

Structure of some peptides isolated from the soluble fraction of the tryptic digest of *S*-sulpho-trypsinogen

The following peptides isolated from the soluble fraction of the tryptic digest of *S*-sulpho-trypsinogen¹ are identical with peptides isolated from the tryptic digest of diisopropylphosphoryl-trypsin²

- T-2: Glu(NH₂)-Thr-Ala-Ileu-Ser-Asp(NH₂)
 T-45: Gly-Asp(NH₂)-Thr-Lys
 T-47: Ser-Ser-Gly-Thr-Ser-Tyr-Pro-Asp-Val-Leu-Lys
 T-52: Ser-Ala-Ala-Ser-Leu-Asp(NH₂)-Ser-Arg
 T-55: Leu-Lys
 T-56: Ser-Gly-Ileu-Glu(NH₂)-Val-Arg
 T-59: Ser-Arg
 T-64: Leu-Lys-Ser-Ala-Ala-Ser-Leu-Asp(NH₂)-Ser-Arg

The structures of some other peptides isolated from the soluble fraction of the tryptic digest of *S*-sulpho-trypsinogen¹, namely those peptides remaining in solution after acidifying the tryptic digest to pH 3.0, are shown in Table I.

TABLE I
STRUCTURES OF THE ISOLATED PEPTIDES

Designation of the peptide	Structure
T-23	Ser-Ser-Cys-Lys
T-24	Val-Ala-Ser-(Leu, Ser, Pro, Ileu, Thr, Ser, Cys, Ala, Gly, Ala, Ser, Thr, Glu, Cys)- Leu-Ileu-Ser-Gly-Try-Gly-Asp(NH ₂)-Thr-Lys
T-26	Ala-Pro-Ileu-Leu-Ser-Asp-Ser-Ser-Cys-Lys
T-27	Ser-Ala-Tyr-Pro-Gly-Glu(NH ₂)-Ileu-Thr-Ser-Asp(NH ₂)
T-30	Cys-Leu-Lys-Ala-Pro-Ileu-Leu-Ser-Asp-Ser-Ser-Cys-Lys
T-37	Val-Asp-Asp-Asp-Asp-Lys-Ileu-Val-Gly-Gly-Tyr-Thr-(Asp, Cys, Thr, Pro, Gly, Ala, Val)-Tyr
T-53	Ser-Ala-Tyr-Pro-Gly-Glu(NH ₂)-Ileu-Thr-Ser-Asp(NH ₂)-Met-Phe-Cys- Ala-Gly-Tyr-Leu-Glu-Gly-Gly-Lys
T-61	Asp(NH ₂)-Lys-Pro-Gly-Val-Tyr-Thr-Lys
T-62	Lys-Pro-Gly-Val-Tyr-Thr-Lys

The structures were established on the basis of cleavage with chymotrypsin (EC 4.5.5.5), pepsin (EC 3.4.4.10), subtilopeptidase A (EC 3.4.4.16), leucine aminopeptidase (EC 3.4.4.1), separation and characterization of fragments by chromatography on ion-exchange resins and on paper and by high-voltage paper electrophoresis. The N- and C-terminal groups were determined by dinitrophenylation and hydrazinolysis. The quantitative amino acid composition was determined by an amino acid analyzer³.

In addition to the above peptides the following three peptides were obtained from the tryptic digest of *S*-sulpho-trypsinogen, *viz.*

- T-1: Asp(NH₂)-(Ser₃, Cys₂, Glu(NH₂), Gly₄, Asp, Pro, Val₂)-Lys
 T-29: Ser-(Ala, Tyr, Pro)-Gly-Glu(NH₂)-Ileu-Thr-Ser-Asp(NH₂)-Met-Phe-(Cys, Ala,
Gly, Tyr, Leu, Glu, Gly, Gly, Lys, Asp(NH₂), Ser, Cys, Glu(NH₂), Gly, Gly, Asp, Ser,
Gly, Pro, Val, Cys, Ser, Gly)-Lys
 T-57: Leu-Glu(NH₂)-Gly-Val-Ser-Ileu-Try-Gly-Ser-Gly-Cys-Ala-Glu(NH₂)-Lys

the structure of which is discussed in another paper⁴.

The peptides T-22 (see ref. 1), T-26, T-27, T-45, T-47, T-52, T-56 and T-62 described here apparently coincide with peptides from the tryptic digest of S-sulphotrypsinogen, the full or partial structure of which has been described by WALSH, KAUFFMAN AND NEURATH⁵.

Peptide T-1 is apparently identical with peptide O-TR-1 as described by DIXON, KAUFFMAN AND NEURATH⁶; however, according to our analysis the peptide contains two valine residues.

Peptide T-37 apparently represents the N-terminal of the trypsinogen molecule. At this N-terminal is located the well-known "activation peptide" Val-Asp₄-Lys⁷⁻⁹ and into this region most probably fits also the peptide (I)Ileu-(Gly₂, Val₂, Thr₂, Pro, Cys, Ala, Asp, Tyr₂)⁵ with the only difference that our peptide T-37 contains three glycine residues. The N-terminal of trypsinogen was formulated by DESNUELLE AND FABRE^{9,10} and by GABELOTEAU AND DESNUELLE¹¹ as the sequence Val-Asp₄-Lys-Ileu-Val-Gly-Glu-Tyr-. According to our analyses the correct formula is that of peptide T-37 which does not contain glutamic acid*.

It further follows from the structures shown here that peptides T-27, T-53 and T-1 represent a part of peptide T-29. As was shown earlier^{1,2}, peptides T-29, T-64, T-24 and T-47 probably follow each other and thus form a sequence of 84 amino acids: Ser-Ala-Tyr-Pro-Gly-Glu(NH₂)-Ileu-Thr-Ser-Asp(NH₂)-Met-Phe-Cys-Ala-Gly-Tyr-Leu-Glu-Gly-Gly-Lys-Asp(NH₂)-Ser-Cys-Glu(NH₂)-Gly-Gly-Asp-Ser-Gly-Pro-Val-Val-Cys-Ser-Gly-Lys-Leu-Lys-Ser-Ala-Ala-Ser-Leu-Asp(NH₂)-Ser-Arg-Val-Ala-Ser-(Leu, Ser, Pro, Ileu, Thr, Ser, Cys, Ala, Gly, Ala, Ser, Thr, Glu, Cys, Leu)-Ileu-Ser-Gly-Try-Gly-Asp(NH₂)-Thr-Lys-Ser-Ser-Gly-Thr-Ser-Tyr-Pro-Asp-Val-Leu-Lys. Details of the work described here shall be published in the Collection of Czechoslovak Chemical Communications.

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* Since completion of this work, we became aware of the publication in September, 1962, by WALSH *et al.*¹² in which the N-terminal sequence of trypsinogen is given as: Val-Asp₄-Lys-Ileu-Val-Gly-Gly-Tyr-Thr-Cys-Gly-Ala-Asp(NH₂)-Thr-(Val, Pro)-Tyr. The partial structure of our peptide T-37 is in agreement with this.