7,800 Da. Various monosaccharides such as glucosamine, galactosamine, and mannose and other polysaccharides including alginates, β-glucan, and hyaluronic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). For bacterium, S. mutans was kindly provided from Prof. Jae-Gyu Jeon (Chonbuk University, Korea). All other reagents were used without further purification and were of the highest grade available.

Preparation of MMWCs
The HMWC with approximately 200 kDa was dissolved in 1 N hydrochloride to be 20 g/L, 50 g/L and 80 g/L in concentration, tentatively named as MMWC1, MMWC2, and MMWC3. Each solution was subjected to partial depolymerization by acid-hydrolysis by reacting at 100°C for 30 min and cooling down at room temperature. After that, ethanol was used to precipitate the medium molecular weight chitosan from the three MMWCs samples, respectively. The precipitates were then continuously washed with the cold-ethanol until the pH value of each MMWCs solution was same as the ethanol. After several washing, the final products were dried by lyophilization and the resulting MMWCs were used for subsequent experiments.

Antimicrobial activity
In order to investigate the bacterial growth inhibitory activity of various sugars against S. mutans, various monosaccharides and other polysaccharides including alginates, β-glucan, hyaluronic acid, HMWC and MMWCs were tested. S. mutans was incubated anaerobically in brain heart infusion (BHI) broth consisting of 1% sucrose and 1% starch at 37°C for 3 days. The assay was carried out as follows: solutions containing various mono- and polysaccharides illustrated above were prepared to 1% (w/v) in distilled water, respectively or acetic acid (1%) for only HMWC. Each solution prepared was added to BHI broth to be final concentration of 0.5% (v/v). The 100 ml of inoculum of S. mutans pre-incubated in BHI broth at the optimized condition for overnight was added in fresh broth in the presence or in the absence of each sample. Each broth prepared was further incubated at 37°C for 12 h anaerobically, by providing 5% CO2 into an incubator. After the incubation, the growth rate of S. mutans was determined by measuring the optical density at 600 nm. Furthermore, aliquots from the culture broth in the presence of chitosan series including MMWCs and HMWC were centrifuged at 13,500 rpm for 5 min and subjected to determine inhibitory effect of the samples on biological functions including (i) growth rate, (ii) synthesis of EPS and (iii) acidification of medium by S. mutans.

Inhibitory effect of MMWCs on biological functions of S. mutans
Chitosan series including HMWC and MMWC were tested to estimate their effectiveness as inhibitory compounds to evaluate their biological functions on synthesizing soluble and insoluble EPS, and acidification of S. mutans. To determine concentration of water soluble and water insoluble EPS synthesized by S. mutans, the aliquots taken from each sample at time intervals of 3, 6, 9 and 12 h were subjected to phenol-sulfuric acid method using glucose as the standard material (18,19). For the determination of influence of chitosan series on glycolytic pH drop of S. mutans, pH meter (HANNA instruments, Korea) was used to measure the pH change of supernatant from each aliquot taken from the same condition aforementioned above. Furthermore, the concentration of protein in supernatant of aliquots was determined by following method of Bradford (20) using bovine serum albumin (BSA) as standard protein.

RESULTS AND DISCUSSION

Effect of bioactive substances on microbial growth
The antimicrobial activity of mono- and polysaccharides toward the growth of S. mutans was determined by measuring the turbidity of each culture medium. As shown in Fig. 1, chitosan among the several polymers tested in this study exhibited a considerable inhibitory activity on growth against S. mutans. Protonation of the number of amino groups linking to C-2 on chitosan backbones may be an important factor in electrostatic interaction between the polycationic structure and the predominantly anionic components of the microorganism’s cell wall, as early study demonstrated (21). The hypothesis suggested is supported by the result elucidating inhibitory effect of monosaccharides on growth of S. mutans. As shown in Fig. 2, among the several monosaccharide tested, specifically amino sugars including glucosamine, mannosamine and galactosamine showed strong inhibitory activity (over 80%) on growth of S. mutans, compared with neutral sugars. On the other hand, neutral sugars including glucose and xylose facilitated growth of S. mutans, compared to control. Although metabolic pathway for utilizing of various amino sugars by S. mutans is no ascertainable yet, these results suggest that S. mutans may not able to utilize or bio-convertible of amino sugars as