Effects of salts on the elicitation of sweetness of sweet-tasting protein

Tetsuya MASUDA and Naofumi KITABATAKE

Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University,

Summarry

Lysozyme is one of the most thoroughly characterized enzymes, and one of the sweet-tasting proteins. However, a detailed explanation of the sweetness of the hen egg white lysozyme (HEL) is as yet unknown.

At first in order to clarify the relationship between the sweetness and enzymatic activities of lysozyme, chemical modification of carboxyl residues were attempted. Modification of lysozyme by glycine methylester or aminomethansulfonic acid resulted in the loss of enzymatic activity. On the contrary the sweetness of lysozyme was fully retained. These results indicate that both enzymatic and sweetness activities were independent each other.

Second, to clarify the functional regions for elicitation of sweetness of lysozyme, the effect of the side chain of lysine was investigated by chemical modification method. Three types of chemically modified variants (guanidination, acetylation, and phosphopyridoxylation) were prepared. These results indicated that positive charges of side chain of lysine residues influenced on the sweetness of lysozyme and blocking the positive charge or introduction of negative charge in lysine residues resulted in the loss of sweetness.

Next, to obtain the detailed information on the sweetness of lysozyme, site-directed mutagenesis studies using *Pichia* expression systems were also performed. A series of lysozyme mutants, single, double, triple mutants at various regions were prepared. Mutations of lysine to arginine residues, lysine to alanine residues, arginine to alanine residues have been performed to elucidate the critical regions of sweetness of lysozyme in detail.

Taken together these results, finally, effects of salts on the elicitation of sweetness of lysozyme were investigated in detail. The results demonstrated that electrostatic interaction would play a significant role in the interaction the sweet-tasting protein and receptor.