

**NUCLEASE-T: AN ACTIVE DERIVATIVE OF  
STAPHYLOCOCCAL NUCLEASE COMPOSED OF TWO  
NONCOVALENTLY BONDED PEPTIDE FRAGMENTS**

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An extracellular nuclease produced by *Staphylococcus aureus*<sup>1-3</sup> has been shown to contain 149 amino acid residues (mol wt, 16,807) and to lack both sulfhydryl groups and disulfide bonds (Fig. 1).<sup>3-5</sup> The enzyme catalyzes the cleavage of both DNA and RNA to yield 3'-nucleotides.<sup>2, 3</sup> The absolute requirement for  $Ca^{++}$ <sup>1, 2, 6</sup> may be explained by the interdependency of  $Ca^{++}$  and nucleotide binding to the protein, and the conformation of the enzyme is stabilized by this reversible ligand interaction.<sup>7, 8</sup> The present communication summarizes experiments on the cleavage of the polypeptide chain by limited digestion with trypsin in the presence of 3',5'-deoxythymidine-diphosphate to yield three peptide fragments: Nase-T-p<sub>1</sub><sup>9</sup> (residues 1-5), Nase-T-p<sub>2</sub> (residues 6-49), and Nase-T-p<sub>3</sub> (residues 50-149). The latter two fragments associate reversibly to form an active complex designated nuclease-T (Nase-T) which possesses approximately 8 per cent of the enzyme activity of the native enzyme.

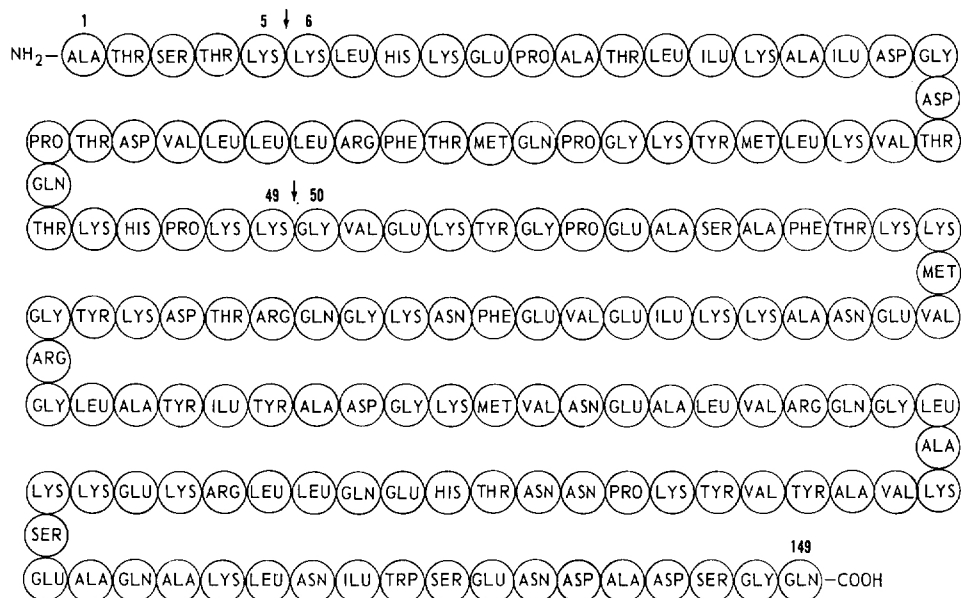


FIG. 1.—The proposed amino acid sequence of staphylococcal nuclease  $V_8$ .<sup>5</sup> The arrows indicate the bonds cleaved by trypsin in the presence of 3',5'-dTDP and  $Ca^{++}$  (see the text). The amino acid sequences of nucleases produced by strains  $V_8$  and Foggi are very similar, if not identical, with respect to  $NH_2$ - and  $COOH$ -termini and peptide maps of the tryptic digests (unpublished results by H. Taniuchi, C. Cusumano, and C. B. Anfinsen). Accordingly, the designations of the spots on the peptide map are the same as described previously.<sup>4</sup>

*Methods.*—Staphylococcal nuclease (Foggi strain), obtained from Worthington Biochemical Corp., was further purified on a phosphocellulose column.<sup>10</sup> Other materials and methods have been reported elsewhere,<sup>3-6</sup> unless otherwise specified. The results of amino acid analyses<sup>11</sup> are presented in  $\mu$ moles.

*Preparation of nuclease-T:* Four ml of a solution at pH 8.0 containing 50 mg nuclease, 5 mg deoxythymidine-3',5'-diphosphate (3',5'-dTDP) (Calbiochem),  $\text{CaCl}_2$  (0.01 *M*),  $\text{NH}_4\text{HCO}_3$  (0.05 *M*), and 0.5 mg trypsin (Worthington, DFP-treated), were incubated at 25° for 2 hr. The reaction was stopped by the addition of 1.5 mg soybean trypsin inhibitor (Worthington, crystallized) and the mixture was lyophilized. The dried material was dissolved in 0.5 ml 0.01 *N* acetic acid-0.1% ammonium acetate and applied to a Sephadex G-50 (medium, Pharmacia) column (1.2  $\times$  22 cm) equilibrated with the same buffer. The column was developed with the buffer at 5-6 ml per hour at 4°. The absorbancies of the fractions were read at 280  $m\mu$ . Two peaks (effluent volumes 10-19 ml and 21-39 ml, respectively) were detected. Fractions comprising the peaks were pooled and lyophilized. The second fraction contained Nase-T-p<sub>1</sub>, free lysine, and 3',5'-dTDP as determined by absorbancy measurements at 280 and 260  $m\mu$  and by peptide mapping (see below). Nase-T-p<sub>1</sub> and lysine were purified by paper electrophoresis.<sup>4</sup> The material in the first peak from the Sephadex column was dissolved in 4 ml 0.3 *M* ammonium acetate, pH 6.0, and applied to a phosphocellulose column (1.2  $\times$  10 cm), equilibrated with the same solution. A gradient elution was performed at 70-80 ml per hour using a Varigrad containing, in the first and second chambers, respectively, 100 ml of 0.3 *M* ammonium acetate, pH 6.0, and 100 ml 1.0 *M* ammonium acetate, pH 8.0. The gradient was followed by additional elution with 50 ml of the second solution. The absorbancies of the fractions, collected every 5 min, were read at 280  $m\mu$ , and each fraction was assayed<sup>7</sup> for enzymic activity. Only one major peak appeared, at the same position as previously observed with native nuclease. Essentially constant specific activities (8-9% of the native value) with both DNA and RNA were observed throughout the fractions constituting this peak. The over-all recovery of Nase-T, based on absorbancy at 280  $m\mu$ , was 45-50%. The active fractions were pooled and lyophilized, dissolved in H<sub>2</sub>O, and lyophilized a second time.

*Separation of Nase-T-p<sub>2</sub> and Nase-T-p<sub>3</sub>:* Nase-T (25 mg), in 1.0 ml 50%  $\text{CH}_3\text{COOH}$  containing phenol red, was applied to a Bio-Gel P-20 (50-150 mesh, Bio-Rad Laboratories) column (2  $\times$  200 cm), equilibrated with 50%  $\text{CH}_3\text{COOH}$  for 48 hr at 4°. The filtration was performed at 5.6 ml per hour at 4° with 50%  $\text{CH}_3\text{COOH}$ . The absorbancies of fractions, collected every 30 min, were read at 280  $m\mu$ . Aliquots of fractions, dissolved in H<sub>2</sub>O after lyophilization, were assayed

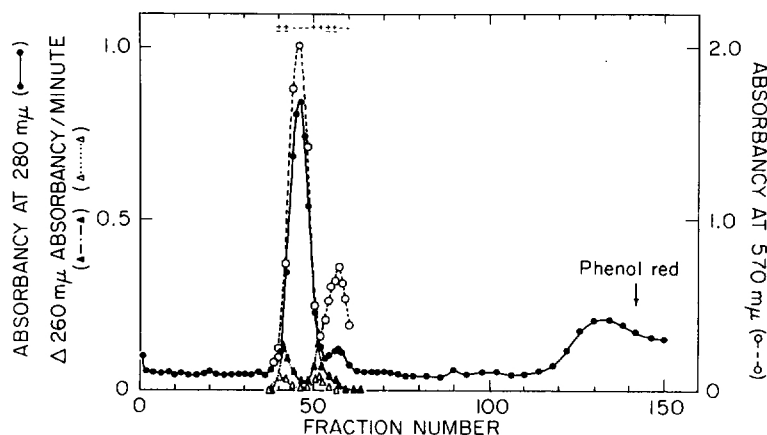


FIG. 2.—Separation of Nase-T-p<sub>2</sub> and p<sub>3</sub>. Details are given in the text. Enzymatic activity was determined against both DNA (▲) and RNA (△). The immunological reactivity with anti-nuclease rabbit serum (presented as +, ±, and -) was carried out with the cooperation of Dr. S. Fuchs.<sup>10</sup> The second activity peak appeared between peptide fractions containing Nase-T-p<sub>2</sub> and p<sub>3</sub>, respectively, and may be due to reassociation of the two peptides. The presence of the two peptide components in this overlapping region was shown by polyacrylamide gel electrophoresis at pH 2.3 and was also consistent with the immunological findings.

for enzymic activities. Total amino acid content was estimated by reaction with ninhydrin after alkaline hydrolysis (Fig. 2). The peak fractions (42-49 and 55-61), constituting Nase-T-p<sub>3</sub> and p<sub>2</sub>, respectively, were pooled and lyophilized.

*Isolation of reconstituted Nase-T (Nase-T')*: A solution at pH 8.0 containing, in 1.5 ml, 0.32  $\mu$ moles Nase-T-p<sub>3</sub>, 1.2  $\mu$ moles Nase-T-p<sub>2</sub>, 1.5 mg 3',5'-dTDP, CaCl<sub>2</sub> (0.01 M) and NH<sub>4</sub>HCO<sub>3</sub> (0.05 M) was incubated with 0.1 mg trypsin at 25° for 3 hr. The subsequent isolation of Nase-T' by chromatography was the same as described above for the isolation of Nase-T. Approximately 0.2  $\mu$ mole of reconstituted Nase-T' was recovered.

*Results*—The native nuclease is highly susceptible to digestion by proteases.<sup>3-5</sup> However, a potent inhibitor, deoxythymidine-3',5'-diphosphate, protected the nuclease from such extensive digestion<sup>12</sup> (Table 1). The progress of limited digestion by trypsin is summarized in Figure 4. A peptide map of the trypsin digest (Fig. 3) showed only two spots and a streak, the latter suggesting the presence of large peptides. One of the two discrete spots was identified as lysine by amino acid analysis (0.3 moles/mole nuclease after 2 hr), and the second as a pentapeptide, Ala-Thr-Ser-Thr-Lys (Nase-T-p<sub>1</sub>). Amino acid analysis of an acid hydrolyzate gave the following results: Lys, 0.03 (1); Thr, 0.047 (2); Ser, 0.022 (1); Ala, 0.021 (1). Leucine amino peptidase digestion for five hours released Ala, 0.006; Thr, 0.004; Ser, Lys, <0.002. Amino acid analysis of a fragment produced by CPase-B digestion and purified by peptide mapping yielded Ala, 0.013 (1); Thr, 0.036 (2);

TABLE 1  
EFFECT OF DEOXYTHYMIDINE-3',5'-DIPHOSPHATE AND Ca<sup>++</sup> ON THE SUSCEPTIBILITY OF NUCLEASE TO PROTEOLYTIC DIGESTION

Proteases	Presence or absence of 3',5'-dTDP and Ca <sup>++</sup>	Per cent of Initial Activity (30 min)	Per cent of Initial Activity (60 min)	Per cent of Initial Activity (120 min)
Trypsin	+	11.7	4.1	6.4
	-	9.8	2.4	0.1
$\alpha$ -Chymotrypsin 4.5% w/w of nuclease	+	75.2	85.8	85.4
	-	38.6	20.2	4.3
Subtilisin 4% w/w of nuclease	+	89.3	90.8	70.2
	-	34.7	22.7	9.1
Pronase 3% w/w of nuclease	+	8.1	5.6	6.7
	-	17.4	4.3	4.9

The components in the incubation (25°) mixtures (0.1 ml total volume) were as described in the section above describing the preparation of Nase-T. Aliquots (5  $\mu$ l) were removed and mixed with 5  $\mu$ l 5 N acetic acid. In the case of trypsin, soybean trypsin inhibitor was added instead of acetic acid. The enzymic activities were examined with suitably diluted aliquots. The presence of both 3',5'-dTDP and Ca<sup>++</sup> was necessary to protect the nuclease from complete digestion by trypsin, but had no effect on the tryptic digestion (1 hr) of apomyoglobin (horse heart), as judged by peptide maps. The relative values with RNA as substrate were parallel to those with DNA.

Ser, 0.018 (1). CPase-A digestion of this fragment for three hours yielded Thr, 67 per cent. Hydrazinolysis of the fragment yielded threonine only.

*Characterization of Nase-T'*: The isolated Nase-T showed a single symmetrical peak upon ultracentrifugation (kindly performed by Dr. E. Steers). In these experiments, samples, dissolved in a solution containing 0.1 M Tris, 0.001 M EDTA, and 0.05 M NaCl at pH 7.5, were centrifuged at 56,000 rpm, 25°. The standard cell contained 1.1 per cent native nuclease and the wedge cell, 0.63 per cent Nase-T. The sedimentation velocity of Nase-T was essentially the same as that of the nuclease.<sup>6</sup> Nase-T was also immunologically identical with the nuclease as examined by the method of Ouchterlony using rabbit antinuclease serum.<sup>10</sup> However, the pattern on polyacrylamide gel electrophoresis was different from that shown by native nuclease (Fig. 5). Two discrete bands with different intensities appeared

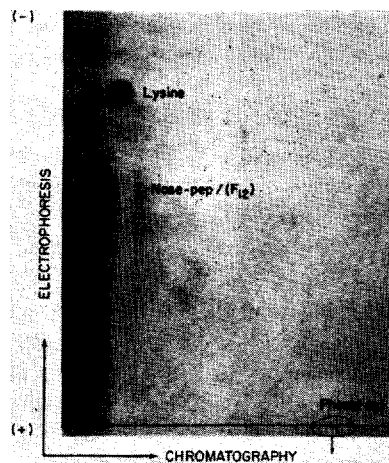


FIG. 3.—Peptide map of a limited tryptic digest (2 hr) of the nuclease (1 mg) in the presence of 3',5'-dTDP and  $\text{Ca}^{++}$ .

on gel electrophoresis at pH 2.3 ("2.3 gel") (Fig. 5). The DNase and RNase activities of Nase-T did not change during incubation for two hours with trypsin in the presence of 3',5'-dTDP and  $\text{Ca}^{++}$ . When both the nuclease and Nase-T (2.5 times excess over the nuclease) were mixed in a solution containing 3',5'-dTDP and  $\text{Ca}^{++}$ , the enzymic activity of the mixture at various times after addition of trypsin was the sum of the control incubations with trypsin of the nuclease and Nase-T alone. In the absence of 3',5'-dTDP and  $\text{Ca}^{++}$ , however, complete digestion occurred.

All major tryptic fragments of nuclease except  $\text{F}_{12}$  (Fig. 3) were present in trypsin digests of Nase-T. Amino acid analysis of Nase-T showed a lower content of threonine and serine than the nuclease (Table 2), and application of Sanger's DNFB method indicated  $\text{NH}_2$ -terminal lysine and glycine. The yield of bis-DNP-lysine was 32 per cent, and of DNP-glycine, much less. No other ether-soluble DNP amino acids were found. Amino end-group analysis of BrCN-treated nuclease and Nase-T produced, qualitatively, DNP-Ala, -Val, -Thr, and -Tyr,<sup>4</sup> and bis-DNP-Lys, DNP-Gly, -Val, -Thr, and -Tyr, respectively. The first stage of Edman degradation of Nase-T produced, qualitatively, lysine and glycine, and the second, leucine and valine. Carboxypeptidase-A released only glutamine (70%, 9 hr) and carboxypeptidase B, only lysine (80%, 9 hr). To prevent digestion by endopeptidase activity contaminating the CPase A and CPase B preparations, 3',5'-dTDP and  $\text{Ca}^{++}$  were added to the mixture. Nuclease, digested under the same conditions, served as the control and yielded COOH-terminal Gln only as reported previously.<sup>4</sup> The enzymic activities did not change significantly during CPase-B digestion (7 hr) in the

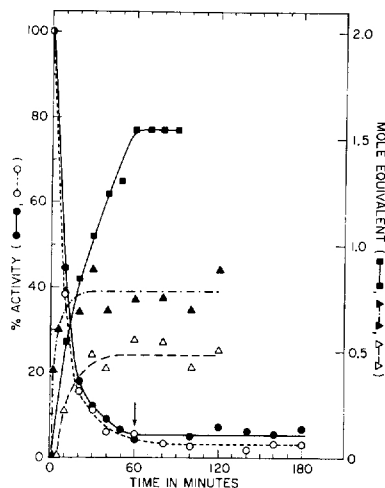


FIG. 4.—The kinetics of limited tryptic digestion of nuclease in the presence of 3',5'-dTDP and  $\text{Ca}^{++}$  (conditions as described in text). The following data are summarized: activities with DNA (●) and RNA (○); determination of alkali (0.1 M NaOH) consumption (■) at pH 8 as followed in the pH stat (radiometer); determination of released Nase-T- $\text{p}_1$  (▲) and lysine (Δ), using purified Nase-T- $\text{p}_1$  as the standard during paper electrophoretic separation at pH 3.6 and estimation using a Beckman analytrol.<sup>4</sup>  $\text{NH}_4\text{HCO}_3$  was omitted in the digestion carried out in the pH stat. Further addition of trypsin after 60 min of incubation (indicated by an arrow) did not cause further change in enzymic activities.

TABLE 2  
AMINO ACID COMPOSITIONS OF NUCLEASE-T<sub>1</sub> AND NUCLEASE-T<sub>1</sub>' AND  
COMPONENT PEPTIDES OF NASE-T

	Nuclease <sup>a</sup>	Nase-T <sub>a</sub>	Nase-T-p <sub>2</sub> <sup>b</sup>	Nase-T-p <sub>3</sub> <sup>b</sup>	Nase-T <sub>1</sub> <sup>c</sup>
Lys	21.4	19.0	7.3 (8)	15.1 (14)	e
His	3.7	3.3	2.7 (2)	1.3 (1)	e
Arg	4.6	4.6	1.3 (1)	4.0 (4)	4.2
Asp	14.2	14.1	2.9 (3)	10.9 (11)	14.1
Thr	9.4	7.1	4.9 (5)	3.1 (3)	7.5
Ser	6.1	4.4	0.2 (0)	3.6 (4)	4.5
Glu	17.5	16.9	2.5 (3)	14.9 (15)	16.0
Pro	6.5	6.0	4.3 (4)	2.2 (2)	6.2
Gly	10.2	9.6	2.0 (2)	8.0 (8)	8.6
Ala	13.1	12.8	2.0 (2)	11.6 (11)	12.7
Cys-	0.0	0.0	0.0 (0)	0.0 (0)	0.0
Val	8.6	9.3	2.0 (2)	6.4 (7)	8.7
Met	3.6	3.3	1.7 (2)	2.1 (2)	3.0
Ile	5.2	4.6	1.9 (2)	2.8 (3)	4.6
Leu	10.3	10.6	6.2 (6)	5.1 (6)	11.2
Tyr	6.9	6.3	1.0 (1)	6.2 (6)	6.1
Phe	(3)	(3)	(1) (1)	(2) (2)	(3)
Trp <sup>d</sup>	1	1	e (0)	e (1)	e

Values are expressed as moles/mole of phenylalanine.<sup>4</sup>

<sup>a</sup> Values are obtained from analyses of aliquots (0.008  $\mu$ mole) hydrolyzed for 26, 52, and 92 hr, respectively.

<sup>b</sup> Theoretical values (from the sequence of nuclease V<sub>8</sub><sup>5</sup>) are given in parentheses. The amounts of Nase-T-p<sub>2</sub> and p<sub>3</sub> analyzed were 0.022 and 0.010  $\mu$ mole, respectively.

<sup>c</sup> Duplicate samples containing 0.003  $\mu$ moles.

<sup>d</sup> Estimated as described in ref. 4.

<sup>e</sup> Not determined.

presence of 3',5'-dTDP and Ca<sup>++</sup>. Hydrazinolysis produced only lysine (24%).

If the amino acid sequences of the nucleases produced by the V<sub>8</sub> and Foggi strains are the same (Fig. 1), these results would suggest the following. Trypsin cleaves bonds between residues 5 and 6 and residues 49 and 50, releasing Nase-T-p<sub>1</sub> (residues 1-5). Nase-T is composed of Nase-T-p<sub>2</sub> (6-49) and Nase-T-p<sub>3</sub> (50-149), which do not dissociate under the conditions of cleavage. The origin of the fractional amount of lysine released during the limited digestion is unknown. Partial removal of residue 49 by trypsin seems a likely explanation. The release of Nase-T-p<sub>1</sub> is complete in 10 minutes while nuclease activity continues to fall for at least 30 minutes (Fig. 4). Therefore, the cleavage of the bond between residues 49 and 50 appears to be responsible for the decrease in activity to 8 per cent of the native level.

The correctness of this interpretation was established by the following observations.

*Nase-T-peptides 2 and 3:* Polyacrylamide electrophoresis of Nase-T on "2.3" gel yielded bands at the positions occupied by separated Nase-T-p<sub>2</sub> and p<sub>3</sub> (Figs. 5 and 6). The NH<sub>2</sub>-terminal sequences of Nase-T-p<sub>2</sub> and p<sub>3</sub> were, qualitatively, Lys-Leu and Gly-Val, respectively, as shown by successive Edman degradation. The COOH-terminal residue of Nase-T-p<sub>2</sub> was lysine by both CPase-B digestion (6 hr, 90%) and hydrazinolysis (19%). No amino acids were released by CPase-A. The COOH-terminal residue of Nase-T-p<sub>3</sub> was glutamine (11%) as shown by CPase-A digestion (6 hr). No free amino acids were found upon hydrazinolysis of Nase-T-p<sub>3</sub>. The amino acid compositions of both peptides accounted for the assigned portions of the sequence of native nuclease (Table 2). The tryptic peptide maps of the fragments did not overlap, and the sum of the components on the maps accounted for all major spots of Nase-T. The locations of major spots on either map were consistent with the peptides derived from the assigned portion of the nuclease sequence.<sup>4, 5, 13</sup> Identification of major spots in digests of Nase-T-p<sub>2</sub> was confirmed by amino acid analyses of eluted samples.<sup>14</sup>

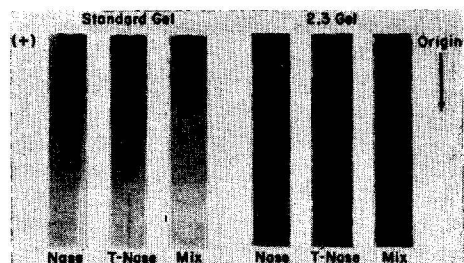


FIG. 5.—Polyacrylamide gel electrophoresis patterns of Nase-T (in this figure and Fig. 6 labeled T-Nase). "Mix" represents the mixture of nuclease and Nase-T. Standard gel, 3 ma, 8 hr; "2.3" gel, 2 ma, 2 hr.

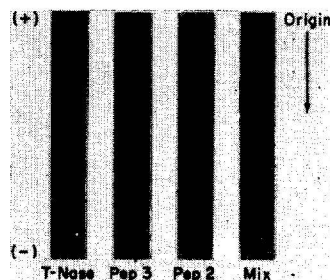


FIG. 6.—Polyacrylamide gel electrophoresis ("2.3" gel, 3 ma, 4 hr) of Nase-T-p<sub>3</sub> (Pep 3), Nase-T-p<sub>2</sub> (Pep 2), and the mixture of p<sub>3</sub> and p<sub>2</sub>.

The recovery of original enzymic activity was less than 10 per cent after Bio-Gel filtration, while the recovery of protein, as judged by measurements of absorbancy at 280 m $\mu$ , was 64 per cent (Fig. 2). Studies of regeneration of activity upon mixing separated Nase-T-p<sub>2</sub> and p<sub>3</sub> (Fig. 7) indicate that neither of the component peptides of Nase-T exhibits enzymic activity alone.

*Properties of Nase-T:* The relative instability of Nase-T to heat, compared with nuclease, was indicated by measurement of the temperature dependence of the reduced mean residue rotation at 233 m $\mu$  (Fig. 8), and by Arrhenius plots of the enzymic activities (Fig. 9). Addition of 3',5'-dTDP and Ca<sup>++</sup> stabilized Nase-T to a greater extent than native nuclease. The optimal concentrations of Ca<sup>++</sup> in the activity assays of Nase-T and nuclease are the same (10<sup>-2</sup> M).

The levels of enzymic activities of Nase-T respond to increasing concentrations of DNA and RNA in a manner similar to native nuclease.<sup>7</sup>

*Reconstituted Nase-T (Nase-T')*: When Nase-T-p<sub>3</sub> was mixed with a three to fourfold excess of Nase-T-p<sub>2</sub>, the regenerated enzymic activities against DNA and RNA were not affected by two hours of incubation with trypsin in the presence of 3',5'-dTDP and Ca<sup>++</sup>. The peptide map of the mixture showed a streak, as shown in Figure 3, and the small tryptic peptides produced were derived only from Nase-T-p<sub>2</sub>. The isolated Nase-T' was identical with the original Nase-T by several

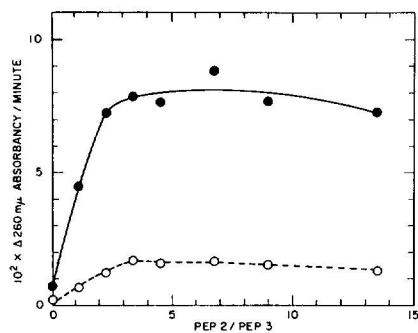
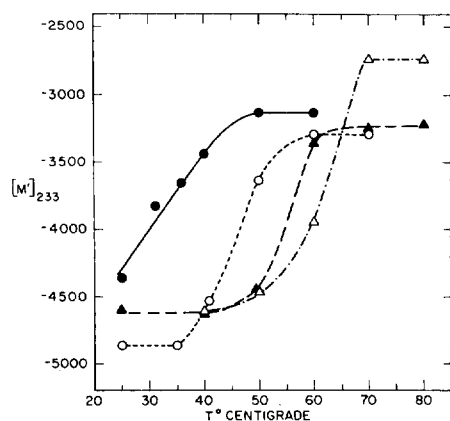
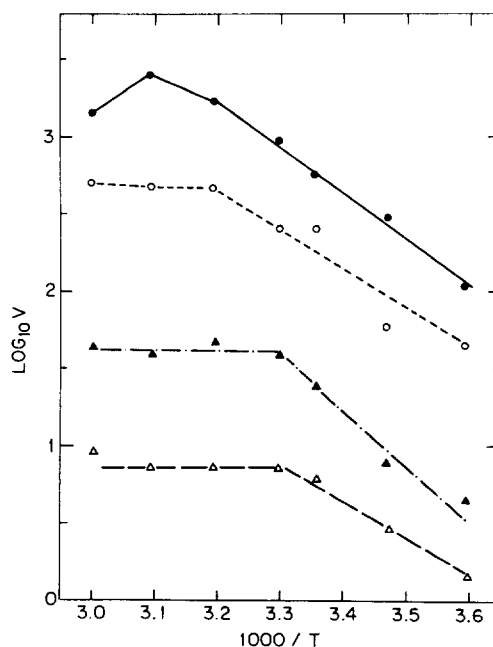


FIG. 7.—Enzymic activity as a function of the ratio of Nase-T-p<sub>2</sub> to Nase-T-p<sub>3</sub> using DNA (●) and RNA (○) as substrates. The concentration of Nase-T-p<sub>3</sub> was constant ( $4.8 \times 10^{-5}$   $\mu$ mole/ml as determined by the amino acid analysis) in all assays. The aqueous solutions of both peptides were mixed before the assay. The regenerated maximum specific activities were  $1.8 \times 10^3$  and  $0.38 \times 10^3$  units/ $\mu$ mole Nase-T-p<sub>3</sub> with DNA and RNA, respectively. (Native nuclease exhibits approximately  $2 \times 10^4$  and  $0.5 \times 10^4$  units/ $\mu$ mole with DNA and RNA, respectively.) The results shown have been corrected for slight endogenous activity in the Nase-T-p<sub>2</sub> preparation employed (accounting for about 5% when maximum activity was obtained). Neither Nase-T-p<sub>2</sub> nor p<sub>3</sub> enhanced the activities of Nase-T or nuclease when added alone at concentrations of  $4 \times 10^{-4}$  and  $8 \times 10^{-5}$   $\mu$ mole/ml, respectively.



(Above) FIG. 8.—Temperature-dependence of reduced mean residue rotations at 233  $m\mu$ ,  $[M]_{233}$ , in 0.05  $M$  Tris, pH 7.0, 0.1  $M$  NaCl of Nase-T (●), Nase-T with 3',5'-dTDP and  $Ca^{++}$  (○), nuclease (▲), and nuclease with 3',5'-dTDP and  $Ca^{++}$  (Δ). 3',5'-dTDP was added in fourfold excess over protein;  $Ca^{++}$ ,  $10^{-2} M$ . The concentration of protein was 0.2 mg per ml or less.



(Right) FIG. 9.—Arrhenius plot of the activities of nuclease with DNA (●), nuclease with RNA (○), Nase-T with DNA (▲), and Nase-T with RNA (Δ). The assays were carried out at 5°, 15°, 25°, 30°, 40°, 50°, and 60°.  $T$ , absolute temperature;  $v$ , specific activity.<sup>7</sup> The concentrations of nuclease and Nase-T were 0.22 and 10.1  $\mu g$  per ml, respectively, for the assays at 5° and 15°. At 25° to 60°, concentrations were 0.065 and 1.0  $\mu g$  per ml, respectively. Temperature was maintained with a Haake (type F) bath for temperatures above 25° and a Formatemp, Jr. (Forma Scientific, Inc. model 2095) bath below 25°. Reaction mixtures were equilibrated for 10–15 min before addition of enzyme.

criteria, including the chromatographic pattern on phosphocellulose, the specific activities with DNA and RNA, sedimentation velocity,<sup>15</sup> amino acid composition (Table 2), electrophoresis on "2.3 gel," and the heat transition of the reduced mean residue rotation at 233  $m\mu$ .<sup>16</sup>

*Discussion.*—F. M. Richards<sup>17, 18</sup> demonstrated ten years ago that bovine pancreatic ribonuclease could be converted by limited digestion with subtilisin to an active derivative consisting of two fragments that were held together by noncovalent interactions. The experimental findings on staphylococcal nuclease described in this paper provide a second example of such a two-component, enzymically active derivative. As in the case of RNase-S, Nase-T affords an excellent test object for direct examination of relationships between structure and function and of the nature of the side-chain interactions underlying the formation and stabilization of three-dimensional structure. Nase-T, devoid of disulfide bond stabilization, offers a particularly striking example of the spontaneous formation of tertiary structure on the basis of the genetic information in the primary sequence of the polypeptide chain. Crystallographic experiments on the three-dimensional structures of nuclease and Nase-T, in collaboration with Drs. Cotton, Hazen, and Richardson<sup>19</sup> of the Massachusetts Institute of Technology, and efforts in this laboratory to synthesize part or all of the sequence of nuclease and Nase-T are now in progress.

We wish to acknowledge the excellent technical assistance of Mr. Clifford Lee in amino acid analyses, and the kindness of Dr. Robert Resnik for use of the Cary model 60 spectropolarimeter.

<sup>1</sup> Cunningham, L., B. W. Catlin, and M. J. Privat de Garilhe, *J. Am. Chem. Soc.*, **78**, 4642 (1956).

<sup>2</sup> Alexander, M., L. A. Heppel, and J. J. Hurwitz, *J. Biol. Chem.*, **216**, 3014 (1961).

<sup>3</sup> Anfinsen, C. B., M. Rumley, and H. Taniuchi, *Acta Chem. Scand.*, **17**, 270 (1963).

<sup>4</sup> Taniuchi, H., and C. B. Anfinsen, *J. Biol. Chem.*, **241**, 4366 (1966).

<sup>5</sup> Taniuchi, H., C. B. Anfinsen, and A. Sodja, *J. Biol. Chem.*, in press.

<sup>6</sup> Heins, J. N., J. R. Suriano, H. Taniuchi, and C. B. Anfinsen, *J. Biol. Chem.*, **242**, 1016 (1967).

<sup>7</sup> Cuatrecasas, P., S. Fuchs, and C. B. Anfinsen, *J. Biol. Chem.*, **242**, 1541 (1967).

<sup>8</sup> *Ibid.*, in press.

<sup>9</sup> Abbreviations used are: Nuclease-T, Nase-T; dinitrophenyl, DNP; nuclease-T-peptides 1, 2, and 3, Nase-T-p<sub>1</sub>, p<sub>2</sub>, and p<sub>3</sub>, respectively; carboxypeptidase A and B, CPase-A and -B; deoxythymidine-3',5' diphosphate, 3',5'-dTDP; reconstituted nuclease-T, Nase-T'; EDTA, ethylenediaminetetraacetate.

<sup>10</sup> Fuchs, S., P. Cuatrecasas, and C. B. Anfinsen, *J. Biol. Chem.*, in press.

<sup>11</sup> Spackman, D. H., S. Moore, and W. H. Stein, *Anal. Chem.*, **30**, 1190 (1958).

<sup>12</sup> This possibility was suggested by Dr. P. Cuatrecasas on the basis of experiments on stabilization of the nuclease with 3',5'-dTDP and Ca<sup>++</sup>.

<sup>13</sup> The addition of 3',5'-dTDP and Ca<sup>++</sup> did not protect either peptide, incubated separately, from complete digestion by trypsin.

<sup>14</sup> The following components were analyzed: residues 7-9, 10-16, 17-24, 25-28; 29-32, 33-35, 36-45, and 46-48 (see refs. 4 and 5).

<sup>15</sup> Standard cell, Nase-T, 0.4%; wedge cell, Nase-T', 0.17%; buffer, 0.05 M Tris, pH 7.0, 0.1 M NaCl; 56,000 rpm, 25°. Nase-T-p<sub>3</sub> had a lower S value than Nase-T under the same conditions; standard cell, Nase-T, 0.74%, 2.06S (uncorrected); wedge cell, Nase-T-p<sub>3</sub>, 0.5%, 1.55S (uncorrected).

<sup>16</sup> No changes were observed in the rotation at 233 m $\mu$  ( $\Delta[m'] < 190^\circ$ ) during temperature increase from 25° to 70° with either Nase-T-p<sub>2</sub> or p<sub>3</sub>. However, reconstituted Nase-T' showed the heat-dependent change in optical rotation at 233 m $\mu$  ( $\Delta[m'] = 1,000^\circ$ ) with the mid-point of the transition similar to that of Nase-T (Fig. 8), indicating the restoration of the secondary structure upon reconstitution.

<sup>17</sup> Richards, F. M., these PROCEEDINGS, **44**, 162 (1958).

<sup>18</sup> Richards, F. M., and R. J. Vithayathil, *J. Biol. Chem.*, **234**, 1459 (1959).

<sup>19</sup> Cotton, F. A., E. E. Hazen, Jr., and D. C. Richardson, *J. Biol. Chem.*, **241**, 4389 (1966).