Partial structure of chymotrypsinogen

The quantitative amino acid analysis of chymotrypsinogen according to the procedure of Moore and Stein was performed independently in two laboratories[1,2]; a comparison of the results reveals that the differences in the data obtained do not exceed one residue in any of the values.

From the results of studies performed during the past few years it has become still more obvious that the number of amino acid residues contained in peptides isolated from partial hydrolysates of chymotrypsinogen approaches the analytical data on the distribution of individual amino acids in its molecule.

In our laboratory, after the characterization of small peptidic fragments arising from acid hydrolysis of chymotrypsinogen[3], the products obtained from cleavage of the protein by proteolytic enzymes have been the object of a systematic investigation[4–8,16]. Since apart from soluble products tryptic hydrolysis yielded a considerable quantity of material insoluble in the acidic pH region (which would usually be called "core"), we set about a systematic fractionation of peptides originating from peptic[9] and chymotryptic[10] cleavage of this insoluble amount of tryptic digest of *S*-sulpho-chymotrypsinogen. In further experiments we succeeded in isolating peptide fragments with intact disulphide bonds from a peptic hydrolysate of chymotrypsinogen; after oxidation the peptides furnished information[11] about the mode of linkage of three of the five disulphide bridges in the molecule. Structures which can be formulated unambiguously are summarized in Table I; the order in which they are linked in the molecule, however, remains as yet undetermined.

The number of residues of individual amino acids contained in the structures and the number of residues derived from the quantitative composition of parent chymotrypsinogen are given side by side in Table II. As may be seen, the values for

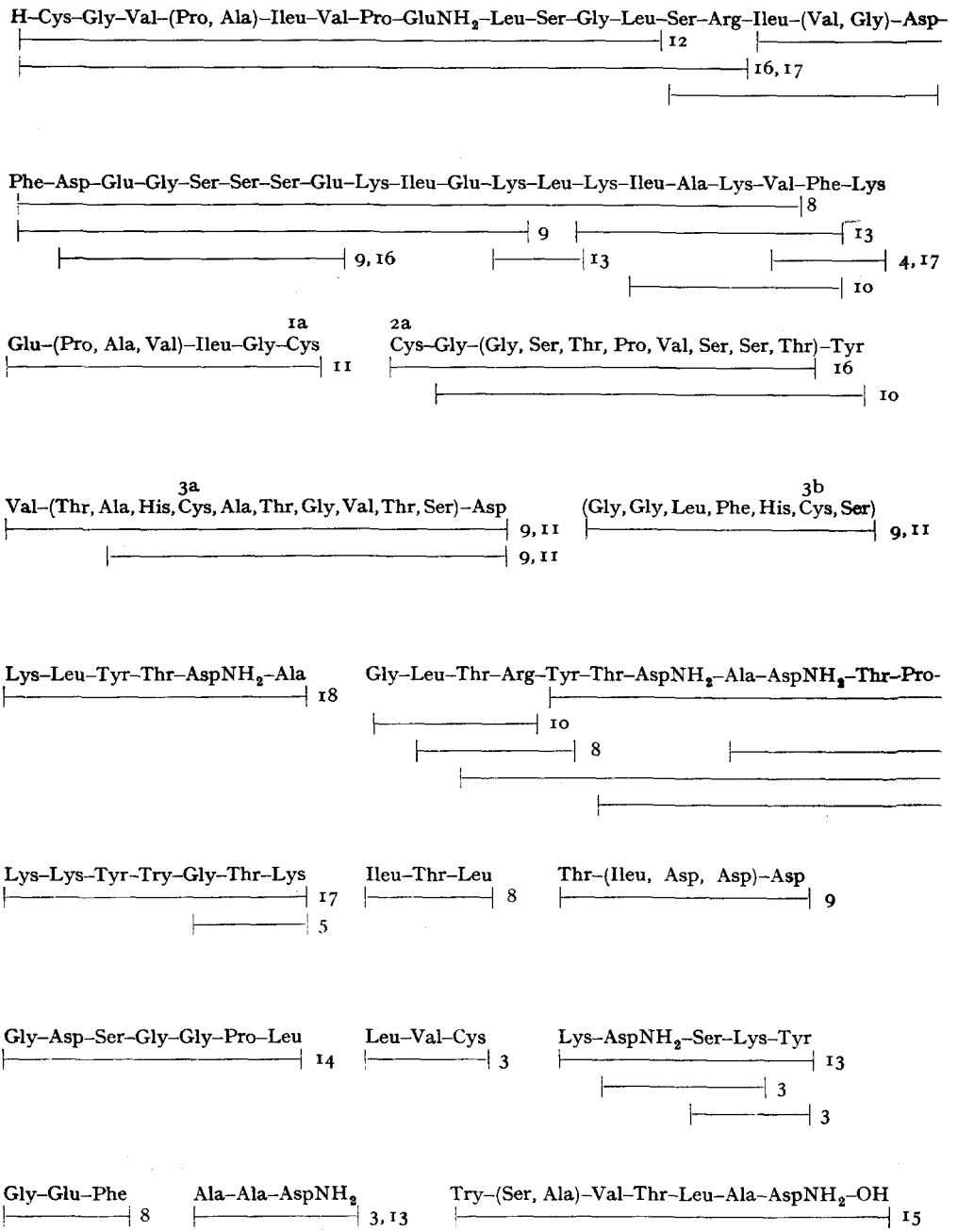
TABLE II

COMPARISON OF ANALYTICAL DATA WITH STRUCTURES

Amino acid	*Number of residues in chymotrypsinogen*		
	Wilcox et al.	*Zmrhal*	*In isolated structures*
Asp	21.8	22.3	20
Thr	23.0	22.0	17
Ser	30.1	29.1	28
Glu	14.2	14.9	16
Pro	8.7	9.1	12
Gly	23.3	22.2	21
Ala	21.7	22.0	22
Val	22.4	22.0	18
Met	1.9	1.8	1
Ile	9.9	9.8	9
Leu	18.8	19.2	19
Tyr	4.1	4.2	4
Phe	6.5	6.2	6
Lys	13.2	13.5	14
His	1.9	1.95	2
Arg	4.0	3.9	4
Try	7.0	6.8	5
Cys	10.0	9.8	10
NH_3 amide	24.0	24.6	16

TAE

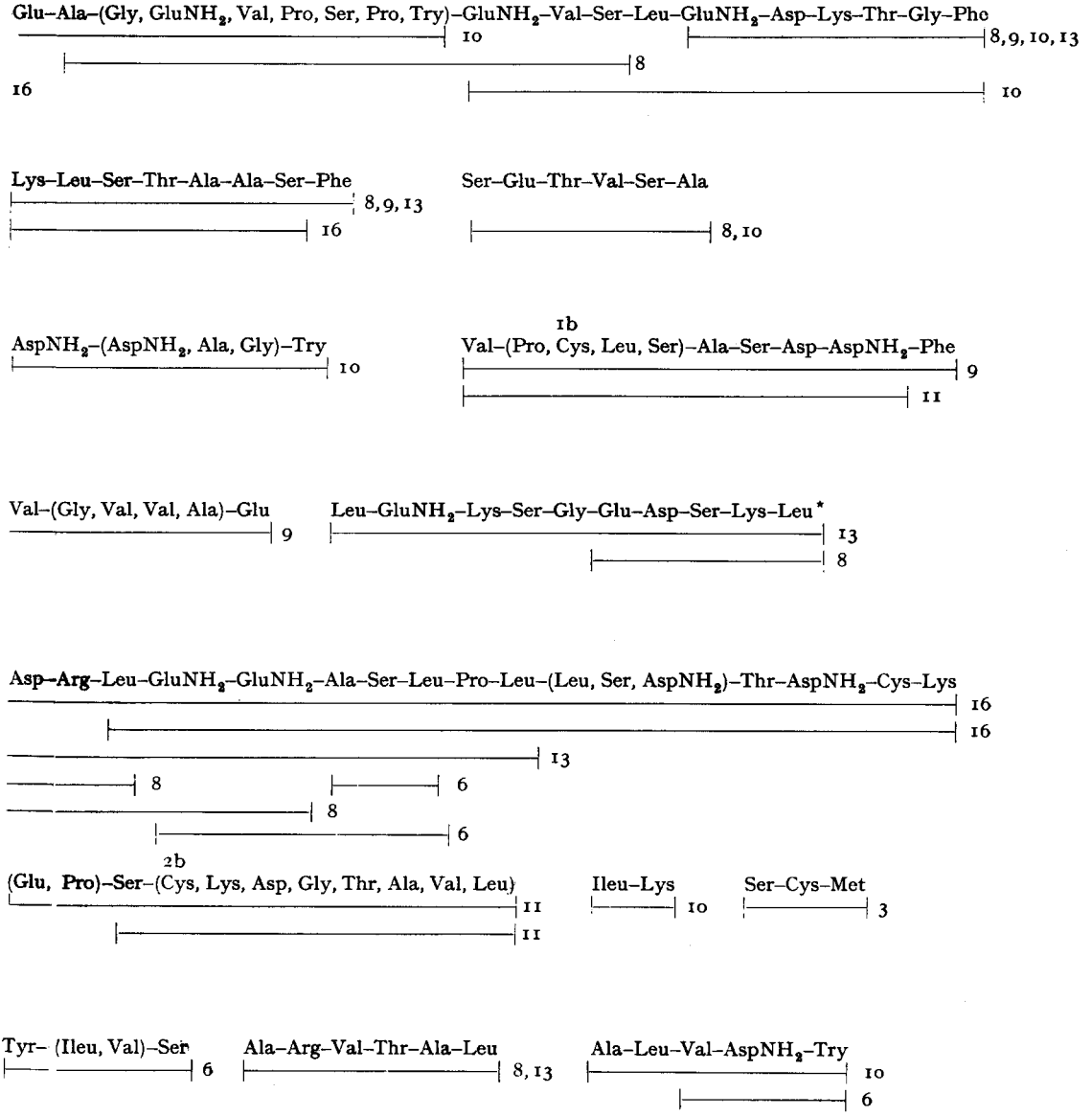
PEPTIDES FROM PARTIAL HYDRO



* in ref. 13: Ileu.

LE I

LYSATES OF CHYMOTRYPSINOGEN



most of the amino acids are either in agreement or differ only slightly from the theoretical analysis; the only considerable difference existing is that pertaining to proline. Obviously, some of the structures comprising cysteine residues joined by disulphide bridges overlap other structures containing proline. These overlappings, however, cannot be defined from the data so far available. Nor is the final account of amides yet complete.

Of the three disulphide bonds established in the partial structures the one worth special interest is that joining the peptides containing the two histidine residues of chymotrypsinogen. Unlike other authors we have found two of the four arginine residues to be bound very close to each other (positions 1 and 10). The accumulation of five lysine residues within a dodecapeptide structure seems to be another characteristic feature of chymotrypsinogen.

The N-terminal portion of the chain of chymotrypsinogen has been satisfactorily defined. The pentadecapeptide terminated with arginine, isolated by us from a tryptic digest of *S*-sulpho-chymotrypsinogen is identical with the A-peptide reported by MEEDOM[12] and extended by the dipeptide sequence Ser–Arg, which is liberated in the course of activation. On the other hand our own peptide structures do not compare favourably with the analysis of the C-chain as reported by HARTLEY[13].

In one case, in addition to the peptide possessing the structure Lys–Leu–Ser–Thr–Ala–Ala–Ser we also isolated the peptide Lys–Leu–Leu–Ser–Thr–Ala–Ala–Ser, *i.e.*, with one recurring leucine residue[16], from the peptic hydrolysate of chymotrypsinogen. The two structures were both as a whole and after subsequent partial hydrolysis with subtilisin characterized by quantitative analysis and the unambiguous determination of the amino acid sequence. In view of the fact that the total account of amino acid residues excludes any possibility of the parallel existence of the two structures in chymotrypsinogen, a hypothesis arises concerning a certain microheterogeneity of the starting material, the cause of which must of course be further explored. This solitary finding is therefore being verified in further experiments.

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The effect of aliphatic amino acids on the activation of trypsinogen and on the stability of trypsin solutions

ε-Aminocaproic acid is known to inhibit the activation of plasminogen and to a lesser degree the fibrinolytic and esterase activity of plasmin[1–3]. Several other aliphatic amino acids with terminal amino groups also inhibit plasminogen activation, but they are less potent than ε-aminocaproic acid. In descending order of effectiveness they are δ-aminovaleric acid, δ-aminolevulinic acid, ω-aminocaprylic acid and γ-aminobutyric acid[4]. The purpose of this study was to find out whether ε-aminocaproic acid and related compounds would interfere also with the activation of trypsinogen. The amino acids were examined for their effect on the autocatalytic activation of trypsinogen as well as on the activation by enterokinase. Besides ε-aminocaproic acid the series included ω-aminocaprylic acid, δ-aminovaleric acid, γ-aminobutyric acid and β-alanine.

To measure the amount of trypsin formed in the activation mixtures, an assay system had to be chosen which itself would not be affected by the inhibitory substances in the concentrations used. An esterolytic assay with TAME as a substrate was found to fulfil this condition and was employed for all determinations. LME, on the other hand, is less suitable as a substrate because the LME esterase activity of trypsin is inhibited, though only mildly, by ε-aminocaproic acid[2] as well as by δ-aminovaleric acid and ω-aminocaprylic acid (Table I).

The assay procedure was as follows: to 0.4 ml of the solution to be tested (diluted, if necessary, to contain approximately 0.25 mg% trypsin) were added 0.8 ml of

TABLE I

INHIBITION OF LME ESTERASE ACTIVITY OF TRYPSIN BY ALIPHATIC AMINO ACIDS

Incubation mixtures of 1.6 ml contained as final concentrations 0.033 *M* lysine methyl ester, 1.25 mg% trypsin (crystalline, salt-free, Mann Res. Lab.) and 0.1 *M* amino acid in 0.1 *M* Tris buffer (pH 7.6, 37°). Incubation for 45 min at 37°.

Amino acid	*inhibition (%)*
β-Alanine	0
γ-Aminobutyric acid	0
ε-Aminocaproic acid	2.9
δ-Aminovaleric acid	14.3
ω-Aminocaprylic acid	14.9

Abbreviations: TAME, *p*-toluenesulfonyl-L-arginine methyl ester; LME, L-lysine methyl ester.