Enhancement of Protease-Catalyzed Peptide Synthesis by Salts

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Summary

In the field of peptide synthesis, enzymatic methods have attracted special interest for preparing food peptides with physiologically functional properties. Protease-catalyzed dipeptide synthesis has mainly been studied fundamentally as a model of peptide synthesis. Although oligomerization of a single substrate has been demonstrated using some proteases, studies on its mechanism based on enzyme kinetics are so far insufficient. In the present study, acylation and aminolysis catalyzed by α-chymotrypsin was kinetically investigated in the initial stage of oligomerization of glutamic acid diethyl ester (Glu-di-OEt) to clarify reaction promoting factors and effects of salts.

The reaction was conducted in 500mM phosphate buffer (pH7.5) containing NaCl by using 100mM Glu-di-OEt and 10μM α-chymotrypsin at 25°C. The overall reaction yield and insoluble product yield increased with an increase in NaCl concentration. Dipeptide formation was deduced to be the key step of the oligomerization by demonstrating that the dipeptide product was converted to highly polymerized products rapidly in an early step of the reaction. Both acylation and aminolysis rates measured in model reaction systems were enhanced with increasing ionic strength, suggesting that hydrophobic interaction between the enzyme and the substrate was important as a driving force of the reaction. From this study, it is concluded that an enhancement of aminolysis is responsible for an increase in the reaction yield, and salt components contribute to promotion of the oligomerization through the activation of aminolysis.