

# The Amino Acid Sequence of an Extracellular Nuclease of *Staphylococcus aureus*

## III. COMPLETE AMINO ACID SEQUENCE

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### SUMMARY

The amino acid sequence of an extracellular nuclease of *Staphylococcus aureus*, strain V8, is presented. The calculated molecular weight of the nuclease is 16,807.

In a previous paper data were presented that permitted the linear arrangement of five fragments produced by cleavage of an extracellular nuclease of *Staphylococcus aureus* with cyanogen bromide (1). The amino acid sequences of the tryptic peptides prepared from these fragments, together with the partial sequences of chymotryptic peptides isolated from digests of the intact nuclease, have also been presented (2).

In the present communication, the tryptic peptides derived from each cyanogen bromide fragment are arranged in linear order on the basis of the amino acid sequences of the tryptic and chymotryptic cleavage products. In order to obtain supplemental information necessary for final alignment, certain tryptic fragments of trifluoroacetylated nuclease were separated and examined. The molecular weight calculated from the deduced sequence is consistent with estimates obtained from physicochemical examination (3) and from x-ray diffraction studies (4).

### EXPERIMENTAL PROCEDURE

The procedures employed have been described in the preceding reports (1, 2), unless otherwise specified.

**Tryptic Digestion of Trifluoroacetylated Nuclease**—The trifluoroacetylation of the nuclease was carried out by the method of Goldberger and Anfinsen (5). Approximately 1  $\mu$ mole of the protein was used for each preparation. After trifluoroacetylation, the reaction mixture (5 ml) was dialyzed against 3 liters of 0.1 M acetic acid for 48 hours with five changes, and lyophilized. The trifluoroacetylated nuclease was digested with trypsin at 37° for 3 hours, and the resulting material was treated with

piperidine as described elsewhere (5). The mixture, containing large tryptic fragments, was lyophilized. Approximately 92% of the lysine residues of the nuclease had been trifluoroacetylated as judged by resistance to deamination with  $\text{NaNO}_2$  (6) (Table I).<sup>1</sup> Separation of the fragments was performed by column chromatography either on carboxymethyl Sephadex C-50 (Pharmacia), 1  $\times$  43 cm, or on phosphocellulose P 11 (Whatman Chromedia), 7.4 meq per g, 1  $\times$  14 cm (7). Separation by two-dimensional peptide mapping on Whatman No. 3 MM paper was utilized for some of the peptides as described previously (1).

The amino acid compositions and  $\text{NH}_2$ -terminal groups of trifluoroacetylnuclease fragments obtained in pure form are presented in Table II.

**Thermolysin Digestion**—Purified thermolysin was kindly donated by Dr. T. H. Jukes (8, 9). The reaction mixture, containing 6 mg of a given peptide, 0.1 mg of thermolysin, and 1 mg of soybean trypsin inhibitor (Worthington) in 1 ml of  $2.5 \times 10^{-2}$  M  $\text{NH}_4\text{HCO}_3$ - $2 \times 10^{-4}$  M  $\text{CaCl}_2$ , pH 8, was incubated for 2 hours at 37°.

### RESULTS

**Linear Arrangement of Tryptic Fragments**—Designations of peptides and their amino acid sequences, and assignments of tryptic peptides to particular cyanogen bromide fragments, are described in preceding reports (1, 2) unless otherwise specified.

**Cyanogen Bromide Fragment A**—The relation of peptides assigned to Fragment A and the deduced amino acid sequence of Fragment A are illustrated in Fig. 1. Peptide T-V-1, containing homoserine, is placed at the COOH terminus. Since the  $\text{NH}_2$ -terminal residue of Fragment A is alanine (1), either Peptide T-V-8a or T-V-2, both of which contain  $\text{NH}_2$ -terminal alanine, should constitute the  $\text{NH}_2$ -terminal sequence of Fragment A. Chymotryptic Peptide C-9c includes Peptide T-V-2, as judged by its partial sequence. It contains the  $\text{NH}_2$ -terminal sequence Ile-Lys, and leucine as the COOH-terminal residue. Upon dilute acid hydrolysis of Peptide C-9c, aspartic acid and glycine

<sup>1</sup> Preliminary results of the amino acid analysis of the tryptic fragments of trifluoroacetylated nuclease were consistent with trifluoroacetylation of almost all lysine residues of the nuclease.

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were liberated in a molar ratio of 2:1, indicating a sequence (Asp)-Gly-(Asp),<sup>2</sup> a sequence found in Peptide T-V-2 (2). Thus Peptide T-V-8a must form the NH<sub>2</sub> terminus of Fragment A. Peptide T-18a, which includes Peptide T-V-7b and contains the

<sup>2</sup> The abbreviations used are: (Glu) and (Asp), Glu or Gln and Asp or Asn, respectively; TFA-nuclease, trifluoroacetylated nuclease.

TABLE I

Deamination of ε-amino groups of lysine residues in nuclease before and after trifluoroacetylation

The amounts of protein used for deamination (see "Experimental Procedure") were 0.5 to 1 mg. After incubation, the mixtures (0.5 ml) were dialyzed against 2 liters of 0.1 M acetic acid at 4° for 24 hours. The dialysates were lyophilized. The dried samples were subjected to acid hydrolysis and amino acid analysis. Representative residue numbers, calculated on the basis of 3 phenylalanine residues per molecule, are presented. Other amino acids were found in proportions essentially the same as in hydrolysates of native nuclease (1). As a control, trifluoroacetylated nuclease was subjected to hydrolysis without treatment with NaNO<sub>2</sub>.

Amino acid	Samples treated with NaNO <sub>2</sub>				Samples not treated with NaNO <sub>2</sub>	
	Native		Trifluoroacetylated		Trifluoroacetylated	
	μmole	residues	μmole	residues	μmole	residues
Lysine.....	0.012	0.48	0.199	18.3	0.400	19.6
Histidine.....	0.080	3.2	0.036	2.9	0.059	2.9
Arginine <sup>a</sup> .....	0.066	2.6	0.028	2.4	0.112	5.5
Methionine <sup>a</sup> .....	0.068	2.7	0.035	3.2	0.083	4.1
Tyrosine <sup>a</sup> .....	0.006	0.25	0.007	0.56	0.147	7.2
Phenylalanine.....	0.076	(3)	0.036	(3)	0.061	(3)

<sup>a</sup> Arginine, methionine, and tyrosine are partially destroyed by NaNO<sub>2</sub>.

TABLE II

Amino acid composition of tryptic peptides obtained from trifluoroacetylated nuclease

Peptides obtained by chromatography on either carboxymethyl Sephadex or phosphocellulose are designated CM and P, respectively. The peptides obtained by the two-dimensional peptide mapping (see "Experimental Procedure") are indicated as M. Other details are given in the text. Amino-terminal groups were determined by the dinitrophenylation procedure (1). When identical fragments were obtained by more than one separation method, only one analysis is presented.

Amino acid	TFA-M7	TFA-P2	TFA-M1	TFA-CM11
Lysine.....	0.015(1)	0.039(1)	0.005(1)	0.052(4)
Histidine.....			0.005(1)	
Arginine.....	0.016(1)		0.005(1)	
Aspartic acid.....	0.020(1)	0.015(0)	0.014(2)	0.040(4)
Threonine.....	0.012(1) <sup>a</sup>		0.008(1)	
Serine.....		0.010(0)		0.024(2)
Glutamic acid.....		0.043(1)	0.015(2)	0.071(5-6)
Proline.....			(1) <sup>b</sup>	0.004(0)
Glycine.....	0.024(1)	0.029(1)		0.006(1)
Alanine.....		0.057(2)		0.032(3)
Valine.....		0.031(1)	0.005(1)	
Isoleucine.....				0.010(1)
Leucine.....		0.037(1)	0.015(2)	0.013(1)
Tyrosine.....	0.014(1)	0.025(1)	0.006(1)	
NH <sub>2</sub> -terminal residue.....	Thr	Glu	Val	Lys
Assigned to cyanogen bromide fragment.....	D	E	E	E

<sup>a</sup> Partial destruction of NH<sub>2</sub>-terminal residue by ninhydrin staining.

<sup>b</sup> Qualitative determination.

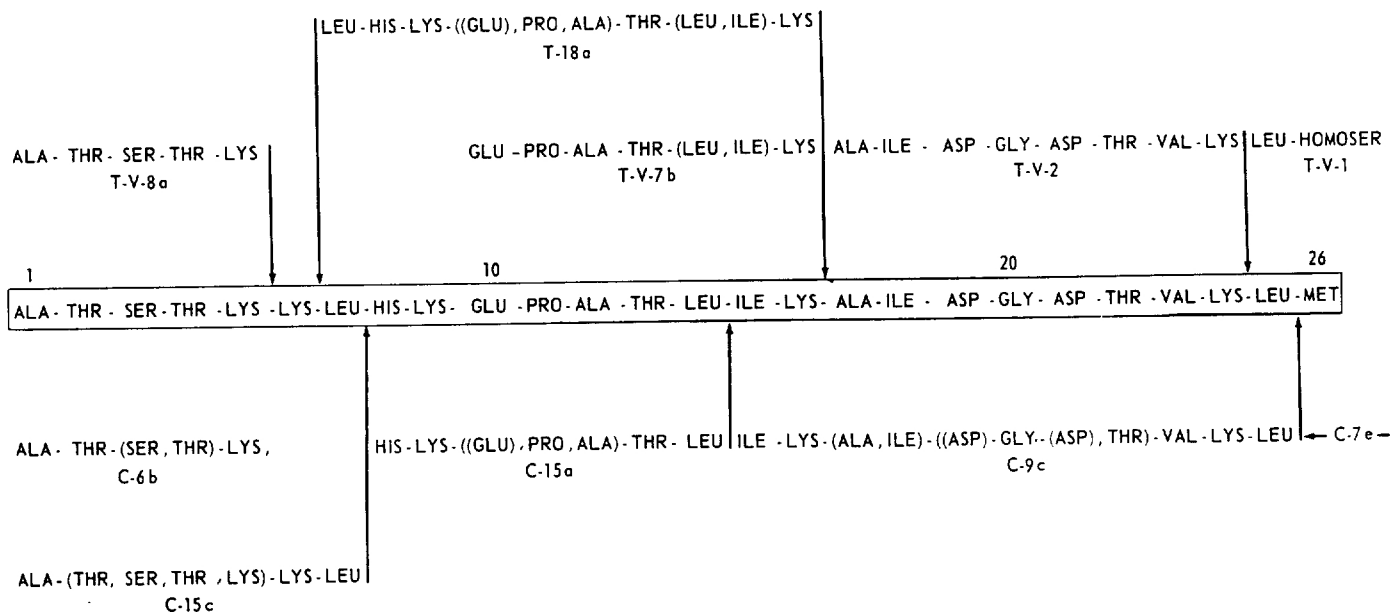


FIG. 1. Diagram of the sequence of cyanogen bromide Fragment A. The deduced sequence is shown in the enclosed middle line. Tryptic and chymotryptic fragments are shown above and below the deduced sequence, respectively. The vertical arrows above and below the sequence indicate the bonds cleaved by trypsin and chymotrypsin, respectively.

NH<sub>2</sub>-terminal sequence Leu-His-Lys, may be arranged in relation to Peptide T-V-8a by consideration of the structure of chymotryptic Peptide C-15c. Chymotryptic Peptide C-6b was identical with tryptic Peptide T-V-8a as judged by the amino acid sequences (2). The reason for the unusual cleavage produced during chymotryptic digestion is unknown.

Chymotryptic Peptide C-15a lacks the COOH terminus of tryptic Peptide T-18a, -Ile-Lys, which is the NH<sub>2</sub>-terminal part of Peptide C-9c as described above. Thus Peptide T-V-7b is connected to Peptide T-V-2, and the COOH-terminal sequence of Peptide T-V-7b is deduced to be -Leu-Ile-Lys. The COOH-terminal leucine residue of Peptide C-9c is assigned as the NH<sub>2</sub> terminus of Peptide T-V-1.

In Fig. 1, the tryptic fragment (Lys, Leu, His, Lys) is indicated as missing. Although adding only tentative evidence, the rather small chromatographic fraction, T-V-18, of the tryptic digest of cyanogen bromide Fragment A (see Reference 1) had the qualitative amino acid composition (Lys<sub>2</sub>, His, Leu), and contained NH<sub>2</sub>-terminal lysine upon dinitrophenylation.

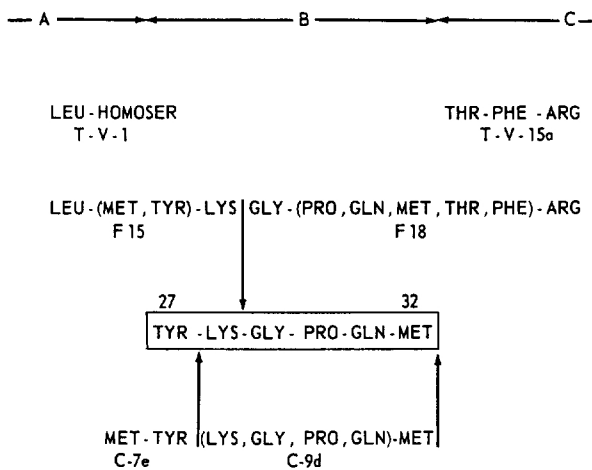


FIG. 2. Diagram of the sequence of cyanogen bromide Fragment B (see legend to Fig. 1). A, B, and C represent cyanogen bromide Fragments A, B, and C.

**Cyanogen Bromide Fragment B**—The partial sequence, Tyr-Lys-Gly ((Glu), Pro) homoserine, of Fragment B was deduced by the arrangement of tryptic Peptides F15 and F18, both of which contain a methionine residue (1) (Fig. 2). The NH<sub>2</sub>-terminal residue was shown to be tyrosine by dinitrophenylation (1).

Edman degradation indicated the NH<sub>2</sub>-terminal sequence to be Tyr-Lys-Gly. Carboxypeptidase A digestion (24 hours, 18% yield) released only homoserine. The electrophoretic mobility of Fragment B at pH 6.5 was consistent with amidation of the glutamic acid residue. The liberation of homoserine by carboxypeptidase A indicated the presence of penultimate glutamic acid or glutamine on the basis of the specificity of carboxypeptidase A, which would not hydrolyze a COOH-terminal bond involving proline (10).

Chymotryptic Peptide C-9d was assigned to Fragment B on the basis of its amino acid composition, and this supported (2) the amidation of glutamic acid and the location of the proline residue (Fig. 2). Another chymotryptic peptide, C-7e, is composed of the carboxyl- and amino-terminal residues of Fragments A and B, respectively (Fig. 2).

**Cyanogen Bromide Fragment C**—Tryptic peptides assigned to this fragment (1, 2) were arranged in the linear order shown in Fig. 3, as follows. Since the NH<sub>2</sub>-terminal residue of this fragment was threonine (1), Peptide T-V-15a occupies the NH<sub>2</sub>-terminal position (1). Peptide T-V-10, containing homoserine, forms the COOH terminus of Fragment C. Tryptic fragments of chymotryptic Peptides C-18a and C-20a had amino acid compositions identical with those of most of the tryptic peptides assigned to Fragment C (1, 2). These fragments could be arranged in order as follows. Peptide C-20a had the same amino acid composition as Peptide C-18a, except that glycine, proline, glutamic acid, 2 alanine residues, serine, and phenylalanine were absent from Peptide C-20a. Since a tryptic fragment (C-18a-TIV) of Peptide C-18a, containing these amino acids in addition to tyrosine, lacked lysine, Fragment C-18a-TIV may be placed at the COOH terminus of Peptide C-18a. Fragment C-18a-TI (C-20a-TI), which lacked the NH<sub>2</sub>-terminal leucine residues of Peptide T-V-5b, is the NH<sub>2</sub> terminus of Peptide C-18a (C-20a). Fragment C-18a-TII (C-20a-TII) was connected to Fragment

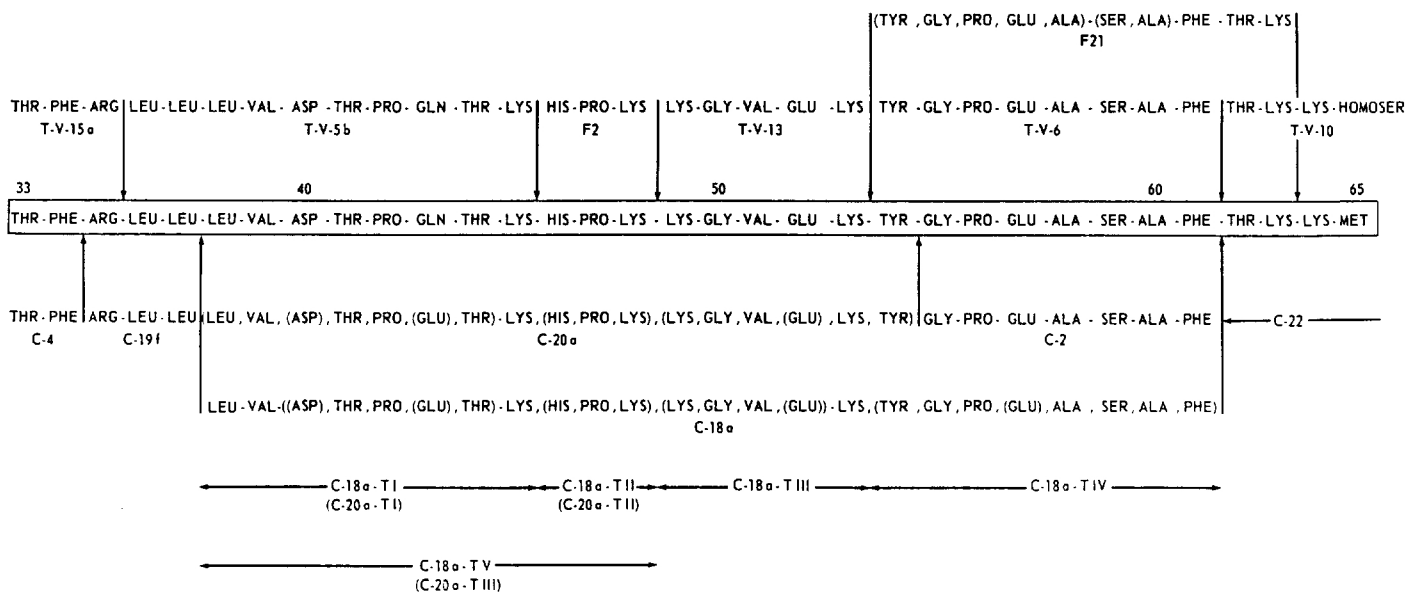


FIG. 3. Diagram of the sequence of cyanogen bromide Fragment C (see legend to Fig. 1)

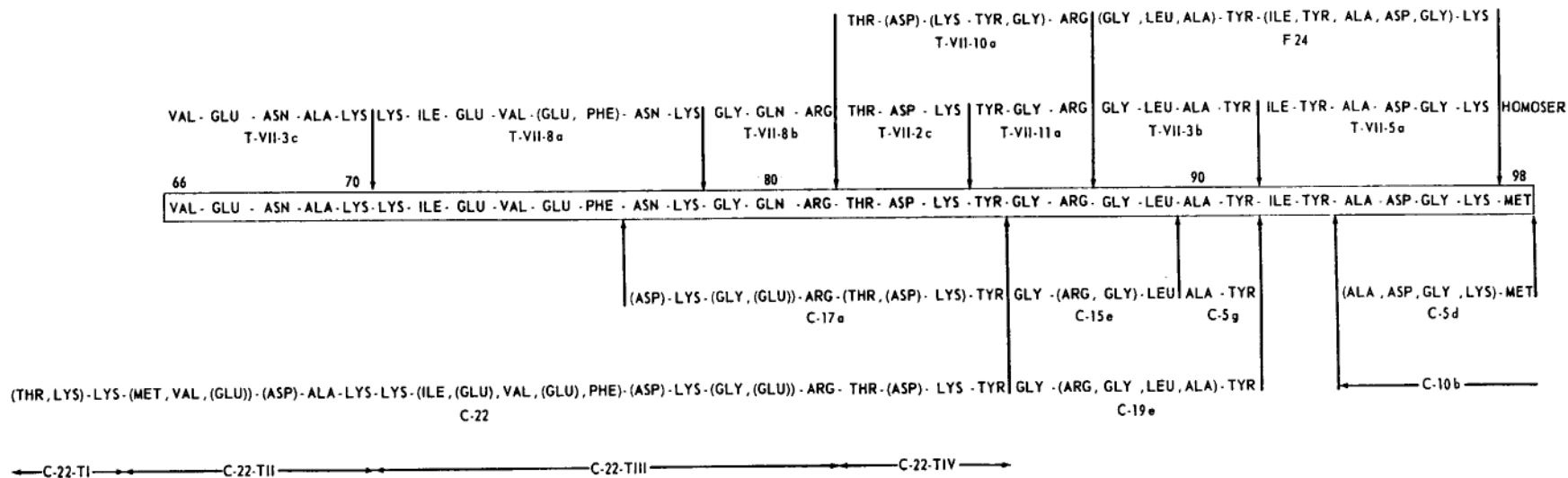


FIG. 4. Diagram of the sequence of cyanogen bromide fragment D (see legend to Fig. 1). Subfragments of chymotryptic peptide C-22 are indicated by horizontal arrows.

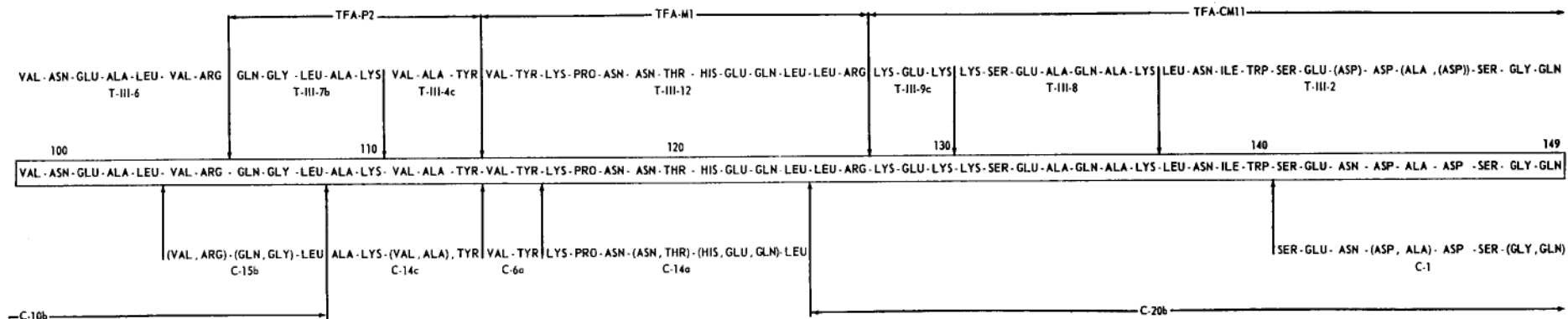


FIG. 5. Diagram of the sequence of cyanogen bromide Fragment E (see legend to Fig. 1). Peptide T-III-12 is described as T-III-12a in the text.

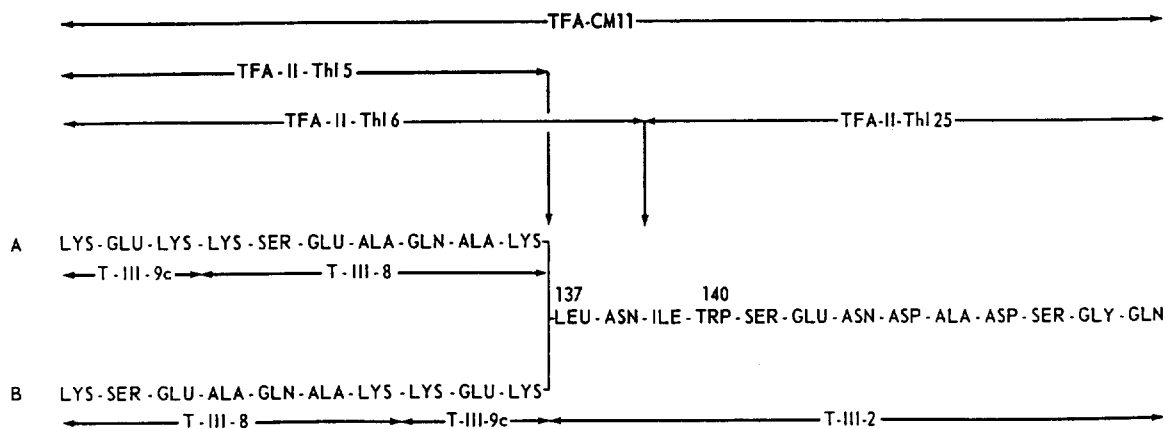


FIG. 6. Diagram of the linear order of Peptides T-III-9c and T-III-8. The bonds cleaved by thermolysin are indicated by the vertical arrows. Other details are given in the text. Fragment TFA-II-Thl-26, which was obtained by the same procedure as that used to prepare Fragments TFA-II-Thl-5, 6 and 25, appeared to be identical with Peptide T-III-2 on the basis of qualitative amino acid composition and position on two-dimensional peptide maps.

TABLE III

*Thermolysin fragments of Peptide TFA-CM11*

The mixture of tryptic fragments obtained from TFA-nuclease was fractionated on Sephadex G-25. The fraction containing Peptide TFA-11 was digested with thermolysin for 2 hours. The fragments thus formed were separated by two-dimensional peptide mapping. An aliquot of the eluate obtained from each ninhydrin-positive spot was analyzed for amino acid composition, and from these results Fragments TFA-II-Thl5, Thl6, and Thl25 were shown to be derived from TFA-11.

Amino acid	TFA-II-Thl-5	TFA-II-Thl-6	TFA-II-Thl-25
Lysine.....	0.016(4) <sup>a</sup>	0.024(4)	
Aspartic acid.....		0.006(1)	0.068(3)
Serine.....	0.005(1)	0.005(1)	0.035(2)
Glutamic acid.....	0.014(3)	0.022(3)	0.050(2)
Glycine.....	0.005(0) <sup>b</sup>	0.004(0) <sup>b</sup>	0.029(1)
Alanine.....	0.011(2)	0.013(2)	0.026(1)
Isoleucine.....			0.015(1) <sup>c</sup>
Leucine.....		0.006(1)	
Tryptophan.....			(1) <sup>c</sup>

<sup>a</sup> Partial destruction by ninhydrin staining.

<sup>b</sup> This amount of glycine was often found as a contaminant in samples eluted from paper.

<sup>c</sup> Determined by the amino acid analysis of a digest by leucine aminopeptidase.

C-18a-TI (C-20a-TI) by Fragment C-18a-TV (C-20a-TIII). Thus the corresponding Peptides T-V-5b, F2, T-V-13, and T-V-6 were placed in order and tyrosine was deduced to be the COOH terminus of Peptide C-20a, consistent with the specificity of chymotrypsin. Chymotryptic Peptide C-19f connected Peptides T-V-15a and T-V-5b. Tryptic Peptide F21 gave the connection between Peptides T-V-6 and T-V-10.

*Cyanogen Bromide Fragment D*—Fig. 4 shows the relations of tryptic and chymotryptic peptides assigned to Fragment D. Digestion of chymotryptic Peptide C-22 with trypsin produced many of the originally isolated tryptic peptides. The amino acid analyses and end groups of these fragments of Peptide C-22 permitted the ordering of the tryptic peptides (2). Thus Peptides T-VII-3c, T-VII-8a, T-VII-8b, and T-VII-2c (see Fig. 4) can be placed in order. The NH<sub>2</sub>-terminal part of Peptide C-22

(Thr, Lys, Lys, Met) was found in the COOH-terminal sequence of Fragment C (see Fig. 3), further confirming the juxtaposition of cyanogen bromide Fragments C and D. Another chymotryptic peptide, C-17a, included Peptides T-VII-8b and T-VII-2c and had, in addition, the NH<sub>2</sub>-terminal sequence (Asp)-Lys (2). Since Peptide T-VII-8a is adjacent to Peptide T-VII-8b (as discussed above), the sequence (Asp)-Lys is the COOH terminus of Peptide T-VII-8a, consistent with the known sequence -Asn-Lys (2). The undetermined part of the sequence of Peptide T-VII-8a (Glu, Phe) could be deduced, since the bond involving the amino group of asparagine should be susceptible to chymotrypsin. Thus phenylalanine, rather than glutamic acid, may be placed adjacent to the asparagine residue.

Peptide T-VII-11a was connected to Peptide T-VII-2c by consideration of the COOH terminus of Peptide C-22. This arrangement was confirmed by an overlapping tryptic peptide, T-VII-10a. Chymotryptic Peptide C-19e (2) connected Peptide T-VII-11a to T-VII-3b, consistent with chymotryptic Peptides C-15e and C-5g. A tryptic peptide (TFA-M14),<sup>3</sup> identical with peptide T-VII-3b, was obtained from tryptic digest of TFA-nuclease, together with TFA-M7 (identical with Peptide T-VII-10a) (Table II). These findings are consistent with the indicated positions of the two arginine residues. Tryptic Peptide F<sub>24</sub> included Peptides T-VII-3b and T-VII-5a. Chymotryptic Peptide C-5d lacked only isoleucine and tyrosine from the amino acid composition of Peptide T-VII-5a. A chymotryptic peptide corresponding to residues 92 and 93, Ile-Tyr (Fig. 4), has not been found.

*Cyanogen Bromide Fragment E*—Fig. 5 summarizes the relationships of tryptic and chymotryptic peptides assigned to Fragment E. Since the COOH-terminal residue of Fragment E was glutamine, Peptide T-III-2 may be assigned to the COOH terminus (1, 2). Chymotryptic Peptide C-15b connected Peptide T-III-7b to Peptide T-III-6, which is assigned to the NH<sub>2</sub> terminus of Fragment E (1). Another chymotryptic peptide, C-10b, included the COOH terminus of cyanogen bromide Fragment D, Peptide T-III-6, and the NH<sub>2</sub> terminus of Peptide T-III-7b, further confirming this arrangement. Chymotryptic Peptide C-5e (2) included residues 94 to 103 (not shown in Fig. 5). Chymotryptic Peptide C-14c connected

<sup>3</sup> Gly, 0.011; Ala, 0.018; Leu, 0.011; Tyr, 0.008.

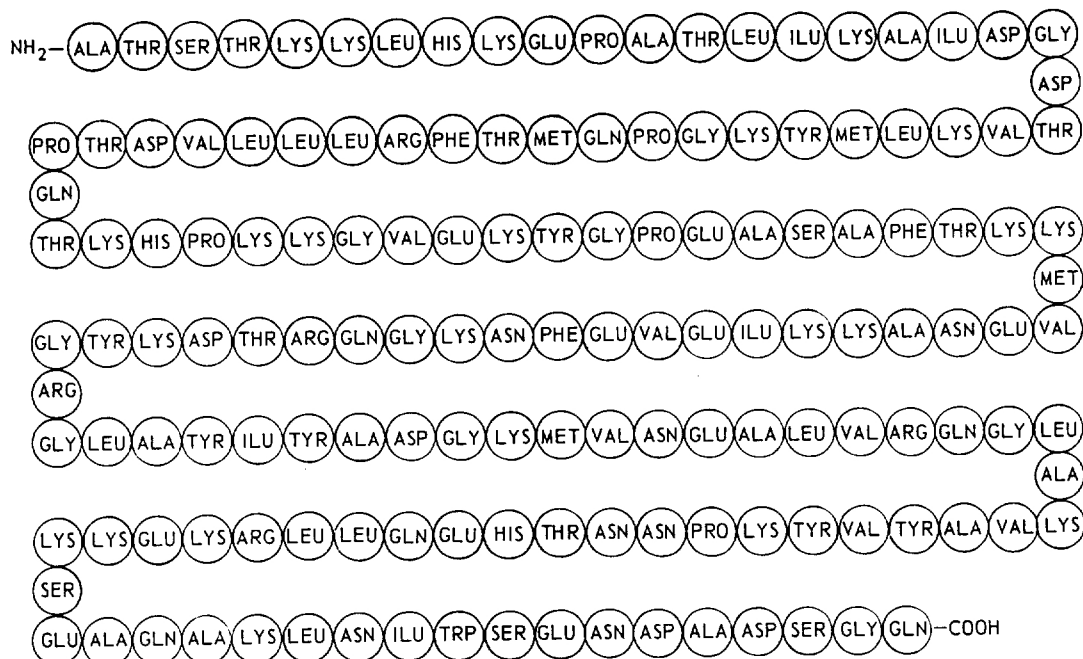


FIG. 7. Proposed amino acid sequence of nuclease V-8

TABLE IV

Amino acid composition of cyanogen bromide fragments of nuclease V8 calculated from sequences

The amino acid compositions reported previously (1) are shown in parentheses.

Amino acid	A	B	C	D	E	Sum	Acid hydroly- sate of nuclease
Lysine.....	5(4-5)	1(1)	6(5-6)	5(4-5)	6(5)	23	(21.4)
Histidine.....	1(1)	0(0)	1(1)	0(0)	1(1)	3	(3.0)
Arginine.....	0(0)	0(0)	1(1)	2(1-2)	2(2)	5	(4.8)
Aspartic acid.....	2(2)	0(0)	1(1)	2(3-4)	2(6-7)	7	(14.6)
Asparagine.....	0(0)	0(0)	0(0)	2(3-4)	5(6-7)	7	
Threonine.....	4(3-4)	0(0)	4(3)	1(1)	1(1)	10	(9.6)
Serine.....	1(1)	0(0)	1(1)	0(0)	3(2-3)	5	(5.1)
Glutamic acid.....	1(1)	0(0)	2(3)	3(4)	5(9)	11	(18.3)
Glutamine.....	0(0)	1(1)	1(3)	1(4)	4(9)	7	
Proline.....	1(1)	1(1)	3(3)	0(0)	1(1)	6	(5.1)
Glycine.....	1(1)	1(1)	2(2)	4(4)	2(2)	10	(7.9)
Alanine.....	3(2-3)	0(0)	2(2)	3(3)	6(6)	14	(14.4)
Half-cystine.....	0(0)	0(0)	0(0)	0(0)	0(0)	0	(0.0)
Valine.....	1(1)	0(0)	2(2)	2(2)	4(3)	9	(9.2)
Methionine.....	1(1) <sup>a</sup>	1(1) <sup>a</sup>	1(1) <sup>a</sup>	1(1) <sup>a</sup>	0(0)	4	(3.5)
Isoleucine.....	2(2)	0(0)	0(1)	2(1-2)	1(1)	5	(5.0)
Leucine.....	3(3)	0(0)	3(2-3)	1(1)	5(5)	12	(11.6)
Tyrosine.....	0(0)	1(1)	1(1)	3(3)	2(2)	7	(6.6)
Phenylalanine.....	0(0)	0(0)	2(1-2)	1(1)	0(0)	3	(3.0)
Tryptophan.....	0(0)	0(0)	0(0)	0(0)	1(1)	1	(1) <sup>b</sup>
Total.....	26	6	33	33	51	149	
Molecular weight.....						16,807 <sup>c</sup>	

<sup>a</sup> Determined as homoserine (1).<sup>b</sup> See Reference 1.<sup>c</sup> On the basis of this molecular weight, the absorbance at 280 m $\mu$  of an aqueous solution containing 1.0 mg of nuclease V8 in 1.0 ml was 0.92 (see References 1 and 11). The concentration of protein was determined by amino acid analysis.

Peptides T-III-7b and T-III-4c. The juxtaposition of Peptides T-III-4c and T-III-12a was discussed in the preceding report (2). Chymotryptic Peptide C-6a should be the NH<sub>2</sub>-terminal part of Peptide T-III-12a, compatible with the above arrangement from the standpoint of the specificity of chymo-

trypsin. A tryptic fragment (TFA-P2) obtained from TFA-nuclease overlapped Peptides T-III-7b and T-III-4c. Another peptide, TFA-M1, was identical with Peptide T-III-12a. The latter peptide furnishes a second example of chymotryptic cleavage between Peptides C-14c and C-6a and further indicates

the intrinsic chymotryptic activity of the trypsin preparation (2). Chymotryptic Peptide C-14a provided confirmation for the distribution of amide groups in Peptide T-III-12a. A large chymotryptic peptide, C-20b, forms the COOH-terminal part of Fragment E, including the COOH terminus of Peptide T-III-12a, together with peptides T-III-9c, T-III-8, and T-III-2. The composition of a tryptic fragment of Peptide C-20b was identical with that of Peptide T-III-2. Peptide TFA-CM11, obtained from TFA-nuclease, appears to overlap Peptides T-III-9c, T-III-8, and T-III-2 (Table II). Since Peptide T-III-2 should be the COOH terminus of Fragment E, only the order of Peptides T-III-9c and T-III-8 remained to be determined. When Peptide TFA-CM11 was digested with thermolysin, which preferentially cleaves bonds involving the NH<sub>2</sub> groups of leucine and isoleucine (9), fragments that overlapped Peptides T-III-9c and T-III-8 were obtained (see Fig. 6 and Table III; Peptides TFA-II-Thl-5 and TFA-II-Thl-6). These fragments were subjected to combined digestion with carboxypeptidases A and B. Lysine, alanine, and glutamine were released with the former peptide, and leucine and asparagine were liberated, in addition to the above amino acids, with the latter peptide. No glutamic acid was released in either incubation.

Peptide TFA-II-Thl-5 (5 hours): Lys, 0.008; Ala, 0.004.

Peptide TFA-II-Thl-6 (8.5 hours): Lys, 0.011; Ser, Gln, and Asn (as serine), 0.010; Ala, 0.006; Leu, 0.008 (Thr, Glu, Gly < 0.003).

These observations suggested the order of Peptides T-III-9c and T-III-8 by the following reason. If Peptide T-III-9c is the NH<sub>2</sub> terminus of Peptide TFA-11, as illustrated in *Sequence A* in Fig. 6, the COOH termini of Fragments TFA-II-Thl-5 and TFA-II-Thl-6 should be -Ala-Gln-Ala-Lys and -Ala-Gln-Ala-Lys-Leu-Asn, respectively. Carboxypeptidases A and B should release these amino acid residues, but not glutamic acid, at least at early stages of digestion. If *Sequence B* in Fig. 6 were the case, carboxypeptidase A and B digestion should release glutamic acid from either Fragment TFA-II-Thl-5 or TFA-II-Thl-6 before the liberation of alanine.

Chymotryptic Peptide C-1 provided information on the distribution of amide groups, not available from studies on Peptide T-III-2. The comparison of the partial sequences of these two peptides permitted construction of the complete sequence shown in Fig. 5.

*Amino Acid Sequence of Nuclease*—The deduction of the amino acid sequence of each cyanogen bromide fragment made it possible to place 149 amino acid residues in order. The

resulting sequence furnishes a working hypothesis for the covalent structure of the nuclease illustrated in Fig. 7. All major fragments obtained from tryptic and chymotryptic digests of the nuclease were found in this structure, as discussed above. The amino acid compositions of the nuclease and of the cyanogen bromide fragments that were reported previously (1) were found to be closely compatible with those calculated from the sequence (Table IV). The difference between the numbers of glycine residues calculated from the reconstructed sequence and from direct amino acid analysis of the nuclease (1) was somewhat large (Table IV). However, the glycine content was well defined in the tryptic peptides and the consistency between the tryptic and chymotryptic peptides that cover the entire sequence was satisfactory.<sup>4</sup>

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<sup>4</sup> Amino acid analyses of 20-hour hydrolysates of several different samples of the nuclease showed 10 moles of glycine per 3 moles of phenylalanine.