


Antimicrobial Activity of Amino-Derivatized Cationic Polysaccharides

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Abstract To improve the antimicrobial property of chitosan, water-soluble chitosan modified in their quaternary ammonium groups were synthesized. The antimicrobial properties were evaluated against *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Candida tropicalis*. The activities increased with increasing cationic charges and the length of the alkyl chain as follows amino-chitosan, dimethylaminoethyl-chitosan, dimethylpropyl amino-chitosan, dimethylamino-1-propyl-chitosan, diethylaminoethyl (DEAE)-chitosan, and quaternized DEAE-chitosan. The modified cationic chitosans showed high antimicrobial property against *B. subtilis*—Gram-positive bacteria, but were less active towards yeast (*C. tropicalis* and *S. cerevisiae*) and *E. coli*—Gram-negative bacteria. The simple structure of the Gram-positive bacteria may explain why the cationic chitosan derivatives are more active towards *B. subtilis* than yeast and *E. coli*. The target sites of the chitosan derivatives are assumed to be the cytoplasmic membranes of microorganisms. The antimicrobial activities were strongly dependent on the cationic

charge and the molecular weight. It can be suggested that these cationic chitosan derivatives have potential as antimicrobial agents.

Keywords Antimicrobial activity · *Bacillus subtilis* · Cationic chitosan derivatives · Gram-negative bacteria · Gram-positive bacteria

Several defense methods have been proven to control the microbial inhibition using antimicrobial agents, including chemically modified composites, probiotics, or quorum sensing-interference [1–4]. In addition, nanoparticles or composite-based materials have shown broad biotechnological applications, including antimicrobial properties, due to their toxic natures [5–9]. Antimicrobial agents have been grouped depending up on their target. Among these, cationic antimicrobial agents are the most frequently employed, especially for external disinfection. These have multiple benefits compared to other disinfectants. They possess higher antimicrobial activity and killing rate, broad spectrum activity, and are less toxic to mammalian cells [10]. Cell envelope of bacteria is the target for the cationic biocides [11]. Polycationic biocides are also known be highly active against diverse bacteria [10, 11]. The polymeric biocides are highly active and are thus powerful candidates for polymeric drugs. Their actions can be assigned to high local density of the active groups.

Chitin is abundantly available in nature but applications are restricted due to its poor reactivity and solubility. However, a simple modification such as deacetylation increases its solubility to specific solvents. The chitosan has important biotechnological and industrial applications, such as wastewater purification, chelation of transition metals, immobilization of whole cells, and coating seeds

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for enhancing yield [10, 12–15]. In addition, chitosan and its derivatives have been shown to have antimicrobial activities [16–18]. Water-soluble chitosan, which have modified pendent antimicrobial active groups can be envisaged to have better antimicrobial properties with lower toxicity. Chitosan exhibit antimicrobial activity primarily under acidic conditions, as their solubility declines above pH 6.5. The need is to prepare water-soluble derivatives of chitosan active under all physiological conditions as polycationic biocides. The cationic biocides act as antimicrobials through the following mechanism: (1) adsorption and diffusion, (2) binding and disruption of the cell membrane, (3) releasing the cell constituents, and (4) leading to cell death [12]. This study revealed the significant effect of the charge density and the hydrophobicity of the amino groups introduced to the chito-san on the antimicrobial activity towards *Bacillus subtilis*, *Candida tropicalis*, *Escherichia coli*, and *Saccharomyces cerevisiae*.

Up to 1000 µg/mL, underivatized chitosan hardly suppressed any growth of *E. coli* and *B. subtilis*. Subsequently, at concentrations of 2000 µg/mL, the underivatized chitosan caused a slight suppression of the growth (Table 1). In the case of *B. subtilis*, a concentration of only 100 µg/mL of diethylaminoethyl (DEAE)-chitosan was enough for growth inhibition. On the other hand, a concentration of 500 µg/mL of DEAE-chitosan completely inhibited growth. Although the antimicrobial activity showed improvement against *E. coli* too, the extent of improvement was lower than that observed against *B. subtilis*. The tendency of antimicrobial property against *E. coli* was like that recorded against *B. subtilis*. However, the activity against *E. coli* was recorded to be relatively lower since it required 1000 µg/mL concentration of quaternized DEAE-chitosan for the complete inhibition of growth. Similarly, the antimicrobial activities against *B. subtilis* and *E. coli* were observed to enhance with an increase in the cationic charge of the substituent. At concentrations of up to 200 µg/mL, amino-chitosan hardly suppressed any growth of *E. coli*, while at 500 and 1000 µg/mL, growth suppression of 27 and 54%, respectively, was observed. On the other hand, 1000 µg/mL of quaternized amino-chitosan completely inhibited the growth of *B. subtilis*. The antimicrobial activity against *E. coli* was lower than that against *B. subtilis*. A concentration of even 1000 µg/mL of

quaternized amino-chitosan could inhibit the growth of *E. coli* only up to 26%. Here, the inhibition can be correlated to the differences in their cell wall structures (Fig. S1) [10]. Similarly, the antimicrobial activity observed in yeast (*C. tropicalis* and *S. cerevisiae*) was like that observed in *E. coli* due to their complex membrane structure which contains glucan, mannan, galactomannan, chitin, etc. The antimicrobial property of amino-chitosan derivatives was comparatively lower than that exhibited by the DEAE-chitosan derivatives, indicating the importance of the hydrophobicity of the substituent.

The effect of hydrophobicity on the antimicrobial property against *B. subtilis* was analyzed in the modified chitosan with different dialkylaminoalkyl groups. At 500 µg/mL, the DEAE-chitosan completely inhibited the growth. However, dimethylamino-1-propyl-chitosan (DMAP)-chitosan, dimethylpropylamine (DMAiP)-chitosan, dimethylaminoethyl (DMAE)-chitosan, and amino-chitosan inhibited the growth by 70, 58, 36, and 0%, respectively. On the other hand, at 1000 µg/mL, DEAE-chitosan and DMAP-chitosan inhibited the growth completely, while DMAiP-chitosan, DMAE-chitosan, and amino-chitosan inhibited the growth by 95, 60, and 42%, respectively. Remarkably, the antimicrobial activity was observed to increase with an increase in the hydrophobicity of the substituent in the order of chitosan < amino- < DMAE- < DMAiP- < DMAP- < DEAE-chitosan.

The degree of deacetylation (DD) on the hydrolyzed chitins and molecular weight (MW) were determined by ¹H NMR and gel permeation chromatography (Table 2). In the 500 MHz ¹H NMR spectrum (Fig. S2), five resonances were recorded. The MW was determined using gel permeation chromatography. Up to 2000 µg/mL of chitosans with DDs of 0.42 (MW, 8 × 10⁵) and 0.71 (MW, 3 × 10⁵) were observed to hardly suppress the growth of *B. subtilis*, while 2000 µg/mL of chitosan with a DD of 0.95 (MW, 1.5 × 10⁵) clearly suppressed the growth. The quaternized DEAE-chitosans with DDs of 0.42, 0.71, and 0.95, caused complete inhibition of growth at 200, 150, and 100 µg/mL, respectively. However, the antimicrobial activity of quaternized DEAE-chitosans with DDs over 0.95 were observed to decrease with the decrease in the MW. At 500 µg/mL of quaternized DEAE-chitosan (DD, 1.00; MW, 45 kD), complete inhibition was observed. Similarly,

Table 1 Minimal inhibitory concentrations of chitosan derivatives against different microbes

Microbes	DEAE-chitosan (µg/mL)	qDEAE-chitosan (µg/mL)
<i>Bacillus subtilis</i>	400	160
<i>Candida tropicalis</i>	800	500
<i>Escherichia coli</i>	2000	1500
<i>Saccharomyces cerevisiae</i>	> 2000	2000

No effect was observed with chitosan in the tested concentrations

Table 2 Effect of treatments on the biochemical properties of chitosan

Deacetylation condition			Deacetylation degree (%)	Nitrogen content (%)	Molecular weight
NaOH (%)	Temp. (°C)	Time (h)			
20	90	10	41.8	6.32	800,000
20	130	10	71.2	6.84	300,000
40	130	10	95.1	7.15	150,000
50	130	10	100.0	7.27	100,000
50	130	20	100.0	7.26	45,000

a chitooligosaccharide (DD, 0.73; MW, $3\text{--}5 \times 10^3$) was prepared by treating chitosan with chitosanase. Up to 2000 $\mu\text{g/mL}$, the chitooligosaccharide slightly suppressed the growth of *B. subtilis*. This antimicrobial activity is like that of high MW chitosan with a similar DD. The quaternized DEAE-chitooligosaccharide showed 6, 22, 76, and 100% growth inhibition at 100, 200, 500, and 1000 $\mu\text{g/mL}$, respectively. The antimicrobial activities of chitooligosaccharide derivatives are much lower compared to high and medium MW chitosan derivatives having similar DDs. The quaternized DEAE-chitosan (DD, 0.71; MW, 3×10^5) completely inhibited the growth *B. subtilis* at 200 $\mu\text{g/mL}$.

In summary, the cationic chitosan derivatives proved to have higher antimicrobial property towards *B. subtilis*—Gram-positive bacteria, in comparison to that recorded against *E. coli*—Gram-negative bacteria and yeasts (*C. tropicalis* and *S. cerevisiae*). The antimicrobial activities were strongly dependent on the cationic charge and the MW. Thus, the chitosan derivative with a MW of 1.5×10^5 (DD, 0.95) had the highest activity among all the derivatives. The phenomenon demonstrates the presence of an optimal MW range as a requirement for antimicrobial action. It thus seems that chitosan derivatives can also be employed as antimicrobials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicting interests associated with this publication.

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