THE SEQUENCE AROUND AN ACTIVE-SITE ASPARTYL RESIDUE IN PEPSIN

J. R. KNOWLES and Grith B. WYBRANDT
The Dyson Perrins Laboratory, University of Oxford, U. K.

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A hepta-peptide of composition Asp, Thr_2, Ser, Gly, Val, Ile, the aspartyl residue of which is required for enzymic activity in pepsin, has recently been isolated [1]. We now report the amino acid sequence of this peptide, along with a convenient and rapid technique for its isolation.

Inhibition of pepsin with the active-site-directed irreversible inhibitor *N*-diazo[1-^{14}C]acetyl-L-phenylalanine methyl ester, results in the rapid and stoichiometric inactivation of the enzyme [1]. Digestion of the acetone-denatured inhibited protein with native pepsin was followed by chromatography on Sephadex G-25 with water as the eluent. The fractions containing radioactive peptide were pooled and freeze-dried [1]. This peptide mixture was subjected to vertical electrophoresis on Whatman No. 3MC paper at pH 3.5 (pyridine-acetate buffer). A guide strip was cut off, and treated with a saturated aqueous solution of redistilled triethylamine. This treatment results in the hydrolysis of the ester link between the aspartyl group of the labelled peptide and the radioactive label. The paper strip was sewn into another sheet of paper and subjected to electrophoresis at pH 3.5 under the same conditions as the first run, but in a perpendicular direction [2]. On staining with ninhydrin-cadmium reagent [3], one major spot slowly appeared off the diagonal, in the position expected if the treatment with triethylamine had unmasked the β-carboxyl group of the aspartyl residue. The off-diagonal peptide was isolated using a method similar to the above [2]. The peptide was eluted from the paper with water and the sequence determined using the dansyl-Edman procedure described by Gray [4]. Dansyl amino acids were identified by thin-layer chromatography on polyamide plates according to the method reported by Woods & Wang [5]. The solvent systems used were those numbered 1 and 2 by Woods & Wang [5], and a system containing ethyl acetate : methanol : glacial acetic acid, 20 : 1 : 1 (v/v) (Dr. B. S. Hartley, private communication). This last solvent system gives better resolution of Thr and Ser, and separates Asp from dansyl sulphonic acid.

Dansyl-Edman degradation on the whole peptide gave Ile-Val-Asp- but the yields of the subsequent dansyl amino acids were rather low (presumably because of some $\alpha \rightarrow \beta$ rearrangement of the aspartyl peptide link during degradation), and a partial acid hydrolysis was performed. The peptide was treated with 1M-acetic acid in a sealed tube at 100° for 15 hr. The resulting hydrolysate was separated into its components by paper electrophoresis on Whatman No. 1 paper at pH 3.5. The following peptides were eluted from the paper, and sequenced as: Ile-Val; Thr-Gly-Thr; Ser. The C-terminal amino acid was determined using a solution of carboxypeptidase-A. The whole peptide (0.01 μmole) was dissolved in 40 μl of 0.02M ammonium bicarbonate solution and 15 μl of a solution of carboxypeptidase-A (1 mg/ml in 0.1M ammonium bicarbonate) added, and the mixture incubated for 6 hr at 37°. Amino acid analysis of the resulting digest showed that Ser was the C-terminal amino acid, with Thr as the penultimate residue.

These data allow the unambiguous assignment of the sequence as: Ile-Val-Asp-Thr-Gly-Thr-Ser.

Using comparable inhibitors, Hamilton and coworkers [6] and Stepanov and Vaganova [7], have recently obtained sequences of Ile-Val-Asp-Thr, and Val-Asp, respectively. It seems very probable that the same essential carboxyl group is involved in all three labelling studies.

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