ASPARTIC ACID RESIDUE No. 177 OF BOVINE TRYPSINOGEN

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1. Introduction

In earlier studies in the elucidation of the complete amino acid sequence of bovine trypsinogen [1, 2], we also determined the position of amide groups in the molecule [3]. One of these groups was found in position no. 177. Recent studies on the models of the active center of trypsin, however, have led to the assumption that this position plays an important role in the binding of substrates or inhibitors of trypsin. From the studies on the possible analogies in the three-dimensional structure of the polypeptide chains of trypsin and chymotrypsin, on a model of chymotrypsin [4], and from a comparison of the amino acid sequence of trypsin and thrombin, Hartley [5] concluded that position no. 177 in trypsin is occupied by an aspartic acid rather than by an asparagine residue. These results led us to reexamine whether position no. 177 is occupied by an asparagine or - according to our present concept of the nature of the bond between the enzyme and the substrate - by an aspartic acid residue.

2. Material and methods

Trypsin was a crystalline preparation of Léčiva, Prague. After recrystallization it was dialyzed and lyophilized.

S-Sulfo-trypsin was prepared by the method of Pechère and co-workers [6]. The dialysis of the reaction mixture after S-sulfonation was replaced by gel filtration over a Sephadex G-25 column equilibrated with 0.1 M ammonium carbonate.

2.1. *Tryptic digestion of S-sulfo-trypsin*

To 45 ml of a solution containing 300 mg of S-sulfo-trypsin, 6 mg of trypsin was added. The reaction mixture was maintained at 37° and pH 8.1 in the thermostated vessel of TTI Autotitrator (Radiometer, Copenhagen) using 0.1 N NaOH as titrant. Digestion was discontinued after 6 hr by the addition of acid to pH 2.7. The precipitate formed was centrifuged off.

2.2. *Isolation of peptide T 1 (amino acid residues no. 177–192)*

The clear supernatant after tryptic digestion was concentrated to a volume of 20 ml in a rotary evaporator and fractionated on a Dowex 50-X2 column (1 × 140 cm) equilibrated with 0.1 M pyridine formate buffer at pH 3.0. A flow rate of 28.4 ml/hr was maintained and fractions were collected every 10 min. Peptide T 1 was eluted as the first fraction in tubes no. 16–36. The pooled fractions were rotary evaporated and the dry residue was purified by high-voltage electrophoresis on paper at pH 1.9 (acetic acid-formic acid (85%)-water, 3:1:16) [7].

The peptide was subjected to Edman degradation by the method described elsewhere [8] and the PTH-amino acids were separated and identified by thin-layer chromatography [9].

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3. Results and discussion

Of the peptides isolated by us from various enzymatic digests of trypsin, the most suitable one for the reinvestigation of the amino acid in position no. 177 is peptide T 1, isolated from the tryptic digest of S-sulfo-trypsin [10]. This peptide, which comprises residues no. 177–192, can be isolated very easily since it emerges as the first fraction when the tryptic digest of S-sulfo-trypsin is chromatographed on Dowex 50-X2 [10]. Another advantage of this peptide is that the residue no. 177 is at the *N*-terminus of the peptide and can thus be identified in one step of an Edman degradation.

When we subjected peptide T 1 to Edman degradation, we were able to identify unambiguously PTH-aspartic acid in the first step and PTH-serine in the second step. In view of these results, the amino acid sequence of peptide T 1 [11] is as follows:

Asp–Ser–Cys–Gln–Gly–Asp–Ser–Gly–Gly–Pro–
177
–Val–Val–Cys–Ser–Gly–Lys
192

The erroneous assignment [3] of an asparagine residue to position no. 177 can obviously be accounted for by the presence of a small amount of asparagine peptides which gave a positive growth-stimulating test with *Lactobacillus casei* and thus simulated the presence of asparagine in peptide T 1.

The aspartic acid residue no. 177, which is only five residues distant from the serine no. 183 of the active site, is most likely the residue which is responsible for the binding of basic substrates of trypsin.

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