

## Comparison of the Salt Effect on the Activity of Metalloproteinases from Human and *Bacillus thermoproteolyticus* and its Application to Food Science

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[Introduction] The activity of a thermostable metalloproteinase from *Bacillus thermoproteolyticus*, thermolysin (TLN) is remarkably enhanced by high concentration of salts (1-5 M), as well as the stability. On the basis of the result, the degradation of untapped proteinaceous resources and the synthesis of the sweetener aspartame, by TLN, have been examined in the presence of salts. In this study, we adopt a collagenase closely related to TLN, human matrix metalloproteinase-7 (matrilysin, MLN) and describe the salt effect on the activity of MLN in comparison with that of TLN. [Experimental procedures] Recombinant human MLN was prepared from the inclusion bodies expressed by *E. coli*. The peptidase and collagenase activity of MLN was measured at pH 7.5 with MOCAC-PLGL(Dpa)AR and azocoll, respectively. [Results] The temperature giving 50% inactivation in a 30-min incubation ( $T_{50}$ ) and the activation energy for the thermal inactivation ( $E_a$ ) of TLN were determined to be 89°C and 134 kJ/mol, whereas those of MLN were 66°C and 237 kJ/mol, respectively. The peptidase activity of TLN measured with Furylacryloyl-Gly-Leu-amide increased in an exponential fashion with an increase in [NaCl], and the degree of activation at x M NaCl was expressed as  $1.9^x$ . The activation was brought about solely through an increase in the catalytic constant ( $k_{cat}$ ). On the other hand, the peptidase activity of MLN was increased in a biphasic exponential fashion, and the activation was brought about solely through a decrease in the Michaelis constant ( $K_m$ ). The degree of activation at x M NaCl was expressed as  $2.1^x$  when [NaCl]<0.5 M and  $1.4^x$  when [NaCl]>0.5 M, suggesting that the decrease in  $K_m$  observed at [NaCl]<0.5 M was derived from the weakened repulsive electrostatic interaction between MLN and MOCAC-PLGL(Dpa)AR and that at [NaCl]>0.5 M was from the strengthened hydrophobic interaction between them with increase in [NaCl]. The collagenase activity was decreased to 40-50% by the addition of 0.2-0.5 M NaCl, increased to 200% with increasing [NaCl] from 0.5 to 3 M, and decreased to 100% with increasing [NaCl] from 3 to 4 M. With an increase in [NaCl] from 0.5 to 3 M, the  $k_{cat}$  and  $K_m$  values increased, and the increment of the former was larger than that of the latter. It was suggested that 3 M NaCl was the most effective concentration in hydrolyzing collagens, whereas the higher the [NaCl], the more effective in hydrolyzing peptides.