

INVESTIGATION OF FRAGMENT B-5 FORMED IN THE CLEAVAGE OF PEPSIN WITH CYANOGEN BROMIDE

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The peptide B-5 formed in the cleavage of reduced carboxymethylated porcine pepsin consists of 40-43 amino acid residues. A characteristic feature of it is the comparatively high content of hydrophilic amino acids. Previously, by the stepwise splitting off of methylthiohydantoin the following N-terminal sequence of the peptide B-5 was established: Asp-Gly-Glu-Thr-Ile- [1]. The action of carboxypeptidase A splits off from the peptide homoserine, which is the C-terminal amino acid, and this is preceded by serine, alanine, and isoleucine.

We hydrolyzed the dinitrophenyl derivative of peptide B-5 with pepsin at pH 2.2 at 37° C for 2 min. From the hydrolysate we isolated the DNP-peptide P-3 with the composition Asp₁, Ser₁, Thr₂, Glu₁, Gly₂, Ala₁, Ile₁, corresponding to the N-terminal sequence of B-5. The hydrolysis of B-5 with pepsin at pH 2.2 and 37° C for 4 hr led to the formation in good yield of the peptide P-1, having the composition Thr₂, Ser₁, Gly₁, Ala₁, Pro₁, Leu₁. It was shown by the DNP method that the N-terminal position in P-1 is occupied by leucine. Hydrolysis with carboxypeptidase A gives the C-terminal sequence Ser-Ala. The stepwise splitting off of methylthiohydantoin [2] enabled the structure of P-1 to be established completely (the yields of methylthiohydantoin referred to the preceding cycle of elimination are shown in parentheses): Leu-Thr(100)-Gly(76)-Pro(65)-Thr(92)-Ser(58)-Ala(142).

On the basis of the results given above, the partial sequence of B-5 can be represented as Asp-Gly-Glu-Thr-Ile-(Thr, Gly, Ser, Ala)-...-Leu-Thr-Gly-Pro-Thr-Ser-Ala-... (Ser, Ala, Ile)-Met.

A comparison of the results obtained with the sequence of amino acids in methionine-containing peptides [3] permits the assumption that in the structure of pepsin the B-5 peptide follows directly after the N-terminal fragment B-2 and corresponds to the central part of the polypeptide chain of the enzyme.

REFERENCES

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