SHORT COMMUNICATIONS

Tryptic Cleavage of Synthetic Poly-ε-aminocaproyl-α-amino Acids

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The syntheses of several linear poly-ε-aminocaproyl-α-amino acids such as poly-ε-aminocaproyl-D, L-alanine (M. W. 21,800)1), poly-ε-aminocaproyl-L-alanine (M. W. 7,000)2), poly-ε-aminocaproyl-L-phenylalanine (M. W. 51,200)3), poly-ε-aminocaproyl-α-aminoisobutyric acid (M. W. 35,000)4), poly-ε-aminocaproyl-L-leucine (M. W. 22.300)4) and poly-ε-aminocaproylglycine (M. W. 19,300)4) have recently been achieved by Noguchi and his coworkers, with the aid of their "N-carbothiophenylamino acid method"5). Among these synthetic polymers, the alanine and the α-aminoisobutyric acid derivatives, like the Bence Jones protein, possess an unusual property; they are reversibly coagulated by heat in acidic or neutral aqueous solutions6). In the present communication, we wish to report that crystalline trypsin of relatively high concentrations can partly hydrolyse these polymers.

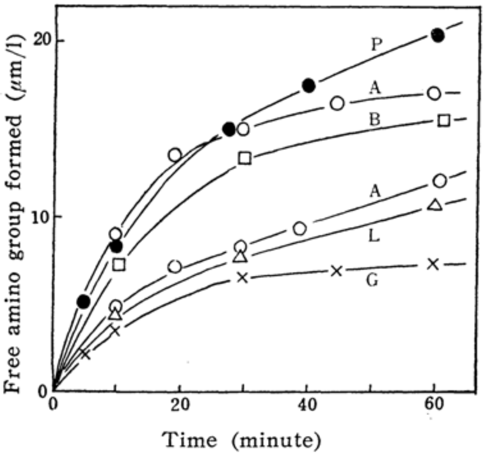

Fig. 1. Hydrolyses of poly-ε-aminocaproyl-α-amino acids by trypsin.
P, poly-ε-aminocaproyl-L-phenylalanine; A, poly-ε-aminocaproyl-D, L-alanine (above); A, poly-ε-aminocaproyl-L-alanine (bellow); B, poly-ε-aminocaproyl-α-aminoisobutyric acid; L, poly-ε-aminocaproyl-L-leucine; G, poly-ε-aminocaproylglycine.

Each reaction mixture contained 0.2 per cent of polymer, 185 μg. of twice recrystallized trypsin, and 0.013 M phosphate buffer (pH 7.1) in a final volume of 5.0 ml. Incubation was carried out at 25°.

As can be seen from Fig. 1, a considerable hydrolysis of several poly-ε-aminocaproyl-α-amino acids has occurred with 37 μg. per ml. of twice recrystallized trypsin, as evidenced in Fig. 1 by the appearance of free amino groups. The free amino groups were determined by the ninhydrin method according to Moore and Stein7). The viscosity of the reaction mixture decreased as the hydrolysis proceeded. No detectable hydrolysis was observed when the concentration of trypsin was reduced to 7 μg. per ml., a concentration of the enzyme suitable for its usual substrates. The polymers were resistant against the action of crystalline α-chymotrypsin and carboxypeptidase of high concentrations under the same conditions.

A search for the cleavage products of poly-ε-aminocaproyl-D, L-alanine by quantitative paper chromatography revealed that about a half mole of ε-aminocaproic acid and alanine, together with a large amount of dialysable fragments, were released from one molecule of the polymer. This suggests that the ε-aminocaproic acid and the alanine were derived from the N-terminal and the next amino acid residues of the polymer, respectively, assuming that the D-linkages were not split by the action of trypsin. The dialysable fragments yielded ε-aminocaproic acid and alanine on hydolysis with hydrochloric

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acid, and their mean degree of polymerization was estimated to be 6.

Information available up to date show that only lysyl and arginyl peptide or ester linkages are susceptible to the tryptic attack[8,9]. The results reported here, however, clearly indicate that poly-ε-aminocaproyl-α-amino acids can also be hydrolysed by the action of the proteolytic enzyme when relatively high concentrations of the enzyme are employed. It is interesting, in this connection, to note that ε-aminocaproic acid possesses a structure corresponding to α-deaminolysine. In the polymers of ε-aminocaproyl-α-amino acids, the ε-amino group, except for that of the N-terminal residue, is not in a free state. This is remarkable since the free amino groups of lysyl and arginyl residues have been believed to be required for the tryptic hydrolysis of the susceptible compounds.

Thus, the finding described above has an important bearing on the substrate specificity of trypsin and calls for a more extensive study on this point, using a number of synthetic substrates. An investigation along this line is now being carried out in our laboratory.

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