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Peptides isolated from peptic hydrolysate of diisopropylphosphoryl-trypsin

In the comparative studies on protein structures¹ which our laboratory has been working on we have been concerned above all with chymotrypsinogen and trypsin. A survey of the peptide sequences in these proteins so far known was published by us earlier².

The present paper gives a preliminary report on results obtained by studying peptides isolated from a peptic hydrolysate of DIP-trypsin. This study permits us to ascertain mutual bonds in the vicinity of the residues of lysine and arginine, which are split on tryptic hydrolysis.

DIP-trypsin (1000 mg) was hydrolyzed by pepsin (enzyme to substrate ratio, 1:50) at 37° and pH 2.0 for 2.5 h. A small quantity of non-dialyzing material was removed from the peptic hydrolysate by dialysis against distilled water. The freeze-dried dialysate was fractionated on a Zerolit 225 X2 ion-exchange resin (100-200 mesh) column of 2.2 × 150 cm at 40° using an elution gradient. For this purpose volatile buffers composed of pyridine and formic acid or pyridine and acetic acid proved satisfactory as in the case of the separation of a peptic hydrolysate of chymotrypsinogen³.

Elution was initiated with the 0.1 M buffer (with respect to pyridine) and the gradient developed by gradually introducing solutions of increasing molarity and pH-value into a mixing vessel (volume, 2200 ml). The solutions used were as follows: 0.2 M, pH 3.1; 1 M, pH 5.1; 2 M, pH 5.2; 2 M, pH 7.0 and 2 M ammonia. The eluates were collected in 20-ml fractions at a flow rate of 1 ml/min.

The course of the separation on the column was checked by submitting dry residues of 0.4-ml aliquots taken from each fraction to descending paper chromatography³ in the system *n*-butanol-pyridine-acetic acid-water (15:10:3:12)⁴. According to the chromatograms, individual fractions obtained from the column were pooled and fractionated further by preparative chromatography on Whatman No. 1 or No. 3 paper in the system⁴ mentioned above. The zones obtained were purified by paper electrophoresis in the system formic acid-acetic acid of pH 1.9 at a potential gradient of 65 V/cm.

A majority of the zones isolated comprised individual peptides as was proved by calculating the molar ratios of amino acids by comparing the peptide hydrolysate

Abbreviation: DIP-, diisopropylphosphoryl-.

with a set of different concentrations of standard samples, and by determining the N- and C-terminal groups by means of the dinitrophenylation technique and hydrazinolysis, respectively. The partial structure and/or the complete amino acid sequence of the peptides has been determined.

Table I presents a survey of peptides in order of emergence from the column.

TABLE I
PEPTIDES ISOLATED FROM A PEPTIC HYDROLYSATE OF DIP-TRYPSIN

AspNH ₂ ·Ser	Lys·(Ser ₂ , Glu, Thr, Ala, Ileu)·Asp
Val·(AspNH ₂ , Glu ₃ , Gly)	Lys·(Ser, Ala)
Val·(Ala, Ser)·Ser	AspNH ₂ ·Ser·Arg·Val·Ala·Ser
Ileu·(Ser, AspNH ₂)·GluNH ₂	Val·(Arg, Leu, Gly, Glu, Asp, (I)Leu, AspNH ₂)
AspNH ₂ ·Ileu	Val·Arg·Leu·(Gly, GluNH ₂)·Asp
Ileu·(AspNH ₂ , Ser)	Arg·Val·Ala·Ser
Ileu·(Ala, Ser)	Ileu·(AspNH ₂ , Ser, Glu, Try)
Asp·(Ala, Ileu)·Ser	(Leu, Lys)·Leu
Val·(Leu, Ser)	Val·(Arg, Leu, Gly)
Ileu·(Ser, Leu)	Ileu·(Ser, Gly)·Try·Gly·(AspNH ₂ , Thr)·
Asp·(Ser ₂ , Tyr)	Lys·(Ser ₃ , Gly, Thr, Pro, Tyr, Val, AspNH ₂)
(I)Leu·(Ser, (II)Leu)	Tyr·(Ser, Val ₂ , Try)
Pro·(Leu, Ileu)	Ileu·(His, Lys ₂ , Ser ₄ , Ala, Pro, Tyr)
(Lys, Ser ₃ , Ala, Ileu)	Ileu·(Ser, Gly)·Try·Gly·(AspNH ₂ , Thr)·Lys
Lys·(AspNH ₂ , Ser, Ala, Leu)·Ser	(Lys, Ser, Ala, Leu)

It can be seen from Table I that, among others, peptides containing lysine and arginine residues in the middle of the chain were isolated from the peptic hydrolysate. Both molecules of arginine, which are present in the molecule of DIP-trypsin, were revealed in the peptides isolated and their sequence established. Of the 3 molecules of histidine we succeeded in finding only one and of the 14 molecules of lysine only 8.

In addition, 3 residues of tryptophan, to which only minor attention has been paid in previous studies, were found in the peptides isolated.

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Présence de deux vasopressines dans la neurohypophyse du poulet

A partir de la post-hypophyse des mammifères, il est possible d'isoler un complexe renfermant les deux peptides *ocytocine* et *vasopressine* et une protéine inerte, la *neurophysine*¹; un procédé de purification simultanée des deux hormones prenant

Biochim. Biophys. Acta, 38 (1960) 571-573