THE BASIC TRYPSIN INHIBITOR OF BOVINE PANCREAS

IV. THE LINEAR SEQUENCE OF THE 58 AMINO ACIDS*

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Recently we reported the amino acid composition and partial sequences of the tryptic peptides of the basic trypsin inhibitor of bovine pancreas (Kassell and Laskowski, Paper III, 1964). This paper presents the remaining sequences of the tryptic peptides, and the composition of the chymotryptic peptides, which complete the arrangement of the amino acids.

Experimental - EC 3.4.4.6, Chymotrypsin B (the most active enzyme obtained by Krehbiel, et al., 1964, Fig. 4) was used to digest the same preparation of reduced carboxymethyl (RCM) inhibitor previously reported (Paper III). Separation of the peptides and amino acid analysis were carried out in the same manner as for the tryptic peptides (to be reported in detail elsewhere). All fractions of 5 mg or more were analyzed. Amide groups were located by the ammonia derived from the individual peptides, and determined with the amino acid analyzer. The sequences were determined on the remaining peptides as described in detail in Paper III, mainly by the subtractive Edman method (Konigsberg, et al., 1962) but in some cases confirmed by other methods quoted in Paper III.

Results and Discussion - Table I summarizes the sequence determinations, and indicates the methods used for each peptide. Fig. 1 gives the

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TABLE I

The Sequences Determined

Arrows indicate the amino acids identified.

Peptide	Sequence	Derivative
T 1-15	Arg-Pro-Asp-Phe-Cys-Leu-Glu-Pro-Pro-Tyr-Thr-Gly-Pro-Cys-Lys	Paper III
T 16-17	Ala-Arg	Trypsin Specificity
T 18-20	Ileu-Ileu-Arg	Trypsin Specificity
T 21-26	Tyr-Phe-Tyr-AsN-Ala-Lys → → → → →	Edman Subtractive and PTH-Asp
T 24-26	AsN-Ala-Lys →	Sanger FDNB
T 27-33	Ala-Gly-Leu-Cys-GlN-Thr-Phe → → →	Edman Subtractive
C 30-33	Cys-GlN-Thr-Phe → → →	Edman Subtractive
T 34-39	Val-Tyr-Gly-Gly-Cys-Arg → → → → →	Edman Subtractive and PTH-derivatives
T 40-42	Ala-Lys-Arg	Paper III
T 43-46	AsN-AsN-Phe-Lys → → →	Edman Subtractive and PTH-derivatives
T 47-53	Ser-Ala-Glu-Asp-Cys-Meth-Arg → → → → → →	Edman Subtractive
C 46-52	Lys-Ser-Ala-Glu-Asp-Cys-Meth ←	Hydrazinolysis
T 54-58	Thr-Cys-Gly-Gly-Ala → → → →	Edman Subtractive and PTH-derivatives

sequence of amino acids and the position and composition of the tryptic and chymotryptic peptides.

There are numerous structural features which may have bearing on the activity of the inhibitor. Amino acids 1-15 were discussed previously (Paper III). Compactness is implied by the distribution of half cystines. From amino acid 8 to 48, there are 8 basic, but no acidic groups, while the end portions each contain 1 excess acidic group. There is a sequence with three bases within 4 amino acids (39-42), and a se-

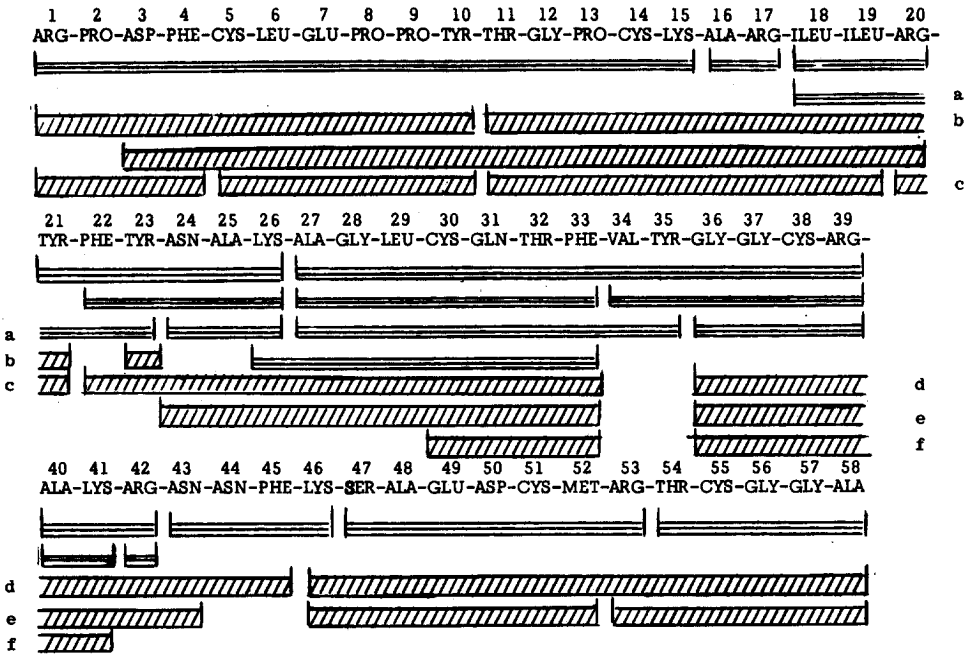

Figure 1. The Amino Acid Sequence. ▭ Tryptic peptides; ▨ chymotryptic peptides; VAL-TYR (#34-35) was lost.

quence of 3 aromatic amino acids (21-23). Of the 3 amino acids present as single residues, methionine (52) has already been shown not to be essential for activity (Kassell, 1964). It is located next to a half cystine making two sulfur containing amino acids in a row. Serine (47) is interestingly in close proximity to some of the other amino acids known to occur near the active serine of trypsin and chymotrypsin. The serine is also next to a lysine. Valine (34) occurs between 2 aromatic amino acids. There are 2 complimentary sequences: Gly-Gly-Cys (36-38) and Cys-Gly-Gly (55-57). Chemical modification versus activity studies are in progress.

Chymotrypsin B, in addition to the expected points of hydrolysis at the aromatic amino acids and at leucine (Fruton, 1948; Enenkel and Smillie, 1963), also cleaved at proline 2, isoleucine 19, asparagine 43,

and methionine 52. Cleavage at asparagine and methionine are known for α-chymotrypsin (Jollès, et al., 1960; Margoliash, 1962).

The additional breaks at arginine 20 and lysine 41 are attributed to the very small amount of trypsin, which was allowed to remain in the activation mixture of chymotrypsinogen B, and indicate extreme sensitivity to trypsin of these 2 bonds of the RCM-inhibitor.

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* Three references were inadvertently omitted from Paper III:

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