LOCALIZATION OF THE ASPARTIC ACID RESIDUE PRESENT IN THE ACTIVE CENTER OF PORCINE PEPSIN

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UDC 547.965

It is known that the catalytic center of porcine pepsin includes the β-carboxy group of aspartic acid. The use of diazoacetyl inhibitors has enabled the amino-acid sequence containing the "active" aspartic acid to be shown [1, 2]: Ile-Val-Asp-Thr-Gly-Thr-Ser. However, the part of the polypeptide chain of pepsin in which this sequence is present has not hitherto been known.

In an investigation of a thermolysin hydrolyzate of the B-3 fragment [3, 4] formed by the cleavage of carboxymethylated pepsin with cyanogen bromide, two peptides were isolated with the following structures:

I. B3Th 624 Ile-Val-Asx-Thr-Gly-Thr-Ser-Leu
1 2 3 4 5 6 7

II. B3Th 611 Gly-Thr-Gly-Ser-Hse.

The sequence 1-7 of peptide B3Th624 coincides with the sequence given above and, therefore, the aspartic acid residue participating in the formation of the active center of pepsin is present in the B-3 fragment. The peptide B3Th611 contains homoserine and is C-terminal in the B-3 fragment. Tang and Hartley [5] have previously reported that pepsin contains a sequence including a methionine residue: Tyr-Gly-Thr-Gly-Ser-Met-Asp-Val-Pro-Thr-Ser. However, Chen and Tang [6] have recently found a different and, in their opinion, more reliable methionine-containing sequence in pepsin:

Phe-Gly-Gly-Met-Asp-Val-Pro-Thr-Ser-Ser-Gly-Gly-Leu.
1 2 3 4 5 6 7 8 9 10 11 12 13

The sequence of residues 5-13 coincides with the N-terminal sequence of fragment B-1, which occupies the C-terminal position in the pepsin molecule [3]; the sequence 2-4 coincides with the C-terminal sequence of the fragment B-4 [7]. Apparently, in the molecule of porcine pepsin the B-4 fragment precedes the B-1 fragment. Tang and Hartley showed that pepsin contains the following methionine-including sequence:

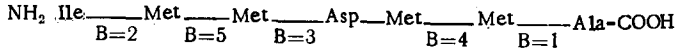
Asp-Ser-Ile-Tre-Met (Asp, Gly, Gly) (Ala, Tyr) [5].
1 2 3 4 5 6 7 8 9 11

Sequence 1-5 coincides with the C-terminal sequence of the N-terminal fragment of pepsin B-2 [3]. The sequence 6-10 partially agrees with the N-terminal sequence of the fragment B-5; NH_2-Asp-Gly-Glu-Thr-Ile [3]. In our laboratory, L. P. Revina, I. B. Pugacheva, and É. A. Vakhitova have isolated the following methionine-containing peptide:

(Ile, Thr, Met, Asp, Gly, Glu, Thr).
1 2 3 4 5 6 7

Sequence 1-3 in it is the C-terminal sequence of fragment B-2, and sequence 4-7 coincides with the N-terminal sequence of fragment B-5. On the basis of these facts, it may be assumed that in the pepsin molecule fragment B-2 is followed by fragment B-5, so that the peptides formed in the cleavage of porcine pepsin by cyanogen bromide are arranged in the polypeptide chain of the enzyme in the following order:

All-Union Scientific-Research Institute of the Genetics and Breeding of Industrial Microorganisms. Institute of Microbiology and Virusology, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 567-568, July-August, 1973. Original article submitted January 14, 1973.



Thus, it can be seen that the aspartic acid residue in the active center of pepsin and reacting selectively with diazoacetyl inhibitors is located close to the C end of the molecule.

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