AN INVESTIGATION OF PEPTIDE B-3 FORMED BY THE CLEAVAGE OF REDUCED CARBOXYMETHYLATED PEPSIN WITH CYANOGEN BROMIDE

Ya. F. Kovalishin, V. I. Ostoslavskaya, and V. M. Stepanov

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In the separation of a cyanogen bromide hydrolyzate of carboxymethylated pepsin on a column of Sephadex G-200 in 6 M urea, we obtained four fractions – 1, 2, 3, and 4 [1]. The results of an investigation of the fragments B-1, B-2, and B-4 have been reported previously [2]. When fraction 3 was rechromatographed on Sephadex G-100 in 8 M urea, it gave two peaks – A and B. When peak B was rechromatographed on Sephadex G-100 in 8 M urea, the peptide that is currently being studied, which we shall subsequently call B-3 was obtained. The N-terminal amino acid of B-3 is apparently blocked and cannot be determined by dinitrophenylation, dansylation by Edman's method, and by leucine aminopeptidase. The N-terminal position in B-3 may possibly be occupied by pyroglutamic acid. However, it is not excluded that the N-terminal amino group underwent carbamoylation on prolonged standing in solutions of urea.

The action of carboxypeptidase A on peptide B-3 (8 mg) at pH 8 for 30 min split off the following amino acids (μmole):

Thr	0,076	Gly	0,037	Leu	0,035
Ser	0,06	Ala	0,069	Tyr	0,025
Hser	0.134	Val	0.035	Phe	0,015.
		Ile	0,072		

The presence of homoserine in the carboxypeptidase hydrolyzate confirms that the peptide B-3 is a product of the specific cleavage of the carboxymethylated pepsin by cyanogen bromide.

The proposed amino-acid composition of the peptide B-3 (Asp_5, Thr_3, Ser_5, Gin_3, Pro_2, Gly_4, Ala_2, Val_2, Ile_3, Leu_2, Tyr_2, Phe_2, Hser), corresponding to 36 amino-acid residues, will be refined in the course of structural investigations.

Peptide B-3 was hydrolyzed with thermolysin in 0.1 M ammonium bicarbonate at pH 8 and 5.7 for 6 h [3]. The hydrolyzate was fractionated on a column of Dowex 50 × 2 resin in a system of pyridine acetate buffers with a linear gradient [4] and then by paper chromatography and electrophoresis. In the homogeneous peptides the following amino-acid sequences were determined by Edman's phenylthiohydantoin method with dansylation (the yields in μmoles per 100 mg of peptide B-3 are given in parentheses):

1. H_2NIle-Gly-Thr-Pro-Ala-Glx-Asx COOH (0.5),
2. H_2NPhe-Asx-Pro(Asp$_2$Ser$_2$Thr) (0.4),
3. H_2NVal-Ile-Phe-Asx-Thr-Gly-Ser-Ser-Asx COOH(0.3),
4. H_2NLeu-Asx-Glx-Glx-TyrCOOH (0.2),
5. H_2NIle-Ley-GlyCOOH(0.8),
6. H_2NVal-Gly-GlyCOOH(1).

Sequences 1 and 2 have also been found in the N-terminal peptide of pepsin B-2, which contains 175 amino-acid residues. A peptide with the sequence Ser-Pro-Ser-Ala-Tyr has been isolated previously in our laboratory from chymotrypsin hydrolyzates of peptides B-2 and B-4. The presence of repeating sequences of amino-acid residues in different parts of the polypeptide chain may be characteristic for pepsin.

All-Union Scientific-Research Institute of the Genetics and Breeding of Industrial Microorganisms.
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This may explain the presence of similar peptides in the enzymatic hydrolyzates of the fragments B-2 and B-3. It is also possible that peptide B-3 was formed in the nonspecific hydrolysis of peptide B-2 in the treatment of the carboxymethylated pepsin with cyanogen bromide. However, an insufficient number of facts has so far been obtained to determine the position of this peptide in the pepsin molecule.

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