Effect of Sodium Chloride on The Antimicrobial Activity of The Partially Unfolded Lysozyme

Supported Researcher: Hisham R. Ibrahim
Co-researcher: Inoue Yoichi
(Kagoshima University, Faculty of Agriculture)

Summary

There is a great interest among consumers in using natural antimicrobial systems for assuring food safety against undesirable microorganisms. These demands encourages exploitation of naturally occurring antimicrobials. Hen egg-white lysozyme is a good candidate to control pathogenic bacteria. However, the limited antimicrobial activity of lysozyme to certain Gram-positive bacteria restricts its implication in formulated food and drug systems. We have attempted several protein modification and genetic engineering approaches to render lysozyme active in killing Gram-negative bacteria. Recently, we found that the controlled thermal denaturation converts lysozyme into a potent bactericidal molecule against Gram-negative and -positive bacteria, regardless of its residual enzymatic activity. The most potent bactericidal lysozyme was produced by heating at 80°C for 20 min at pH 6.0 (HLz80/6) retaining 54 % of the enzyme activity. The antimicrobial mechanism of HLz80/6 was found to operate through enhanced interaction to the bacterial membranes and subsequent permeabilization.

The present study was undertaken to determine the effect of NaCl, a frequent food ingredient regarded as antimicrobial inhibitor to lysozyme action, on the novel bactericidal activity of HLz80/6 and to verify possible antimicrobial synergism with glycine. HLz80/6 showed remarkably stronger bactericidal activity than the native (NLz) lysozyme against both Gram-positive *S. aureus* and Gram-negative *E. coli* at any salt (NaCl, $CaCl_2$, and $MgCl_2$) concentration up to 0.1 %, suggesting its potential use in food and drug systems which requires moderate ionic balance. A similar promising trend was observed by testing the bactericidal effect of HLz80/6 against other bacterial strains such as *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Bacillus cereus*. Addition of 0.5 % and 0.1 % NaCl concentration suppressed the bactericidal activity of HLz80/6 against *S. aureus* and *E. coli*, respectively. However, glycine at 0.4 % concentration exhibited good antimicrobial synergy with HLz80/6 and thus shifted the suppressive dose of NaCl to 1 % and 0.5 % against *S. aureus* and *E. coli*, respectively. Further increase in glycine concentration up to 1.6 % favorably synergized with HLz80/6, but not NLz, against both strains even in the presence of the inhibitory high doses of NaCl, suggesting a greater opportunity for application of HL80/6 with glycine in formulated food systems. Thus, the results introduce an interesting finding that partial denaturation of lysozyme can induce its antimicrobial specificity to include the food-borne Gram-negative pathogens and heralded fascinating opportunities for application of HL80/6 with glycine in formulated food systems. Considering the defects in flavor and taste caused by the addition of potentiator like glycine, HL80/6 greatly reduced the minimal bacteriostatic concentration of glycine, while glycine potentiated the bactericidal action of HL80/6 to circumvent the inhibitory effects of salt.