Lumry, R., and Smith, E. L. (1955), *Disc. Far. Soc. 20*, 105.
Manning, D. T., and Niemann, C. (1958), *J. Am. Chem. Soc. 80*, 1478.
Marburg, S., and Jencks, W. P. (1962), *J. Am. Chem. Soc. 84*, 232.
McDonald, C. E., and Balls, A. K. (1957), *J. Biol. Chem. 227*, 727.
Mounter, L. A. (1958), *Biochim. Biophys. Acta 27*, 219.
Mounter, L. A., and Whittaker, V. P. (1953), *Biochem. J. 54*, 551.
Nath, R. L., and Rydon, H. N. (1954), *Biochem. J. 57*, 1.
Neurath, H., and Hartley, B. S. (1959), *J. Cellular and Comp. Physiol. 54*, suppl. 1, 179.
Nimmo-Smith, R. H. (1960), *Biochem. J. 75*, 284.
Pandit, U. K., and Bruice, T. C. (1960), *J. Am. Chem. Soc. 82*, 3386.
Pauling, L. (1960), The Nature of the Chemical Bond, ed. 3, Ithaca, New York, Cornell University Press, p. 260.
Schoellman, G., and Shaw, E. (1962), *Biochem. Biophys. Res. Commun. 7*, 36.
Schonbaum, G. R., Nakamura, K., and Bender, M. L. (1959), *J. Am. Chem. Soc. 81*, 4746.
Spencer, T., and Sturtevant, J. M. (1959), *J. Am. Chem. Soc. 81*, 1874.
Stadtman, E. R. (1954), *in* Symposium on the Mechanism of Enzyme Action, McElroy, W. D., and Glass, B., editors, Baltimore, Johns Hopkins Press, p. 581.
Timm, E. W., and Hinshelwood, C. N. (1938), *J. Chem. Soc.* 862.
Tommila, E., and Hinshelwood, C. N. (1938), *J. Chem. Soc.* 1801.
Viswanatha, T., and Lawson, W. B. (1961), *Arch. Biochem. Biophys. 93*, 128.
Weil, L., James, S., and Buchert, A. R. (1953), *Arch. Biochem. Biophys. 46*, 266.
Whitaker, J. R., and Jandorf, B. J. (1956), *J. Biol. Chem. 223*, 751.

The Amino Terminal Sequence of Bovine Trypsinogen*

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As part of an investigation of the amino acid sequence of bovine trypsinogen, three peptides have been isolated which appear to be derived from the amino terminus of the molecule. These peptides have been obtained from tryptic and chymotryptic digests of *S*-sulfotrypsinogen and from chymotryptic digests of trypsinogen. Integration of their sequences establishes the N-terminal 20 amino acids of trypsinogen.

Studies of the structure and activation of bovine trypsinogen have established that the N-terminal residue is valine (Rovery *et al.*, 1953) and that the N-terminal sequence is Val.Asp.Asp.Asp.Asp.-Lys.Ileu.Val.Gly (Davie and Neurath, 1955; Desnuelle and Fabre, 1955). During the activation process, the peptide bond between lysine and isoleucine is opened, giving rise to the hexapeptide valyl-(aspartyl)$_4$-lysine (Davie and Neurath, 1955) and the enzymatically active protein, trypsin, with an N-terminal isoleucine (Rovery *et al.*, 1953). This single hydrolytic event is accompanied by an appreciable decrease in the levorotation of the molecule (Neurath *et al.*, 1956; Pechère and Neurath, 1957), which has been taken as an indication of changes in the secondary or tertiary structure of the molecule during its activation. The hypothesis has been put forward by Neurath and Dixon (1957) that such conformational changes might include a reorientation of the N-terminal region of the molecule which, by bringing histidine into juxtaposition with a particular DFP-sensitive[1] serine, might play a role in giving rise to the active center.

In the course of a program designed to elucidate the complete amino acid sequence of trypsinogen, peptides have been isolated from enzymatic digests of *S*-sulfotrypsinogen (Walsh *et al.*, 1961, and unpublished experiments) which permit the deduction of the amino acid sequence of the N-terminal twenty amino acids. The sequence is presented here prior to determination of the entire primary structure of the protein with the object of extending the detailed knowledge of the particular region of the molecule which is modified in the course of activation.

Materials and Methods

Crystalline bovine trypsinogen, purchased from Worthington Biochemicals (Lot TG711, contain-

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[1] The following abbreviations are used: DFP, diisopropylphosphofluoridate; Tris, tris(hydroxymethyl)aminomethane; FDNB, 1-fluoro-2,4-dinitrobenzene; DNP-, dinitrophenyl-.

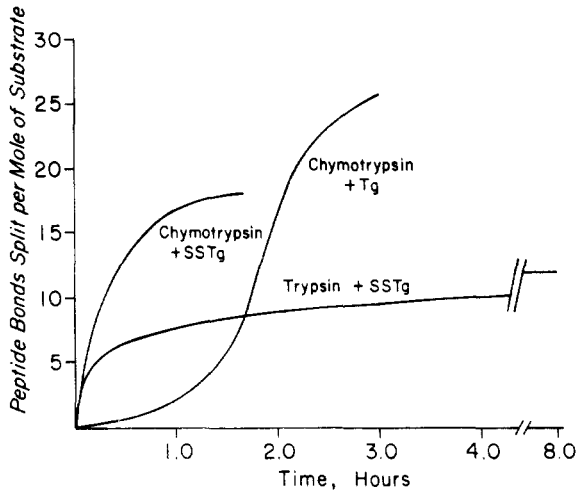

FIG. 1.—Progress of enzymatic digestion of *S*-sulfotrypsinogen and of trypsinogen. The number of bonds split per mole of substrate are calculated from the *p*H-stat measurements, assuming the mean *pKa* of liberated α-amino groups to be 7.0. Experimental details of each digest are given in the text.

ing 50% magnesium sulfate) was used throughout these studies. Solutions were prepared by dissolving the protein in 0.01 M HCl and dialyzing against 0.001 M HCl at 3°. The protein proved to be 95% homogeneous by moving boundary electrophoresis at a concentration of 11 mg per ml in a buffer or Tris chloride of ionic strength 0.1, *p*H 8.0, containing 0.23 mg of soybean trypsin inhibitor (Worthington Biochemicals) per ml. The trypsinogen contained less than 0.24% active trypsin and could be 92% activated in 0.1 M Tris chloride containing 0.05 M calcium chloride, *p*H 8.1, as judged by its activity toward benzoyl-L-arginine ethyl ester, using the zero order reaction rate constant of 0.36 moles liter^{-1} min.$^{-1}$ mg N (Green and Neurath, 1953). *S*-sulfotrypsinogen was prepared from trypsinogen by the method described by Pechère *et al.* (1958).

Trypsin was prepared by the activation of trypsinogen as indicated above. The activated mixture was then adjusted to *p*H 3.0, and the solution was dialyzed against 0.001 M HCl and stored in frozen form.

Chymotrypsin was thrice-crystallized α-chymotrypsin obtained from Worthington Biochemicals. Nagarse (a crystalline protease from *B. subtilis*) was purchased from the Biddle Sawyer Corp., and elastase from Worthington Biochemicals. Carboxypeptidase A was prepared from acetone powder of beef pancreas glands (Allan *et al.*, 1962), and was treated with DFP immediately prior to use to eliminate any endopeptidase activity. Carboxypeptidase B was prepared from pork pancreas by the method of Folk *et al.* (1960). Leucine aminopeptidase, prepared by the Folk *et al.* (1959) modification of the method of Hill *et al.* (1958), was the generous gift of Dr. P. E. Wilcox. The enzyme was activated with $MnCl_2$ immediately prior to use (Spackman *et al.*, 1955).

Tryptic digestion of 2.10 g of *S*-sulfotrypsinogen was carried out in 1025 ml of a solution containing 0.02 M $CaCl_2$, 0.01 M indole, and 0.11 g of trypsin and maintained at 26°, *p*H 8.0, in a *p*H-stat for 8 hours (Fig. 1). At the end of the period, trypsin was inactivated by reducing the *p*H to 3.2 with formic acid. After removal of insoluble material, the solution of tryptic peptides was extracted with cyclohexane to remove the indole. Calcium was removed as the insoluble fluoride by the addition of the calculated amount of 10% hydrofluoric acid. The supernatant was lyophilized and the dry product dissolved in 200 ml of 0.05 M pyridine adjusted to *p*H 2.3 with formic acid, and the mixture on peptides was resolved on a chromatographic column of Dowex 50×2 (200–400 mesh) with volatile pyridine-formic-acetic buffers (Walsh *et al.*, 1961, and unpublished data).

The chymotryptic digest of 580 mg of *S*-sulfotrypsinogen was prepared in 145 ml of a solution containing 6.0 mg of chymotrypsin and maintained at *p*H 7.8, 37°, for 100 minutes (Fig. 1). The digest was then adjusted to *p*H 3.5 and a trace of insoluble material removed by centrifugation. The supernatant was subjected to gradient elution chromatography on DEAE-sephadex with ammonium and pyridinium acetate buffers ranging from *p*H 8.0 to 6.5 (Walsh, Kauffman, and Neurath, unpublished data).

Trypsinogen was also digested with chymotrypsin without prior reduction of the disulfide bonds. In this case, 31.6 mg of trypsinogen was digested for 2 hours at *p*H 7.8, 37°, with 0.38 mg of chymotrypsin. The reaction was terminated by adjusting the *p*H to 3.5 with acetic acid. The peptides were purified by paper ionophoresis at *p*H 6.5 and the acidic peptides collected.

The sigmoid form of the progress curve (Fig. 1) would be consistent either with autocatalytic activation accompanied by autolysis or with a slow preliminary opening of the structure by hydrolysis of peptide bonds, followed by a rapid digestion of the "opened" structure. The latter explanation is favored by the observation that less than 0.3% of the trypsinogen was in the form of active trypsin at the end of the digestion.

The fractions obtained by ion exchange chromatography of digests of *S*-sulfotrypsinogen all contained mixtures of peptides. These were easily resolved by ionophoresis at 34 volts per cm on Whatman #3 paper in volatile buffers (*p*H 6.5, 3.6, and 2.1) by adaptations of the techniques of Michl (1953) and Naughton *et al.* (1960), and by paper chromatography in butanol-acetic acid–water mixtures (3:1:1) as needed. The peptide zones were located by staining a narrow guide strip with either ninhydrin or hypochlorite-tolidine (Greig and Leaback, 1960). The final purity in each case was judged by homogeneity during ionophoresis and chromatography, by end-group analysis with FDNB (Fraenkel-Conrat

et al., 1955), and by observations that within the limits of experimental error the measured ratios of amino acid residues were integral numbers.

Amino acid compositions were determined in acid hydrolysates prepared in 6 N HCl *in vacuo* at 105° overnight. Peptides with both isoleucine and valine were hydrolyzed an additional 24 hours to break any isoleucyl valine bonds (*cf.* Harfenist, 1953). Prior to hydrolysis, *S*-sulfocysteine was converted to cysteic acid by oxidation of the peptide with performic acid by the technique of Pierce (1955). Amino acids were separated by the method of Richmond and Hartley (1959) and quantitated by the method of Tigane *et al.* (1961). Lysine was separated from hydroxylysine on Reeve Angel SA-2 paper (Amberlite IR-120, resin-loaded) by use of 0.2 N sodium citrate buffer, *p*H 4.25, yielding relative mobilities of arginine, lysine, and hydroxylysine of 0.23, 0.58, and 1.0 respectively (L. H. Ericsson, unpublished data). Leucine and isoleucine were separated in the *t*-amyl-phthalate system of Blackburn and Lowther (1951), with two modifications. The quality and reproducibility of the chromatogram was considerably improved, first, by including versene as recommended by Meloun *et al.* (1958) and, second, by eluting the paper with the buffer for 48 hours and then drying before applying the sample.

Amino acid sequences were determined by conventional methods of end-group analysis and partial, selective hydrolysis with acid or enzymes. N-terminal amino acids were determined with Sanger's FDNB reagent (Fraenkel-Conrat *et al.*, 1955). In most cases the identity of the DNP-amino acid in the ether extract of the hydrolysate was confirmed by quantitative analysis of the aqueous phase. N-terminal sequences were also deduced from the order of liberation of amino acids by leucine aminopeptidase. The C-terminal amino acids were removed by digestion with carboxypeptidase A or B in 0.1 M ammonium acetate buffer, *p*H 8.5, for various lengths of time. The liberated amino acids were then identified on paper and quantitated in the usual system for amino acid separations. In some case, the peptide spots were also eluted, hydrolyzed, and quantitated. Partial and selective hydrolyses of peptides were accomplished either by limited exposure to HCl or by digestion with one of the proteases—Nagarse, elastase, trypsin, and chymotrypsin. The reactions were stopped by heat-inactivation of the enzyme and the resultant peptide mixtures separated by paper ionophoresis or chromatography.

Results

Of the many peptides isolated from the tryptic and chymotryptic digests of *S*-sulfotrypsinogen (Walsh *et al.*, 1961, unpublished data), only three could be directly related to the N-terminus of trypsinogen. Peptide T-10, obtained from a tryptic digest, was identical in composition with the N-terminal hexapeptide of Davie and Neurath (1955). Peptide C-17a, obtained from a chymotryptic digest, proved to contain this unusual hexapeptide and, in addition, the amino acids tryosine, glycine, isoleucine, and valine—the last three of which would be expected from the work of Desnuelle and Fabre (1955). The third relevant peptide (T-9a) was the only peptide in the tryptic digest with an N-terminal sequence Ileu.Val.Gly. No other peptides in these digests contained the known N-terminal sequences of either trypsinogen or trypsin. The compositions and yields of these three purified peptides are given in Table I. The composition of a peptide isolated from the chymotryptic digest of trypsinogen is also presented in this Table for comparison with peptide C-17a.

Table I

Compositions of Selected Peptides from Enzymatic Digests of *S*-sulfotrypsinogen (SSTg) and Trypsinogen (Tg)

	Tryptic Peptides from SSTg		Chymotryptic Peptides	
	T-10	T-9a	from SSTg C-17a	from Tg
Valine	1.0	2.0	1.9	2.0
Aspartic acid	4.0	1.0	4.3	3.8
Lysine	1.1		1.0	1.1
Isoleucine		0.8	0.7	0.9
Glycine		3.0	2.2	2.0
Tyrosine		2.1	0.9	0.4
Threonine		1.9		
Cysteic acid		1.2		
Alanine		1.0		
Proline		0.9		
Yield (%)	19	13	17	23

Sequence of Peptide T-10: Val.Asp.Asp.Asp.-Asp.Lys.—Dinitrophenylation of peptide T-10 yielded DNP-valine. Digestion of 0.2 μmoles of T-10 with 0.014 mg of carboxypeptidase B in 0.1 M ammonium acetate at *p*H 8.5 yielded complete recovery of lysine as measured after purification by ionophoresis at *p*H 6.5. Subsequent chromatography on SA-2 paper verified that it was indeed lysine, not hydroxylysine. Digestion of 0.1 μmole T-10 with 0.01 mg of leucine aminopeptidase in 0.1 M ammonium acetate, *p*H 8.6, for 4 hours at 37° yielded only one amino acid: valine. Deduction of the sequence of T-10 is summarized in Table II.

Table II

Summary of Evidence for Sequence of T-10

Dinitrophenylation	Val.
Leucine aminopeptidase	Val.
Carboxypeptidase B	.Lys
Sequence	Val.Asp.Asp.Asp.Asp.Lys

Sequence of Peptide C-17a: Val.Asp.Asp.Asp.-Asp.Lys.(Ileu,Val,Gly)(Gly,Tyr). — Dinitrophenylation yielded DNP-valine in the ether extract; the aqueous phase contained ε-DNP-lysine, *O*-DNP-tyrosine, and the following ratios of amino acids (after 16 hours of hydrolysis): aspartic acid 3.9, glycine 2.4, valine 0.8, isoleucine 0.6.

Two μmoles of C-17a was digested with 0.5 mg of trypsin in 0.7 ml of 0.12 M ammonium acetate, *p*H 8.6, containing 0.005 M calcium acetate at room temperature for 20 minutes, and the resultant peptide mixture was resolved at *p*H 6.5 by ionophoresis. Two new peptide spots appeared: One was an acidic spot with the same mobility as T-10, containing valine, aspartic acid, and lysine in the ratios 1.0:4.0:1.0. The other, a neutral spot at *p*H 6.5, was purified again by butanol–acetic–water chromatography to yield a peptide containing (after 28 hours of hydrolysis) the amino acid ratios isoleucine 0.6, valine 0.7, glycine 2.4, and tyrosine 1.0.

Overnight digestion of 0.2 μmoles of C-17a with 0.24 mg of carboxypeptidase A yielded the following amino acids (in μmoles): tyrosine 0.16, glycine 0.24, valine 0.09, isoleucine 0.10. The deduction of the sequence of C-17a is summarized in Table III.

TABLE III
SUMMARY OF EVIDENCE OF SEQUENCE OF C-17a

Dinitrophenylation	Val.
Trypsin	((Val,Asp_4).Lys)(Ileu,Val,-Gly_2Tyr)
Carboxypeptidase A	(Val,Asp_4,Lys)(Ileu,Val,Gly)-(Gly,Tyr)
Sequence	Val.Asp.Asp.Asp.Asp.Lys.-(Ileu,Val,Gly)(Gly,Tyr)

Sequence of Peptide T-9a: Ileu.Val.Gly.Gly.-Tyr.Thr.Cys.Gly.Ala.Asp(NH_2).Thr.(Val, Pro).-Tyr.—Dinitrophenylation of T-9a yielded DNP-isoleucine in the ether extract of the hydrolysate, and this was verified by finding the following ratios of amino acids in the aqueous phase: valine 1.8, glycine 3.2, *O*-DNP-tyrosine (not quantitated), threonine 1.8, half-cystine 1.0, alanine 1.0, aspartic acid 1.2, and proline 0.7.

Digestion of 0.1 μmoles with either 0.001 mg or 0.01 mg of leucine aminopeptidase in 0.1 M ammonium acetate at *p*H 9 for 2 hours at 37° yielded the amino acids and μmolar values described in Table IV.

Treatment of 0.2 μmoles of T-9a with 0.05 mg of carboxypeptidase A in 0.1 M ammonium acetate buffer at *p*H 8.5, 37°, overnight released 0.2 μmoles of tyrosine and no other amino acid.

Two enzymatic digestions were carried out in which 1.0 μmole of T-9a was incubated with either 1 mg of chymotrypsin or 0.1 mg of Nagarse in 0.1 M ammonium acetate, *p*H 8.5, at room temperature for 16 hours. Each digest was resolved by paper chromatography with butanol–acetic–water solvent, and four peptides were found in each case. The yields and compositions of these peptides are summarized in Table V.

TABLE IV
AMINO ACIDS LIBERATED FROM T-9a BY LEUCINE AMINOPEPTIDASE
The conditions of incubation are described in the text.

	Amount of Leucine Aminopeptidase	
	0.001 mg	0.01 mg
	μmoles	
Isoleucine	0.012	0.074
Valine	0.005	.070
Glycine		.040
Tyrosine		.022
Threonine		.016

Sequence of Peptide T9a-C4: Ileu.Val.Gly.-Gly.Tyr.—Incubation of 0.2 μmoles of this peptide with 0.025 mg of carboxypeptidase A in 1 M ammonium acetate, *p*H 9, released all of the tyrosine and no other amino acid in 1 hour at room temperature.

One half μmole of T9a-C4 was partially hydrolyzed with 6 N HCl for 4 hours at 110°, and a peptide was isolated from the hydrolysate by paper chromatography in butanol–acetic–water, where it had the mobility of 1.3 relative to leucine. After 40 hours of hydrolysis of the isolated peptide under the same conditions, isoleucine and valine were found in equal concentration, representing a 50% yield from T9a-C4. Since isoleucine was known to be N-terminal in T-9a, the sequence Ileu.Val must be N-terminal in T9a-C4.

Peptide T9a-C2: Thr.(Cys,Gly,Ala,Asp,Thr,-Pro,Val,Tyr).—Dinitrophenylation gave DNP-threonine in the ether extract of the hydrolysate and the aqueous phase contained amino acids in the following ratios: valine 1.1, glycine 1.2, *O*-DNP-tyrosine (not quantitated), threonine 1.0, alanine 1.0, aspartic acid 1.0, proline 0.3.

Peptide T9a-C1: (Thr,Cys).Gly.Ala.Asp[NH_2].—Incubation of 0.2 μmoles of T9a-C1 with 0.05 mg of carboxypeptidase A in molar ammonium acetate, *p*H 9, released 0.04 μmoles of asparagine in 2 hours and 0.14 μmoles of asparagine, 0.13 μmoles of alanine, and 0.05 μmoles of glycine in 20 hours.

Peptide T9a-N1: (Thr,Cys,Gly)(Ala,Asp[NH_2]).—Six tenths μmole of T9a-N1 was incubated with 0.08 mg of elastase in 0.1 M ammonium acetate, *p*H 8.5, for 1 hour at 37°. Ionophoresis at *p*H 2.1 separated free alanine, free asparagine, and a peptide (in 60% yield), with a mobility of 0.14 relative to lysine, containing equal quantities of cysteic acid, threonine, and glycine.

Peptide T9a-N3: Thr.(Pro,Val).Tyr.—Dinitrophenylation yielded DNP-threonine in the ether extract and valine, proline, and tyrosine in the aqueous phase.

Incubation of 0.2 μmoles with 0.2 mg of carboxypeptidase A yielded complete recovery of

TABLE V

PEPTIDES FROM CHYMOTRYPTIC AND NAGARSE DIGESTS

	Chymotryptic Peptides				Nagarse Peptides			
Peptide	T9a-C1	T9a-C2	T9a-C3	T9a-C4	T9a-N1	T9a-N2	T9a-N3	T9a-N4
Mobility[a]	0.18	0.45	0.99	1.07	0.18	0.62	0.99	1.10
Per cent yield	33	46	Trace[b]	50	84	37	84	[b]
Isoleucine				0.6				
Valine		1.0	+	1.0			1.1	
Glycine	1.2	1.1		2.0	1.1	2.0		
Tyrosine		1.0	+	1.0		1.0	1.0	
Threonine	0.8	1.9	+		0.9		1.0	
Half-cystine[c]	0.8	0.4			1.4			
Alanine	1.1	0.9			1.0			
Aspartic	1.0	1.2			0.9			
Proline		0.8	+				1.0	

[a] Mobility of peptide, relative to a marker of leucine, during chromatography in butanol–acetic–water. [b] Neither T9a-C3 and T9a-C4 nor T9a-N3 and T9a-N4 were well separated during chromatography. [c] Determined as cystine, the hydrolysis product of *S*-sulfocysteic acid.

TABLE VI

SUMMARY OF EVIDENCE FOR SEQUENCE OF T-9a

Dinitrophenylation	Ileu.
Leucine amino peptidase	Ileu.Val(Gly,Gly,Tyr,Thr)
Peptides from chymotryptic and Nagarse digests:	
T9a-C4	Ileu.Val.Gly.Gly.Tyr
T9a-C2	Thr(Cys,Gly,Ala,Asp,Thr,Val,Pro,Tyr)
T9a-N3	Thr(Val,Pro)Tyr
T9a-C1	(Thr,Cys)Gly.Ala.Asp[NH_2]
T9a-N1	(Thr,Cys,Gly)(Ala,Asp[NH_2)]
T9a-N2	(Gly,Gly,Tyr)
Carboxypeptidase A	.Tyr
Sequence	Ileu.Val.Gly.Gly.Tyr.Thr.Cys.Gly.Ala.Asp[NH_2].Thr.(Val,Pro).Tyr

tyrosine in 16 hours at room temperature. A peptide which separated from tyrosine during paper chromatography with butanol–acetic–water had the composition (Thr,Val,Pro).

The evidence for the complete sequence of the peptide T-9a is summarized in Table VI.

DISCUSSION

Evidence has been presented for the following amino acid sequences in three peptides isolated from enzymatic digests of *S*-sulfotrypsinogen.

From tryptic digests the following two peptides were obtained: T-10: Val.Asp.Asp.Asp.Asp.-Lys; T-9a: Ileu.Val.Gly.Gly.Tyr.Thr.Cys.Gly.-Ala.AspNH_2.Thr(Val,Pro)Tyr.

Chymotryptic digests yielded one peptide, C-17a: Val.Asp.Asp.Asp.Asp.Lys.(Ileu,Val,Gly)-(Gly,Tyr).

A reasonable working hypothesis may be developed that peptides T-10 and T-9a occupy adjacent positions in trypsinogen and that this combination in fact comprises the N-terminal eicosopeptide sequence of the molecule. Even without prior knowledge that the N-terminal sequence is Val.Asp_4.Lys.Ileu.Val.Gly (Desnuelle and Fabre, 1955; Davie and Neurath, 1955), the highly unusual grouping of the aspartyl residues in the hexapeptide T-10 and its appearance in C-17a provide a strong argument that C-17a overlaps T-10. When this is considered together with the fact that no peptide other than T-9a with the N-terminal sequence Ileu.Val.-Gly.Gly.Tyr is found in the tryptic digest of *S*-sulfotrypsinogen, the conclusion seems inescapable that the N-terminal sequence is Val.Asp_4.Lys.-Ileu.Val.Gly.Gly.Tyr.Thr.Cys.Gly.Ala.AspNH_2.-Thr.(Val,Pro).Tyr.

This sequence as presented appears to be in conflict with a report of a peptide isolated from an autolysate of trypsin by Gabeloteau and Desnuelle (1958). This peptide had the sequence Ileu.Val.Gly.Glu(or Glu[NH_2]).Tyr, and the authors proposed that this pentapeptide might be the N-terminal sequence of active trypsin because it contained the N-terminal sequence Ileu.Val.-Gly. It is probable, however, that their location of glutamic acid in this pentapeptide is in error, because the recent sequence determinations of Walsh *et al.* (1961 and unpublished data) account

for all nine of the tyrosine residues in trypsinogen without including the sequence described by Gabeloteau and Desnuelle.

Although trypsin is known to exhibit a rigid specificity toward peptide bonds at the carboxyl side of basic residues, examination of the peptide T-9a reveals that trypsin appears to have hydrolyzed a peptide bond at the carboxyl side of tyrosine. It should be pointed out that this was not the only deviation from the accepted specificity of trypsin. An asparagine-methionine bond was also hydrolyzed in the same digest, and the C-terminal asparagine peptide was isolated in 18% yield (Walsh *et al.*, 1961, and unpublished data). Several other seemingly anomalous cleavages occurred, but to a much smaller extent. For example, a peptide identical to T9a-N3 was isolated in 4% yield and another identical to T9a-C2 in 1.6% yield from the same digest. In these examples an Asp[NH_2].Thr and a Tyr.Thr bond must have been broken in addition to the C-terminal tyrosine. It is not known whether these bonds were split, under the conditions of digestion employed, by trace contaminants of enzymes (*e.g.* chymotrypsin or elastase) or whether the intrinsic "acetyltyrosine - ethyl - ester splitting" character of trypsin was itself responsible (Inagami and Sturtevant, 1960; Cole and Kinkade, 1961). Similar problems have been encountered by Bromer *et al.* (1957) and by Hartley (1961).

Knowledge of the more extended N-terminal sequence of trypsinogen provides a factual basis for any extension of the mechanism of activation of the zymogen. It does draw attention to the striking contrast between the highly polar character of the N-terminal hexapeptide of trypsinogen (six charged groups on as many residues) and the largely nonpolar N-terminal sequence of the activated enzyme. Any further interpretation of the conformational change accompanying activation must lie within the framework of restrictions imposed by the structure of this sequence.

References

Allan, B. J., Keller, P. J., and Neurath, H. (1962), in preparation.

Blackburn, S., and Lowther, A. G. (1951), *Biochem. J. 48*, 126.

Bromer, W. W., Staub, A., Sinn, L. G., and Behrens, O. K. (1957), *J. Am. Chem. Soc. 79*, 2801.

Cole, R. D., and Kinkade, J. M., Jr. (1961), *J. Biol. Chem. 236*, 2443.

Davie, E. W., and Neurath, H. (1955), *J. Biol. Chem. 212*, 515.

Desnuelle, P., and Fabre, C. (1955), *Biochim. Biophys. Acta 18*, 49.

Folk, J. E., Gladner, J. A., and Viswanatha, T. (1959), *Biochim. Biophys. Acta 36*, 256.

Folk, J. E., Piez, K. A., Carroll, W. R., and Gladner, J. A. (1960), *J. Biol. Chem. 35*, 2272.

Fraenkel-Conrat, H., Harris, J. I., and Levy, A. L. (1955), *in* Methods of Biochemical Analysis, Vol. II, Glick, D., editor, New York, Interscience Publishers, Inc., p. 359.

Gabeloteau, C., and Desnuelle, P. (1958), *Bull. Soc. Chim. Biol. 40*, 35.

Green, N. M., and Neurath, H. (1953), *J. Biol. Chem. 204*, 379.

Greig, C. G., and Leaback, D. H. (1960), *Nature 188*, 311.

Harfenist, E. J. (1953), *J. Am. Chem. Soc. 75*, 5528.

Hartley, B. S. (1961), Vth International Congress of Biochemistry, Symposium IV, Moscow.

Hill, R. L., Spackman, D. H., Brown, D. M., and Smith, E. L. (1958), *Biochem. Preps. 6*, 35.

Inagami, T., and Sturtevant, J. M. (1960), *J. Biol. Chem. 235*, 1019.

Meloun, B., Vanecek, J., and Sorm, F. (1958), *Coll. Czech. Chem. Comm. 23*, 1788.

Michl, H. (1953), *Naturwiss. 40*, 390.

Naughton, M. A., Sanger, F., Hartley, B. S., and Shaw, D. C. (1960), *Biochem. J. 77*, 149.

Neurath, H., and Dixon, G. H. (1957), *Fed. Proc. 16*, 791.

Neurath, H., Rupley, J. A., and Dreyer, W. T. (1956), *Arch. Biochem. Biophys. 65*, 243.

Pechère, J.-F., Dixon, G. H., Maybury, R. H., and Neurath, H. (1958), *J. Biol. Chem. 233*, 1364.

Pechère, J.-F., and Neurath, H. (1957), *J. Biol. Chem. 229*, 389.

Pierce, J. G. (1955), *J. Am. Chem. Soc. 77*, 184.

Richmond, V., and Hartley, B. S. (1959), *Nature 184*, 1869.

Rovery, M., Fabre, C., and Desnuelle, P. (1953), *Biochim. Biophys. Acta 12*, 547.

Spackman, D. H., Smith, E. L., and Brown, D. M. (1955), *J. Biol. Chem. 212*, 255.

Tigane, E., Wade, E. H. M., Wong, J. T., and Hanes, C. S. (1961), *Can. J. Biochem. Physiol. 39*, 427.

Walsh, K. A., Kauffman, D. L., and Neurath, H. (1961), *Fed. Proc. 20*, A385c.