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Structural Principles of a Halophilic Enzyme, Thermolysin, and Molecular Aspects of the Enzymatic Function

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Summary

Thermolysin is a neutral metallo-endopeptidase produced by Bacillus thermoproteolyticus. Enzymatic aspects of this enzyme including the 3-D structure and enzyme reaction mechanism have been studied extensively. We have found that the enzyme is activated remarkably (10-50 times) by the addition of multimolar (1-5 M) salts, and have reported that thermolysin is a typical halophilic enzyme. In this study, we aimed to reveal the structural features of thermolysin, from the view point of halophilicity, and the molecular aspects of the enzyme function.

The enzyme activity shows a bell-shaped pH dependence, with optimum pH at 7.0. The acidic pKa shifts from 5.4 to 6.7 with an increase in [NaCl] from 0 to 4 M, whereas the alkaline pKa is left unaltered (pKa=7.8). The ratio of the enzyme activity at 4 M NaCl to that at 0 M NaCl is defined as the degree of activation (DOA). The pH-dependence of DOA was also shown to be bell-shaped, and the optimum pH is at 7.0, where the DOA value is 16. The value is 2 at pH 6 and 9. When temperature is shifted from 5 to 37℃, the DOA decreases from 20 to 4, and when alcohol is added to the reaction medium, the DOA also decreases significantly, suggesting that an decrease in the dielectric constant of the medium decreases DOA. When negative charges are introduced onto tyrosyl residues, the DOA is reduced. From these lines of evidence, the electrostatic interactions on the surface of thermolysin were considered to be significant in the halophilicity of the enzyme.