The Amino Acid Sequence of T4 Phage Lysozyme

I. Tryptic Digestion*

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SUMMARY

A total of 38 peptides have been isolated from a trypsin digest of T4 phage lysozyme by the methods of column and paper chromatography and paper electrophoresis. A determination of the amino acid sequence of 26 of these peptides accounts for a total of 164 amino acid residues in the enzyme. Utilizing information obtained from the other incompletely digested peptides, i.e. those which contain two tryptic peptides or more, it has been possible to reassemble the data to represent 20 unique fragments of this enzyme.

T4 phage lysozyme is an enzyme which is produced under the control of phage DNA in Escherichia coli cells after infection with bacteriophage T4.

This enzyme has been shown to be a muramidase-like egg white lysozyme (1), and its primary structure has been reported in preliminary fashion (2, 3). Moreover, lysozymes have been isolated from various mutant strains carrying double frame shift mutations (4-12) or amber mutations (13-16) in the lysozyme locus of the phage genome, and several features of genetic information such as codon sequences utilized in vivo (4-16) and reading direction of mRNA (17) have been revealed from the structural studies of the mutant lysozymes.

The present paper deals with tryptic digestion of T4 phage lysozyme, separation of tryptic peptides, and determination of their amino acid sequences. Twenty-six nonoverlapping peptides, seven overlapping peptides, and five peptides which seem to be obtained by chymotryptic-like action of the trypsin preparation have been recovered. These peptides account for a total of 164 amino acid residues in T4 phage lysozyme. Utilizing information obtained from the incompletely digested peptides, i.e. those which contain two tryptic peptides or more, it has been possible to assemble the data to represent 20 unique fragments of this enzyme.

* This work was supported by grants from Japanese Ministry of Education, the Jane Coffin Childs Memorial Fund for Medical Research, and the National Institutes of Health (Grant GM-10982).
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tryptic digestion, peptic digestion, and dilute acid hydrolysis of T4 phage lysozyme are reported. From these data the complete amino acid sequence of the enzyme has been deduced.

MATERIALS AND METHODS

T4 Phage Lysozyme

The lysozyme was purified from the lysate of E. coli B/I infected with bacteriophage T4 agg e as described previously (1).

Tryptic Digestion

Digestion of T4 phage lysozyme was carried out with 2% by weight of trypsin at 37° and the pH was maintained at 7.4 by the addition of 0.2 N NaOH with an autotitrator. When the digestion had reached apparent completion (1 to 1.5 hours), another aliquot of trypsin (1% by weight of T4 phage lysozyme) was added and the digestion was continued for 2 to 4 hours more. The reaction was stopped by lowering the pH to 3 by the addition of formic acid, and the precipitate formed was collected by centrifugation. It is designated as the core peptide. The soluble portion of the digest was lyophilized.

In earlier work, trypsin (twice crystallized, Worthington) was treated with sufficient diisopropyl fluorophosphate to inhibit about 10% of the tryptic activity. However, since chymotryptic activity in the trypsin preparation was not completely eliminated by diisopropyl fluorophosphate treatment, trypsin in later work was treated with tosyl-L-phenylalanine chloromethyl ketone (21) as follows: 10 mg of trypsin were dissolved in 5 ml of H2O and the pH was adjusted to 7.2 with aqueous ammonia. After the addition of 0.1 ml of TPCK® solution (4 mg per ml of methanol) and 0.01 ml of 1 M CaCl2, the mixture was incubated at 30° for 1 hour. The pH was then lowered to 4 by adding formic acid and the solution was stored at -20°.

In a typical experiment 40 to 100 mg (about 2 to 5 μmoles) of T4 phage lysozyme were used. In some cases, the protein was oxidized with performic acid (22) or carboxymethylated with iodoacetic acid prior to the digestion. Carboxymethylation of the protein was carried out by the method of Crestfield, Moore, and Stein (23) except that the reaction was performed at pH 7.5 to prevent the precipitation of the protein in alkaline pH.

1 The abbreviations used are: TPCK, L-1-tosylamino-L-phenyl-ethyl chloromethyl ketone; PTH-, phenylthiohydantoin derivative of amino acid; DNP-, 2,4-dinitrophenyl-, N.D., not determined; (NH2), amide content; Met(O)2, methionine sulfone; DAH, dilute acid hydrolysis (designated in sequences); Cys(SO3), cysteic acid.
The carboxymethylated protein was freed of excess reagent with a column of Sephadex G-50.

### Column Chromatography of Tryptic Digests

The digests were chromatographed on a Dowex 50-X2 (200 to 400 mesh, pyridine form) column (0.9 × 150 cm). Later it was found that a column 0.9 × 75 cm could be used without any distinct difference from the longer one. The digest was suspended in a small volume of the starting buffer; the pH was adjusted to about 2.5 with formic acid and applied to the column which had been previously equilibrated with the starting buffer. A gradient elution of increasing pH and ionic strength was established with a nine-chamber Varigrad apparatus. The composition of the buffers was as described by Canfield (24). For the longer column, each flask contained 400 ml of buffer; 200 ml of buffer were used in each for the shorter column. The first two chambers contained the starting buffer and the remaining seven contained the buffers as described (24). Final elution was performed with a gradient between 200 ml of the eighth buffer and 200 ml of the ninth buffer. The composition of the ninth buffer was 30% pyridine solution, pH 6.0, adjusted with acetic acid. The elution was carried out at 37° with a flow rate of 30 to 40 ml per hour and fractions of 3 ml (5 ml, in the case of a column 0.9 × 150 cm) were collected. Aliquots from alternate tubes were subjected to alkaline hydrolysis (in 2.5 N NaOH) as described by Hirs, Moore, and Stein (25) and were assayed by the ninhydrin reaction (26).

### Purification of Peptides

After an elution pattern was obtained, each peak fraction was lyophilized and an aliquot from each was used for examination of purity by paper chromatography (1-butanol-acetic acid-water-pyridine, 30:6:20:24, v/v, descending) (27) and high voltage paper electrophoresis (3000 to 4000 volts; 10% acetic acid solution, the pH of which was adjusted to 3.6 with pyridine (28), or 10% pyridine solution, the pH of which was adjusted to 6.5 with acetic acid (28)). Those fractions which were found to have more than one peptide component were further purified by the appropriate method, i.e. either by paper electrophoresis or paper chromatography or a combination of the two if necessary (see Table I). Samples of peptide (0.1 to 0.5 μmole per cm) was loaded on the filter paper (Toyo Roshi Company, Japan, No. 51A) and the conditions for chromatography were standardized by using marker spots of neutral red (migrating 27 cm, with the mobility of peptides expressed as Rf value, i.e. a ratio of mobility of peptide to the mobility of neutral red). Picric acid, which migrates 10 to 15 cm, was used for standardization of electrophoresis. The peptides were located by staining guide strips with 0.5% ninhydrin in acetic acid or with hypochlorite-starch reagent (29), and the peptide bands were eluted with 2 to 5 ml of 50% acetic acid. The eluates were evaporated to dryness over P2O5 and NaOH flakes in a vacuum desiccator.

### Amino Acid Analysis

Appropriate amounts of peptides (about 0.01 μmole) were hydrolyzed at 105–106° in constant boiling HCl for 24 hours in sealed, evacuated tubes. After hydrolysis, the hydrolysate was evaporated to dryness at 80° within 5 min with the use of a rotary evaporator and the residue was dissolved in 1.1 ml of citrate buffer, pH 2.2 (30). For hydrolysis of peptides having bonds more resistant to acid hydrolysis, 6 N HCl containing 50% acetic acid (concentrated HCl-glacial acetic acid, 1:1 by volume) was used instead of 6 N HCl (see details in the explanation for Peptide T24 in the text) (10).

Amide contents of certain peptides were determined as follows. The sample was boiled with 0.5 ml of 0.9 N borate buffer at pH 9.0 for 10 min and dried over H2SO4 in a vacuum desiccator. It was then hydrolyzed as described above. A blank experiment (without sample) was performed in parallel. The amide content was estimated for the amount of ammonia in the sample after a correction for the control value.

Amino acid analysis was carried out with a Yanagimoto amino acid automatic analyser LC-5 (Kyoto, Japan) and a Beckman-Spinco model 120B automatic amino acid analyzer with 5- and 50 cm columns.

### Tryptophan Content

An aliquot from each purified peptide was spotted on a filter paper and the presence of tryptophan was detected with the Biurich reagent (31). When the reaction was positive, tryptophan content was further estimated quantitatively by the method of Spies and Chambers (32).

### Determination of Amino Acid Sequences of Peptides

**Edman Degradation**—An aliquot of the peptide (about 0.1 to 0.2 μmole) was treated according to the method of Konigsberg and Hill (33), modified as follows. The reaction was carried out in 0.5 ml of 50% pyridine solution (unbuffered) with 0.015 ml of phenylisothiocyanate at 37° for 2.5 hours. After the reaction, excess phenylisothiocyanate was extracted twice with 1 ml of cyclohexane and then twice with 1 ml of benzene, and the solution was then completely dried. Cyclization was carried out with 0.2 ml of trifluoroacetic acid at room temperature for 1 hour. After removal of trifluoroacetic acid, 0.4 ml of 0.2 N acetic acid was added and the phenylthiohydantoin derivative of the amino-terminal amino acid was extracted three times with 1 ml of benzene and once with 1 ml of ethyl acetate. Extracts were combined and evaporated to dryness, and the PTH-amino acid was identified by paper chromatography with Solvent F described by Edman and Sjöquist (34). PTH was detected by the iodine azide method (35). An aliquot of the recovered peptide (lacking the amino-terminal amino acid) was then taken for amino acid analysis without additional purification. The remaining portion was dried and subjected to the next cycle of the Edman degradation.

**DNP Method**—The NH2-terminal residue of some peptides was determined by the DNP-method (36).

**Aminopeptidase Digestion**—Pig kidney aminopeptidase M (a gift from Röhm and Haas, Darmstaff) was used to determine amino acid sequences of NH2-terminal portions of peptides (37, 38). Digestion was carried out in 1 ml of 0.067 M phosphate buffer, pH 7.0, at 37° for 5 hours with 10 μg of aminopeptidase M per 0.01 μmole of peptide sample. After the digestion, the pH was lowered with 1 drop of acetic acid and the mixture was directly analyzed with an amino acid analyzer.

**Carboxypeptidase Digestion**—The sample was digested with carboxypeptidase A or B, or both (treated with diisopropyl fluorophosphate, Worthington), in 1 ml of 0.2 M sodium bicarbonate buffer, pH 8.0, containing 0.1 M NaCl at 37° for 3 hours unless otherwise indicated (substrate-enzyme molar ratio, 50:1). Certain peptides which contained acidic amino acid residues were...
Fractions were examined for their purities as described under "Materials and Methods." When the fractions were found to contain more than one peptide, purification of the peptides was performed by paper chromatography or paper electrophoresis (or both).

The amino acid compositions of these peptides are presented in Table I, where the methods used for purification of peptides, approximate yields of peptides, and integer values of each amino acid are also noted. Yields were calculated from the amino acid content of peak fractions.

**Amino Acid Sequences of Tryptic Peptides**

The procedures used for the determination of amino acid sequences of peptides are summarized in the tables. In each table, the amino acid sequence deduced from the present studies is described. General rules used for tables are described in the footnotes to Table I, including notes for abbreviations used. The tryptic peptides are arranged from T1 to T26 in the order of their sequence from the NH₂ to the COOH terminus of the protein. Numbers written after the amino acid sequences indicate sequential positions of the amino acid residues in the protein. The amino acid sequences which have not been determined in the present paper are indicated in parentheses.

The tabular data under the sequence of each peptide refer to the results of amino acid compositions of the intact peptide and of the residue remaining after each step of the Edman degradation. The PTH-amino acids, identified by paper chromatography after extraction, are listed in the column (PTH-). The amino acid that was apparently removed at each step is indicated by **boldface** type. The values less than 0.1 were listed as 0.

### Peptide T1 (1-8): Met-Asn-Ile-Phe-Glu-Met-Leu-Arg (NH₂)

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**Edman degradation**

1. Step 1: 1.0 1.2 0.7 0.8 1.2 1.0 N.D.
2. Step 2: 0.8 0.4 0.7 0.6 1.1 1.0 N.D.
3. Step 3: 0.9 0.4 0.3 1.1 1.2 1.0 N.D.
4. Step 4: 0.9 0.3 0.1 0.3 1.3 1.0 N.D.
5. Step 5: 0.9 0.4 0.2 0.2 0.6 1.0 N.D.
6. Step 6: 0.3 0.2 0.2 0.5 0.9 1.0

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2 Amino acid composition is expressed by molar ratio and, in cases of aminopeptidase and carboxypeptidase digestion, the values listed indicate molar recovery of liberated amino acids. In Edman degradation, the amino acid that was apparently removed at each step is indicated by **boldface** type. The values less than 0.1 were listed as 0.
### Table 1

**Amino acid compositions and purification methods of tryptic peptides of T4 phage lysozyme (in molar ratio)**

Numbers in parentheses indicate integer values of amino acid compositions. Values less than 0.1 were not listed.

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<td>T16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.76</td>
<td>0.98</td>
<td>1.97</td>
<td>2.72</td>
<td>0.75</td>
<td>1.96</td>
<td>1.79</td>
<td>1.10</td>
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<td>1.79</td>
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<td>0.22</td>
<td>23</td>
</tr>
<tr>
<td>T16-1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.99</td>
<td>1.11</td>
<td>2.30</td>
<td>2.04</td>
<td>1.17</td>
<td>1.30</td>
<td>1.11</td>
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<tr>
<td>T16&lt;sup&gt;H&lt;/sup&gt;,&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.96</td>
<td>0.77</td>
<td>0.85</td>
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<tr>
<td>T19</td>
<td>2.01</td>
<td>1.23</td>
<td>2.91</td>
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<tr>
<td>T20</td>
<td></td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T21</td>
<td>1.94</td>
<td>0.95</td>
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<td>0.89</td>
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<td></td>
<td></td>
<td>0.96</td>
<td>1</td>
</tr>
</tbody>
</table>
The amino acid sequence of this peptide was determined by Edman degradation up to the 6th amino acid residue, methionine. The specificity of trypsin requires that the COOH-terminal residue be arginine. The carboxypeptidase (A + B) liberated arginine (0.7), leucine (0.6), and methionine (0.1). This results indicated that COOH-terminal sequence to be \(-\text{Met-Leu-Arg}\). Dilute acid hydrolysis of the peptide gave methionine (0.4) and aspartic acid (1.0), which confirmed the NH\(_2\)-terminal sequence, \(\text{Met-Asn-}\), since only peptide bonds with aspartic acid or asparagine are hydrolyzed by this treatment.

The sequence was further confirmed by chymotryptic fragments of this peptide. After chymotryptic digestion, the following two fragments, T1-C-1 and T1-C-2, were isolated by paper electrophoresis, at pH 3.6. (The yield of the purified peptide is indicated in parentheses.)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Asp</th>
<th>Thr</th>
<th>Ser</th>
<th>Gln</th>
<th>Pro</th>
<th>Gly</th>
<th>Ala</th>
<th>Cys</th>
<th>Val</th>
<th>Met</th>
<th>Leu</th>
<th>Tyr(^a)</th>
<th>Phe</th>
<th>Trp</th>
<th>Tyr</th>
<th>Lys</th>
<th>His</th>
<th>Arg</th>
<th>(NH(_2))</th>
<th>No. of residues</th>
<th>Purification method(^c)</th>
<th>(R_e)</th>
<th>Yield(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T21-1(^e)</td>
<td>1.86</td>
<td>0.96</td>
<td> </td>
<td> </td>
<td>1.00</td>
<td>0.97</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>0.70</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>0.81</td>
<td>3.27</td>
<td>7</td>
<td>PC</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>T22</td>
<td> </td>
<td> </td>
<td>0.83</td>
<td> </td>
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<td> </td>
<td> </td>
<td> </td>
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<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>1.00</td>
<td>(1)</td>
<td>2</td>
<td>PC, PE</td>
<td>0.30</td>
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</tr>
<tr>
<td>T23</td>
<td> </td>
<td> </td>
<td> </td>
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<td> </td>
<td> </td>
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<td>(1)</td>
<td>1</td>
<td>PE</td>
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<tr>
<td>T22-23(^f)</td>
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<td> </td>
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<td>0.95</td>
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<td>0.54</td>
<td> </td>
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<td> </td>
<td> </td>
<td> </td>
<td>0.54</td>
<td>(2)</td>
<td>3</td>
<td>PE</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>T24</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>0.30</td>
<td> </td>
<td> </td>
<td>1.00</td>
<td> </td>
<td> </td>
<td> </td>
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<td> </td>
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<td>(1)</td>
<td>6</td>
<td>PC</td>
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<tr>
<td>T23-24(^g)</td>
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<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>1.00</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
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<td>1.00</td>
<td>(1)</td>
<td>7</td>
<td>PE</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>T25</td>
<td> </td>
<td> </td>
<td>1.00</td>
<td>1.60</td>
<td> </td>
<td> </td>
<td> </td>
<td>0.54</td>
<td> </td>
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<td> </td>
<td>0.83</td>
<td> </td>
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<td> </td>
<td> </td>
<td> </td>
<td>0.33</td>
<td>(1)</td>
<td>8</td>
<td>PC, PE</td>
<td>0.65</td>
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<tr>
<td>T26</td>
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<td>1.00</td>
<td>(2)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>0.98</td>
<td>(1)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
<td>1.03</td>
<td>(1)</td>
<td>2</td>
<td>PC, PE</td>
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</tr>
<tr>
<td>Total(^h)</td>
<td>22</td>
<td>11</td>
<td>6</td>
<td>13</td>
<td>3</td>
<td>11</td>
<td>15</td>
<td>2</td>
<td>9</td>
<td>5</td>
<td>10</td>
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<td>6</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>1</td>
<td>164</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\(^{a}\) Recoveries of Tyr in some peptides were unexpectedly low. The reasons have been discussed in the referenced articles (42).

\(^{b}\) Ratio of the mobility of the peptide to that of neutral red in paper chromatography.

\(^{c}\) Approximate yields were calculated from the amino acid compositions of the pooled fractions based on the amino acids italicized. Also they are expressed as moles per mole (per cent) of the starting protein. Yields of the peptides obtained from the digest of the performic acid-oxidized protein are in parentheses.

\(^{d}\) Chymotryptic fragments of tryptic peptides derived by chymotryptic activity in the trypsin preparation used in an experiment.

\(^{e}\) Overlapping tryptic peptides.

\(^{f}\) Obtained from the digest of the performic acid-oxidized protein. Cysteine content was determined from the amount of cysteic acid.

\(^{g}\) Obtained from the digest of the carboxymethylated protein. Cysteine content was determined from the amount of carboxymethyl cysteine.

\(^{h}\) Total amino acid residues of nonoverlapping peptides. Therefore, the peptides marked \(e\) and \(f\) are not included.

The NH\(_2\)-terminal glutamic acid residue was determined by Edman degradation.

All together, the asparagine residue at the second and the glutamic acid residue at the fifth position were identified as PTH-amino acids and this is also compatible with the amide content (1.2 moles per mole) of this peptide. In tryptic digestion of the performic acid-oxidized protein, the oxidized Peptide T1(0) was eluted together with Peptides T14 and T12 (see Fig. 1). The T1(0) was purified by paper chromatography (1-butanol:2-butanol:2 N pyridine, 3:1:4, upper layer) and the identical sequence was confirmed by the same methods as described above.

In case of the lysozyme in the frame shift mutant strain eJ16edID12, the asparagine residue in the second position has been found to be replaced with a dipeptide, Glu-Tyr (5).
Peptide T2 (9-14): Ile - Asp - Glu - Gly - Leu - Arg (NH₃)

Step 2: 0 0.2 1.0 1.1 1.0 0.9
Step 3: 0 0.3 0.4 1.4 1.0 1.3
Step 4: 0 0.2 0.3 0.3 1.0 0.0
Step 5: N.D. N.D. N.D. N.D. N.D. N.D.

Carboxypeptidase: A + B

PHT-amino acids were identified to be Ile or Leu for Step 1, Asp for Step 2, Glu for Step 3, Gly for Step 4, and Ile or Leu for Step 5, respectively.

Aspartic acid and glutamic acid residues were identified by paper chromatography as PTH-amino acids, and this is compatible with the fact that Peptide T2 was acidic by paper electrophoresis and did not contain amide ammonia.

Peptide T3 (15-16): Leu-Lys—The specificity of trypsin suggests that lysine is the COOH-terminal residue. The NH₂-terminal leucine was confirmed as both DNP-leucine and PTH-leucine.

Peptide T4 (17-18): Ile-Tyr-Lys

Composition 0.8 0.8 1.0 (PTH-)
Edman degradation
Step 1 0 1.0 0.9 Ile
Step 2 0 0 N.D. Tyr
Carboxypeptidase B (1.5 hours)

The NH₂-terminal amino acid was also determined by the DNP-method.

Peptide T5 (20-35): Asp-Thr-Glu-Gly-Tyr-Thr-Ile-Gly-Ile-Gly-His-Leu-Leu-Thr-Lys (Table II)—This sequence was determined by Edman degradation and by chymotryptic digestion of the peptide followed by separation of fragments by paper electrophoresis (pH 3.6). The following three peptides were recovered:

T5-C-1: (Asp, Thr, Glu, Gly, Tyr, Thr)
T5-C-2: Thr-Ile-Gly-(Ile,Gly)-His
T5-C-3: Leu-Leu-Thr-Lys

As for T5-C-1, only amino acid composition was determined because its composition was the same as that of T5-C-2 and T5-C-3, their amino acid sequences were studied as shown below.

Peptide T5-C-2 (26-31): Thr-Ile-Gly-(Ile,Gly)-His

Composition 0.7 2.0 2.0 0.9
Edman degradation
Step 1 0 1.9 2.0 N.D.
Step 2 0 1.4 2.0 N.D.
Step 3 0 1.0 1.2 0.7
Aminopeptidase M 0.8 1.5 1.0 0.5
Carboxypeptidase A 0 0 0 0.3

The presence of aspartic acid and glutamic acid residues in Peptide T5 (but not their amides was also confirmed by the amide content of this peptide, T5 (0.2 mole per mole).

In the case of the lysozyme of the frame shift mutant strain, eJD5/eJD201, the amino acid sequence Glu-Gly-Tyr-Tyr (22-25) has been replaced by Arg-Leu-Leu-His as a result of frame shift mutations (4).

Peptide T5-C-3 (32-35): Leu-Leu-Thr-Lys

Composition 2.0 1.0 1.0 0.4

TABLE II

<table>
<thead>
<tr>
<th>Composition</th>
<th>Asp-Thr-Glu-Gly-Tyr-Thr-Ile-Gly-Ile-Gly-His-Leu-Leu-Thr-Lys (NH₃) (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td></td>
</tr>
<tr>
<td>Step 1</td>
<td>0 2.9 1.0 3.0 1.9 1.8 N.D. 1.9 N.D.</td>
</tr>
<tr>
<td>Step 2</td>
<td>0 1.8 1.1 2.6 1.9 2.0 N.D. 2.3 N.D.</td>
</tr>
<tr>
<td>Step 3</td>
<td>0 2.0 0.4 3.0 2.0 1.4 N.D. 1.8 N.D.</td>
</tr>
<tr>
<td>Step 4</td>
<td>0 1.9 0.2 2.1 2.0 2.2 N.D. 2.0 N.D.</td>
</tr>
<tr>
<td>Step 5</td>
<td>0 0 0 0 0 0.3 0.7 0.0 1.0</td>
</tr>
<tr>
<td>Carboxypeptidase A + B</td>
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</tr>
<tr>
<td>10 min</td>
<td>0 0 0 0 0 0.1 0.2 1.0</td>
</tr>
<tr>
<td>6 hrs</td>
<td>0 0 0 0 0 0.1 0.2 1.0</td>
</tr>
</tbody>
</table>

Chymotryptic digestion

| Asp-Thr-Glu-Gly-Tyr-Thr-Ile-Gly-Ile-Gly-His-Leu-Leu-Thr-Lys (NH₃) |
|-----------------|----------------------------------------------------------------|
| T5-C-1 (62%) (90%) |
| 1.0 0.1 0.3 2.0 |
| T5-C-2 (62%) (90%) |
| 0 0 0 0 0 0.7 2.0 2.0 |
| T5-C-3 (35%) (90%) |
| 0 0 0 0 0 2.0 1.0 1.2 |

* The high Gly value may be caused by contamination from papers used.

Peptide T5-C-1 (62%) 1.2 0.8 1.0 1.4 2.0 0 0 0 0
Peptide T5-C-2 (62%) 0 0 0 0 0 0 0 0
Peptide T5-C-3 (35%) 0 0 0 0 0 0 0 0

As for T5-C-1, only amino acid composition was determined because its composition was the same as that of T5-C-2 and T5-C-3, their amino acid sequences were studied as shown below.

Peptide T5-C-2 (26-31): Thr-Ile-Gly-(Ile,Gly)-His

Composition 0.7 2.0 2.0 0.9
Edman degradation
Step 1 0 1.9 2.0 N.D.
Step 2 0 1.4 2.0 N.D.
Step 3 0 1.0 1.2 0.7
Aminopeptidase M 0.8 1.5 1.0 0.5
Carboxypeptidase A 0 0 0 0.3

Peptide T5-C-3 (32-35): Leu-Leu-Thr-Lys

Composition 2.0 1.0 1.0 0.4

The presence of aspartic acid and glutamic acid residues in Peptide T5 (but not their amides was also confirmed by the amide content of this peptide, T5 (0.2 mole per mole).

In the case of the lysozyme of the frame shift mutant strain, eJD5/eJD201, the amino acid sequence Glu-Gly-Tyr-Tyr (22-25) has been replaced by Arg-Leu-Leu-His as a result of frame shift mutations (4).

Peptide T5-C-1 (62%) 1.2 0.8 1.0 1.4 2.0 0 0 0 0
Peptide T5-C-2 (62%) 0 0 0 0 0 0 0 0
Peptide T5-C-3 (35%) 0 0 0 0 0 2.0 1.0 1.2

As for T5-C-1, only amino acid composition was determined because its composition was the same as that of T5-C-2 and T5-C-3, their amino acid sequences were studied as shown below.

Peptide T5-C-2 (26-31): Thr-Ile-Gly-(Ile,Gly)-His

Composition 0.7 2.0 2.0 0.9
Edman degradation
Step 1 0 1.9 2.0 N.D.
Step 2 0 1.4 2.0 N.D.
Step 3 0 1.0 1.2 0.7
Aminopeptidase M 0.8 1.5 1.0 0.5
Carboxypeptidase A 0 0 0 0.3

Peptide T5-C-3 (32-35): Leu-Leu-Thr-Lys

Composition 2.0 1.0 1.0 0.4

The presence of aspartic acid and glutamic acid residues in Peptide T5 (but not their amides was also confirmed by the amide content of this peptide, T5 (0.2 mole per mole).

In the case of the lysozyme of the frame shift mutant strain, eJD5/eJD201, the amino acid sequence Glu-Gly-Tyr-Tyr (22-25) has been replaced by Arg-Leu-Leu-His as a result of frame shift mutations (4).

Peptide T5-C-1 (62%) 1.2 0.8 1.0 1.4 2.0 0 0 0 0
Peptide T5-C-2 (62%) 0 0 0 0 0 0 0 0
Peptide T5-C-3 (35%) 0 0 0 0 0 2.0 1.0 1.2

* The high Gly value may be caused by contamination from papers used.

Peptide T5-C-1 (62%) 1.2 0.8 1.0 1.4 2.0 0 0 0 0
Peptide T5-C-2 (62%) 0 0 0 0 0 0 0 0
Peptide T5-C-3 (35%) 0 0 0 0 0 2.0 1.0 1.2

* The high Gly value may be caused by contamination from papers used.

Peptide T5-C-1 (62%) 1.2 0.8 1.0 1.4 2.0 0 0 0 0
Peptide T5-C-2 (62%) 0 0 0 0 0 0 0 0
Peptide T5-C-3 (35%) 0 0 0 0 0 2.0 1.0 1.2

* The high Gly value may be caused by contamination from papers used.
TABLE III
Structural studies on Peptide T5-II

<table>
<thead>
<tr>
<th>Composition</th>
<th>Thr-Ile-Gly-Ile-Gly-His-(Leu,Leu,Thr)-Lys (PTH-)</th>
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</thead>
<tbody>
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<td>1.7 1.9 2.1 1.0 2.0 1.0</td>
</tr>
<tr>
<td>Step 1</td>
<td>1.0 1.6 1.9 0.8 2.0 0.7 Thr</td>
</tr>
<tr>
<td>Step 2</td>
<td>1.0 1.0 2.1 N.D. 2.0 N.D. Ile or Leu</td>
</tr>
<tr>
<td>Step 4</td>
<td>0.8 1.1 1.2 N.D. 2.0 N.D. Gly</td>
</tr>
<tr>
<td>Step 5</td>
<td>1.0 0 0.1 N.D. 2.0 N.D. Gly</td>
</tr>
<tr>
<td>Carboxypeptidase A + B</td>
<td>0 0 0 0.5 2.0 1.0</td>
</tr>
</tbody>
</table>

TABLE IV
Structural studies on Peptide T4-6

<table>
<thead>
<tr>
<th>Composition</th>
<th>Ile-Tyr-Lys-(Asx,Thr,Glx,Gly,Tyr,Tyr,Thr,Ile,Gly,Ile,Gly)-His-(Leu,Leu,Thr)-Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td>2.0 2.6 N.D. 1.2 2.8 1.2 5.0 N.D. N.D. Ile or Leu</td>
</tr>
<tr>
<td>Step 2</td>
<td>1.9 1.9 N.D. 1.2 2.9 1.4 3.2 N.D. N.D. Tyr</td>
</tr>
<tr>
<td>Aminopeptidase M</td>
<td>1.0</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>0</td>
</tr>
<tr>
<td>3 hrs</td>
<td>0 0 1.0 0 1.1 0 0 0 1.7</td>
</tr>
<tr>
<td>6 hrs</td>
<td>0 0 1.0 0 0.0 0 0 0 0 4.1 1.7</td>
</tr>
</tbody>
</table>

In earlier work (2), the 2nd amino acid residue from the NH₂ terminus was deduced to have been glutamine, since it was difficult to remove by Edman degradation owing to the formation of pyrroloidone carboxylic acid. However, when the Edman degradation of the second step was performed quickly after cyclization of the first step by the method of Königsberg and Hill, modified as described under "Materials and Methods," the degradation was successfully achieved and the PTH-amino acid of this step was identified as PTH-glutamic acid, not glutamine. Furthermore, the peptide was found to be acidic by electrophoresis at pH 3.6, suggesting that there were 2 acidic amino acid residues in the peptide. This was confirmed by determination of the amide content of the peptide (0.2 mole per mole).

The amino acid sequence of Peptide T7 was further confirmed by fragmentation by dilute acid. An acidic peptide Ser-Glu-Leu (composition 0.8, 1.0, 1.0) was recovered in about 90% from paper electrophoresis at pH 3.6, in addition to each mole of free aspartic acid and lysine. The residual amino acid compositions of the first step of Edman degradation were Ser(0), Glu(1.0), Leu(0.7) and in the second step Ser(0), Glu(0), Leu(0.7). In the second step PTH-glutamic acid was also identified.
Carboxypeptidase A for 3 hours liberated only Leu(0.6) from this peptide. Thus the sequence of this peptide was concluded to be Ser-Glu-Leu [44-46], indicating that it is the NH₂-terminal part of Peptide T7.

**Peptide T8 (40-52):** Ala-Ile-Gly-Arg

**Composition**

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Ile</th>
<th>Gly</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

**Edman degradation**

<table>
<thead>
<tr>
<th>Step</th>
<th>Ala</th>
<th>Ile</th>
<th>Gly</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Carboxypeptidase A + B 0 0 0 0.8

In addition to the above structural studies, NH₂-terminal alanine was also identified by the DNP- method.

**Peptide T9 (53-60):** Asn-Cys-Asn-Gly-Val-Ile-Thr-Lys

<table>
<thead>
<tr>
<th>Edman degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
</tr>
<tr>
<td>Step 2</td>
</tr>
<tr>
<td>Step 3</td>
</tr>
<tr>
<td>Step 4</td>
</tr>
<tr>
<td>Step 5</td>
</tr>
</tbody>
</table>

Carboxypeptidase A + B (6 hours) 0 0 0 0.2 0 0

Dinitrophenylation of this peptide yield DNP-aspartic acid. Absence of amide groups in the peptide was shown both by the identification of PTH-amino acids and by amide analysis (0.1 mole per mole).

**Peptide T9-10 (61-65):** Asp-Glu-Ala-Glu-Lys

<table>
<thead>
<tr>
<th>Edman degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
</tr>
<tr>
<td>Step 2</td>
</tr>
<tr>
<td>Step 3</td>
</tr>
<tr>
<td>Step 4</td>
</tr>
</tbody>
</table>

Carboxypeptidase A + B 0 0 0 0.2 0 0

Dilute acid hydrolysis:

| DAH-1 (42%) | Gly | 3.0 1.0 | 0.1 0.3 0.3 0.9 1.0 |
| DAH-2 (44%) | 0 0 | 0.9 0.3 0.4 0.9 1.0 |

**Table V**

<table>
<thead>
<tr>
<th>Structural studies on Peptide T9</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn-Cys(SO₃)⁻-Asn-Gly-Val-Ile-Thr-Lys (NH₄) (PTH⁻)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Asn</th>
<th>1.9 1.0</th>
<th>1.0 0.6 0.8 1.0 1.0</th>
</tr>
</thead>
</table>

**Table VI**

<table>
<thead>
<tr>
<th>Structural studies on Peptide T9-10</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn-Cys(SO₃)⁻-Asn-Gly-Val-Ile-Thr-Lys-Asp-Glu-(Ala,Glu)-Lys (NH₄) (PTH⁻)</td>
</tr>
</tbody>
</table>

|   | Asn | 3.0 1.0 | 1.1 0.5 0.4 1.0 2.1 2.3 1.1 2.1 |
|---|-----|---------|---------------------|---------------------|

**In the composition Asn is observed as Asp.**
from the digest of the performic acid oxidized protein. The presence of 1 mole each of valine and isoleucine was clearly shown from the result of acid hydrolysis in 6 N HCl containing 50% acetic acid.

The peptide was partially hydrolyzed in 0.03 N HCl for 20 hours. In addition to 1 free mole of cysteic acid and 3 moles of aspartic acid, two peptide fragments were isolated by paper electrophoresis, at pH 3.6.

From a peptide, T9-10-DAH-1, Edman degradation followed by identification of PTH-amino acid resulted in PTH-glutamic acid as the first NH2-terminal amino acid and PTH-valine as the second one. The carboxypeptidase (A + B) liberated lysine (0.7), threonine (0.4), and isoleucine (0.2). These results concluded the structure to be Gly-Val-Ile-Thr-Lys.

From the other peptide T9-10-DAH-2, the first step of Edman degradation resulted in PTH-glutamic acid and the carboxypeptidase (A + B) liberated only 0.4 mole of lysine. The partial sequence was concluded to be Glu-(Ala, Glx)-Lys. From the similarity in the carboxypeptidase reaction on the T9-10 and on T9-10-DAH, the latter peptide is located at the COOH terminus of T9-10. The liberation of cysteic acid and aspartic acid indicated the sequence Asx-Cys-Asx to be present, as in the case of T9. The other aspartic acid is thought to be located between T9-10-DAH-1 and T9-10-DAH-2 from the specificity of the dilute acid hydrolysis, and thus Asx-Cys-Asx-(T9-10-DAH-1)-Asx-(T9-10-DAH-2) is suggested as the sequence of Peptide T9-10. Also, it can be concluded that this peptide results from incomplete tryptic digestion of the protein, and includes both Peptide T9 and Peptide T10.

**Peptide T11 (66-76): Leu-Phe-Asn-Gln-Asp-Val-Asp-Ala-Val-Arg (Table VII)**—The asparagine residue at the third, the glutamine at the fourth, and the aspartic acid residue at the fifth position from the NH2 terminus were deduced from the amino acid composition and by identification of PTH-amino acids after the corresponding steps of Edman degradation. By carboxypeptidase digestion at pH 6, another alanine and aspartic acid were released in addition by digestion at pH 8. This might be explained as follows. The aspartic acid residue is mostly ionized at pH 8 and the ionized β-carboxyl group inhibits the release of not only aspartic acid itself but also of alanine, next to the aspartic acid residue. They might, however, be released at pH 6, since the extent of the ionization of aspartic acid is reduced at this pH. The fact that 3 moles of aspartic acid and 1 mole of glutamic acid and valine were released by dilute acid hydrolysis of the COOH-terminal region was determined by peptic digestion and dilute acid hydrolysis. From the peptic digest, the following two peptides were recovered: T11-P-1: (Leu,Phe,Asx,Glx, Asx,Val,Asx,Ala) and T11-P-2: Ala-Val-Arg. Only composition was studied on T11-P-1. T11-P-2 was composed of Ala(1.0), Val(0.8), and Arg(0.8).

Edman degradation resulted in Ala(0), Val(1.0), Arg(1.0) (83%) after the first step and Ala(0), Val(0), Arg(1.0) (45%) after the second. The PTH-amino acid released in the first step was identified as alanine. After dilute acid hydrolysis of the original Peptide T11, T11-DAH-1 (Ala, Ala, Val, Arg) was recovered. The amino acid sequence of this peptide was deduced to be Ala-Ala-Val-Arg since it contains 1 more alanine residue than T11-P-2 (Ala-Val-Arg).

In the frame shift mutant, eL28eL24, the amino acid sequence Ala-Val-Arg (74-76) has been found to be replaced by Gly-Cys-Cys-Cys (12).

**Peptide T9-10-11 (83-76): Asn-Cys-Asn-(Gly,Val, Ile, Thr, Lys, Asx, Glx, Ala, Glx, Lys, Leu, Phe, Asx, Glx, Asx, Val, Asx, Ala)-Ala-Val-Arg (Table VIII)—This peptide was obtained from the digest of the carboxymethylated protein. From the amino acid composition and the results of Edman degradation up to the third step and carboxypeptidase digestion, it can be concluded that the peptide was obtained as a result of incomplete tryptic digestion of the protein and consists of Peptides T9, T10, and T11 in this order.

**Peptide T12 (77-80): Gly-Ile-Leu-Arg (PTH-)**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leu-Phe-Asn-Gln-Asp-Val-Asp-Ala-Val-Arg (NH2) (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation Step 1</td>
<td>1.0 1.0 1.0 1.0</td>
</tr>
<tr>
<td>Step 2</td>
<td>0.4 1.0 1.0 1.0</td>
</tr>
<tr>
<td>Step 3</td>
<td>0.2 1.0 1.2 1.0</td>
</tr>
</tbody>
</table>

At the third step of Edman degradation, the sample was analyzed without hydrolysis and free arginine was recovered. Carboxypeptidase (A + B) liberated each 1 mole of arginine and leucine. Thus the sequence was concluded to be Gly-Ile-Leu-Arg.

**Peptide T13 (81-85): Asn-Ala-Lys (NH2) (PTH-)**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Asn-Ala-Lys (NH2) (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation Step 1</td>
<td>1.0 1.1 1.0</td>
</tr>
<tr>
<td>Step 2</td>
<td>0.1 1.0 0.5</td>
</tr>
</tbody>
</table>

**Table VII**

**Structural studies on Peptide T11**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leu-Phe-Asn-Gln-Asp-Val-Asp(Ala, Ala, Val)-Arg (NH2) (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxypeptidase A + B (pH 8) 6 hrs</td>
<td>0.0 0.0 0.0 1.0 1.0</td>
</tr>
<tr>
<td>(pH 6) 17 hrs</td>
<td>0.0 0.0 0.9 2.1 1.2 1.0</td>
</tr>
<tr>
<td>Dilute acid hydrolysis</td>
<td>0.1 0.0 3.0 0.7 0.9 0.0</td>
</tr>
</tbody>
</table>
Amino Acid Sequence of T4 Phage Lysozyme. I

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TABLE VIII
Structural studies on Peptide T9-1941

<table>
<thead>
<tr>
<th>Composition</th>
<th>Asp Cys(Cm) Gly Val8 Ile8 Thr Lys Glu Ala Leu Phe Arg PTH-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td>6.0 0.7 1.1 2.4 0.4 0.8 1.8 3.0 3.1 1.0 1.0 0.8</td>
</tr>
<tr>
<td>Step 1</td>
<td>0.9 2.2 0.3 0.7 N.D. 3.1 3.0 0.8 1.1 N.D. Asn</td>
</tr>
<tr>
<td>Step 2</td>
<td>1.0 1.8 0.2 0.7 N.D. 3.0 2.2 0.8 0.8 N.D. Cys(Cm)</td>
</tr>
<tr>
<td>Step 3</td>
<td>1.1 2.3 0.3 0.8 N.D. 3.0 2.5 0.8 0.8 N.D. Asn</td>
</tr>
<tr>
<td>Carboxypeptidase A + B</td>
<td>0 0 0 0 0 0 0 0 0.2 0 0 1.0</td>
</tr>
</tbody>
</table>

a Cm, carboxymethyl.

b The low yield of Ile was conceived as the resistance of the Val-Ile bond for the 8 N acid hydrolysis.

TABLE IX
Structural studies on Peptide T14

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td>1.9 1.0 1.1 1.7 1.0 2.0 1.0 1.0 0.8</td>
</tr>
<tr>
<td>Step 1</td>
<td>1.0 N.D. 0.8 1.8 0.7 2.1 1.0 1.0 N.D. Leu</td>
</tr>
<tr>
<td>Step 2</td>
<td>1.0 0 1.0 1.5 0.8 1.8 1.1 1.0 1.0 N.D.</td>
</tr>
<tr>
<td>Step 3</td>
<td>1.3 0 2.0 1.0 2.0 1.0 1.0 1.1 Pro</td>
</tr>
<tr>
<td>Step 4</td>
<td>0.8 N.D. 0 0.5 0.5 1.0 0.9 1.0 N.D. Val</td>
</tr>
<tr>
<td>Step 5</td>
<td>1.0 N.D. 0 0.6 0 1.8 1.0 1.0 N.D. Tyr</td>
</tr>
<tr>
<td>Step 6</td>
<td>1.1 N.D. 0 0.7 0 1.1 1.0 1.0 N.D. Asp</td>
</tr>
<tr>
<td>Step 7</td>
<td>0.9 N.D. 0 0.9 0 0.9 0.6 1.0 N.D. N.D.</td>
</tr>
<tr>
<td>Step 8</td>
<td>0.3 N.D. 0 0.7 0 1.0 0.2 1.0 N.D. Leu</td>
</tr>
<tr>
<td>Carboxypeptidase A + B</td>
<td>0 0 0 0 0 0 0 0.1 0.8 1.0</td>
</tr>
</tbody>
</table>

Chymotryptic digestion

T14-C-1 (30%)

| 0.2 0.3 0 | 1.0 0.7 0.8 | 0.3 0.2 |

T14-C-2 (30%)

| 0.3 0.1 0 | 0 0 0.1 | 1.0 1.0 0.7 0.6 |

a The low yield of Tyr in amino acid composition may be derived from modification or decomposition (42).

TABLE X
Structural studies on Peptide T14-16

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg-Arg (NH3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxypeptidase A + B</td>
<td>1.2 0.9 0.9 2.0 0.9 2.0 2.0 1.1 1.7 1.9 0.4</td>
</tr>
<tr>
<td>(pH 8) 3 hrs</td>
<td>0 0 0 0 0 0 0 0 0 0 0 1.4</td>
</tr>
<tr>
<td>(pH 6) 6.5 hrs</td>
<td>0 0 0 0 0 0 0 0 0 0 0 1.4</td>
</tr>
<tr>
<td>(pH 6) 17 hrs</td>
<td>0 0 0.1 0.1 0.2 0.5 0.6 0.7 0.7 2.0</td>
</tr>
</tbody>
</table>

In addition to the Edman degradation, dinitrophenylation of this peptide yielded DNP-aspartic acid. Carboxypeptidase B liberated only lysine (0.4).

Peptide T14 (84-96): Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg (Table IX)—Failure of trypsin to hydrolyze the lysyl bond is due to the presence of an adjacent proline residue.

The amino acid sequence from the 1st to the 8th residue was determined by Edman degradation, and the sequence of the COOH-terminal region was analyzed by carboxypeptidase digestion and chymotryptic digestion. From the chymotryptic digest of this peptide, the two peptides T14-C-1 and T14-C-2 were isolated by paper electrophoresis at pH 3.6.

The composition of Peptide T14-C-1 was Asp, Ser, and Leu. The NH2 terminus was determined by Edman degradation followed by identification of PTH-aspartic acid. Carboxypeptidase A digestion liberated only leucine (0.5). Thus the sequence can be concluded to be Asp-Ser-Leu.

The T14-C-2 peptide was composed of Asp, Ala, Val, and Arg. The first step of Edman degradation resulted in PTH-aspartic acid. The residual amino acid composition was Asp(0.3), Ala(1.0), Val(0.6), and Arg(0.8) and in the second step PTH-Ala was identified. Carboxypeptidase (A + B) digestion liberated arginine (0.7). The sequence of the tetrapeptide was established as Asp-Ala-Val-Arg.

Peptide T14-15 (85-96): Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg-Arg (Table X)—The presence of 2 aspartic acid residues (but not asparagine) in the peptide was deduced from the amide content (0.4 mole per mole). Carboxypeptidase digestion of the peptide at pH 8 yielded only arginine and valine, while alanine and aspartic acid were obtained in addition by digestion at pH 6 (see the case of T11).
TABLE XI

<table>
<thead>
<tr>
<th>Composition</th>
<th>Cys(SO₃) Ala Leu Ile Asx Met(O)₂ Val Phe Gly Gly Thr Ser Arg (PTH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td>0.8 2.0 1.7 1.1 1.5 1.5 2.0 2.7 2.0 1.0 0.9</td>
</tr>
<tr>
<td>Step 1</td>
<td>0.2 1.8 1.0 0.8 1.0 1.5 3.0 3.0 1.5 1.3 N.D. N.D.</td>
</tr>
<tr>
<td>Step 2</td>
<td>1.2 1.6 1.1 1.6 2.0 1.8 2.4 3.0 1.5 0.6 N.D. Ala</td>
</tr>
<tr>
<td>Step 3</td>
<td>1.2 0.6 1.1 1.6 2.0 2.0 1.8 2.4 3.1 1.5 0.7 N.D. N.D.</td>
</tr>
<tr>
<td>Carboxypeptidase A + R</td>
<td>0 0 0.3 0 0 0 0 0.1 0.4 0.3</td>
</tr>
</tbody>
</table>

*Asn and Ser were found in the same position in an amino acid analyzer and they were not separated further.*

After dilute acid hydrolysis, the peptide fragments were isolated by paper electrophoresis at pH 3.6, along with about 2 moles of aspartic acid (1.4 moles per mole). The amino acid sequences were determined as follows:

T14-15-DAH-2 (48%): Leu-Lys-Pro-Val-Tyr (PTH-)
Composition 1.0 1.1 1.3 1.0 0.6
Edman degradation
Step 1 0.2 1.0 0.9 1.0 1.2 Leu
Step 2 0.3 0 1.1 1.0 0.5 N.D.
Carboxypeptidase A 0 0 0 0 0.9

The COOH-terminal region was deduced to be Pro-Val-Tyr from the required specificity of carboxypeptidase, since tyrosine would not be released by carboxypeptidase if the proline residue were next to the tyrosine residue at the COOH terminus.

T14-15-DAH-3 (60%): Ser-Leu—After one cycle of the Edman degradation, free leucine was recovered without hydrolysis.

T14-15-DAH-4 (56%): Ala-Val-Arg-Arg (PTH-)
Composition 0.7 1.0 2.0
Edman degradation
Step 1 0.3 1.0 1.7 Ala
Step 2 0.1 0.1 2.0 Val
Step 3 (37%) 0 0 1.0 N.D.

The above data from DAH-peptides together with the results of carboxypeptidase digestion of T14-15 were used to deduce the sequence. From a comparison of Peptide T14 with Peptide T14-15, it can be concluded that the latter peptide is longer by 1 arginine residue at the COOH terminus.

Peptide T16 (97-119): Cys-Ala-Leu-Ile-Asx-Met-Val-Phe-Gln-Met-Gly-Glu-Thr-Arg (Table XI)—This peptide precipitated after trypsin digestion of the protein at pH 3.2, whether the protein was oxidized or not. In some cases, it was obtained in a pure state by washing it with water. However, when the peptide was found by amino acid analysis to contain an impurity, it was purified by chromatography on Dowex 1-X2. After digestion of the unoxidized protein, the core fraction was oxidized by performic acid prior to column chromatography. The oxidized fraction was suspended in 0.1 N acetic acid and then applied to a Dowex 1-X2 column (0.9 × 75 cm) and the column was washed thoroughly with 0.1 N acetic acid. Elution was carried out with use of a three-portion Varigrad apparatus with 100 ml of 0.1 N acetic acid in the first chamber and 100 ml of 2 N acetic acid in both the second and the third chambers. Final elution was performed with 200 ml of 50% acetic acid. The elution was carried out at 37 °C with a flow rate of about 30 ml per hour.

Fractions of 5 ml were collected. An elution pattern is shown in Fig. 2. The peptide was recovered in the last peak fraction, which was eluted by about 50% acetic acid.

The above data from DAH-peptides together with the results of carboxypeptidase digestion of T14-15 were used to deduce the sequence. From a comparison of Peptide T14 with Peptide T14-15, it can be concluded that the latter peptide is longer by 1 arginine residue at the COOH terminus.

**Fig. 2.** Column chromatography of the core fraction of the tryptic digest on Dowex 1-X2. Elution was carried out with 500 ml of 0.1 N acetic acid followed by gradient elution with a three-chambered Varigrad apparatus. The first chamber was filled with 100 ml of 0.1 N acetic acid and both the second and the third chamber were filled with 100 ml of 2 N acetic acid. Elution with 200 ml of 50% acetic acid was then performed. Column, 0.9 × 75 cm; one fraction, 5 ml; flow rate, 30 ml per hour; 37 °C. The elution pattern was obtained by measuring absorbance at 570 nm after alkaline hydrolysis of aliquots from alternate tubes followed by ninhydrin reaction.

Fractions of 5 ml were collected. An elution pattern is shown in Fig. 2. The peptide was recovered in the last peak fraction, which was eluted by about 50% acetic acid.

The above data from DAH-peptides together with the results of carboxypeptidase digestion of T14-15 were used to deduce the sequence. From a comparison of Peptide T14 with Peptide T14-15, it can be concluded that the latter peptide is longer by 1 arginine residue at the COOH terminus.
Edman degradation gave free glycine without hydrolysis as well as PTH-threonine and PTH-methionine sulfone, identified by paper chromatography.

The peptide sequence, Val-Ala-Gly, was determined by Edman degradation. Free glycine was recovered without hydrolysis after the second step of Edman degradation.

Val-Ala-Gly
Composition 0.7 1.0 1.4
Edman degradation
Step 1 0.7 1.0 1.4
Step 2 0.0 0.0 1.0

The peptide Gly-Phe was analyzed by Edman degradation; free phenylalanine and PTH-glycine were identified.

T16-C-3 Thr-Asn-Ser-Leu-Arg (PTH-)
Composition 0.8 1.0 0.9 1.0 1.1
Edman degradation
Step 1 0.0 0.0 0.0 0.0 0.0
Step 2 0.0 0.0 0.0 0.0 0.0

Dilute acid hydrolysis gave only free aspartic acid (1.0) and threonine (0.6) in 24% yield. Therefore, it can be concluded that the NH₃-terminal sequence is Thr-Asx. The Asx was confirmed to be asparagine, since the peptide was found to be basic by paper electrophoresis.

T16-C-4: Thr-Asx—Amino acid analysis after the first step of Edman degradation resulted in the disappearance of threonine and gave only aspartic acid.

T16-C-5: Ser-Leu-Arg—After the first step of Edman degradation resulted in the disappearance of threonine and gave only aspartic acid.

The order of chymotryptic peptides of Peptide T16 was concluded to be H₂N-(T16-C-1)-(T16-C-2)-(T16-C-3)-COOH, since both T16-C-1 and T16 had cysteine residues at the NH₂ terminus, and T16-C-3 contained an arginine residue at the COOH terminus. These three peptides account for total amino acid composition of T16.

After cyanogen bromide cleavage of the protein, the following peptide was recovered: Gly-Thr-Gly-Val-Ala-(Gly, Phe, Thr, Asx, Ser)-Leu-Arg-homoserine. This peptide probably represents the COOH-terminal portion of T16, plus homoserine.

Peptide T16-I (97-114): Cys(Ala, Leu, Ile, Asx, Met, Val, Phe, Glx, Met, Gly, Glx, Thr, Val, Ala, Gly)-Phe (Table XIV)

The presence of 2 glutamine residues was deduced from the amide content of the peptide (2.1 moles per mole), together with the results of Edman degradation and carboxypeptidase digestion.

### Table XII

<table>
<thead>
<tr>
<th>Structural studies on Peptide T16-C-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>Cys(SON)-Ala - Leu - Ile - Asn - Met(O)-Val - Phe (PTH-)</td>
</tr>
<tr>
<td>0.9 1.1 1.0 1.0 1.1 0.9 0.9 0.9</td>
</tr>
<tr>
<td><strong>Edman degradation</strong></td>
</tr>
<tr>
<td>Step 1</td>
</tr>
<tr>
<td>0.2 1.1 1.0 0.8 1.0 0.7 1.0 1.0 N.D.</td>
</tr>
<tr>
<td>Step 2</td>
</tr>
<tr>
<td>0.1 0.2 1.0 1.0 1.1 0.9 0.9 0.6 Ala</td>
</tr>
<tr>
<td>Step 3</td>
</tr>
<tr>
<td>0.0 0.4 0.3 1.0 1.1 0.8 0.9 0.7 N.D.</td>
</tr>
<tr>
<td>Step 4</td>
</tr>
<tr>
<td>0.3 0.4 0.4 0.1 1.1 0.9 1.0 0.7 N.D.</td>
</tr>
<tr>
<td>Step 5</td>
</tr>
<tr>
<td>N.D. N.D. N.D. N.D. N.D. N.D. N.D. N.D. Asn</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
</tr>
<tr>
<td>0 0 0 0 0 0 0.3 0.9</td>
</tr>
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</table>

### Table XIII

<table>
<thead>
<tr>
<th>Structural studies on Peptide T16-C-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>Gln - Met(O)₂-Gly - Glu(Thr, Gly, Val, Ala, Gly)-Phe (PTH-)</td>
</tr>
<tr>
<td>2.0 0.9 2.0 1.0 0.8 1.0 0.8</td>
</tr>
<tr>
<td><strong>Edman degradation</strong></td>
</tr>
<tr>
<td>Step 1</td>
</tr>
<tr>
<td>1.4 0.9 2.7 0.9 0.9 1.0 0.7 Gln</td>
</tr>
<tr>
<td>Step 2</td>
</tr>
<tr>
<td>1.5 0.4 2.7 0.9 0.9 1.0 0.8 Met(O)₂</td>
</tr>
<tr>
<td>Step 3</td>
</tr>
<tr>
<td>1.5 0.4 2.3 0.9 0.9 1.0 0.8 Gly</td>
</tr>
<tr>
<td>Step 4</td>
</tr>
<tr>
<td>N.D. N.D. N.D. N.D. N.D. N.D. N.D. Glu</td>
</tr>
<tr>
<td>Aminopeptidase M</td>
</tr>
<tr>
<td>0.7 0.7 0.5 0.3 0.2 0.1 0.1 0</td>
</tr>
<tr>
<td>Carboxypeptidase A + B</td>
</tr>
<tr>
<td>0 0 0 0 0 0 0.7 0</td>
</tr>
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</table>

Unpublished data.
TABLE XIV

Structural studies on Peptide T16-I

<table>
<thead>
<tr>
<th>Composition</th>
<th>Cys(Cm)</th>
<th>Ala</th>
<th>Leu</th>
<th>Ile</th>
<th>Asp</th>
<th>Met</th>
<th>Val</th>
<th>Phe</th>
<th>Gly</th>
<th>Glu</th>
<th>Thr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation, Step 1</td>
<td></td>
<td>0.7</td>
<td>2.0</td>
<td>1.1</td>
<td>0.7</td>
<td>1.0</td>
<td>1.3</td>
<td>1.2</td>
<td>1.5</td>
<td>5.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td></td>
<td>0</td>
<td>1.9</td>
<td>0.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.9</td>
<td>0.1</td>
<td>1.4</td>
<td>2.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Cm, carboxymethyl.

TABLE XV

Structural studies on Peptide T18-19-C-1

<table>
<thead>
<tr>
<th>Composition</th>
<th>Arg-Trp-(Asx,Glx)-Ala-(Ala, Val)-Asn-Leu-Ala-Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation, Step 1</td>
<td></td>
</tr>
<tr>
<td>Aminopeptidase M</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase A (pH 6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8 ±* 2.0</td>
</tr>
<tr>
<td></td>
<td>1.0 1.2 1.0</td>
</tr>
<tr>
<td></td>
<td>0.8 1.0</td>
</tr>
<tr>
<td></td>
<td>0.3 0 0</td>
</tr>
<tr>
<td></td>
<td>0 1.3 1.1</td>
</tr>
<tr>
<td></td>
<td>0 0 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Detected by the Ehrlich reagent (31).
* Analyzed as asparagine.

Peptide T18-19 (135-155): Arg-Trp-(Asx,Glx)-Ala-(Ala, Val)-Asn-Leu-Ala-Lys—The results of Edman degradation, carboxypeptidase, and aminopeptidase digestion of this peptide were reported in the previous paper (3). Dilute acid hydrolysis of this peptide yielded the following peptides: (Asx,Glx, Ala, Ala, Val) and (Leu, Ala)-Lys. From the chymotryptic digest of T18-19, the following two peptides were isolated:

T18-19-C-1: Leu-Ala-Lys (PTH-)
Composition 0.9 1.0 1.0
Edman degradation, Step 1 0.2 1.0 0.6 Leu
Step 1 0 0 0.9
Carboxypeptidase A + B

T18-19-C-2: Arg-Trp-(Asx,Glx)-Ala-(Ala, Val)-Asn—(Table XV)—The specificity of chymotrypsin requires an asparagine residue in the COOH-terminal position.

Peptide T19 (145-155): Trp-Asp-Gln-Ala-Ala-Val-Asn-Arg-Trp—Ala-Lys—The sequential analysis of this peptide was reported in the previous paper (3). This peptide was shorter by 1 arginine residue than Peptide T18-19 at the NH2 terminus.

Altogether, the studies on T18-19 and T19 concluded the sequence as described above.

Peptide T20 (156-157): Ser-Arg—The first step of Edman degradation resulted in residual arginine (0.9 mole per mole) and dinitrophenylation yielded DNP-Ser.

Peptide T21 (139-145): Tyr-Asn-Gln-Thr-Pro-Asn-Arg—The sequential studies on this peptide were also published previously (3). In the four frame shift mutant strains, eJD2, eJD3, eJD20, and eJD24 (8), Peptide T21 was found to be altered to Met-Val-Tyr-Gln-Thr-Pro-Asn-Arg, Trp-Cys-Ile-Ile-Glu-Thr-Pro-Asn-Arg, Trp-Tyr-Ile-Ile-Gln-Thr-Pro-Asn-Arg, and Trp-Tyr-Lys-Ile-Gln-Thr-Pro-Asn-Arg, respectively, where the italicized sequences indicate the altered residues.

Peptide T21-I (159-165): Tyr-Asn-Gln-Thr-Pro-Asn-Arg—The details of the sequential studies on this peptide were also published in the previous paper (3). This peptide was isolated in earlier work. The NH2-terminal tryptophan residue of Peptide T21 seemed to be split off by chymotryptic activity which remained in the trypsin preparation.

Peptide T22 (146-148): Ala-Lys—Edman degradation and dinitrophenylation of this peptide yielded PTH-alanine and DNP-alanine, respectively.

Peptide T23 (144-150): Ala-Lys—Edman degradation and dinitrophenylation of this peptide yielded PTH-alanine and DNP-alanine, respectively.

Peptide T24 (140-154): Val-Ile-Thr-Thr-Phe—Arg (PTH-)
Composition 6 N HCl (24 hours) 0.3 0.5 1.7 1.0 1.0

![Fig. 3. Recoveries of valine and threonine from Peptide T24 by acid hydrolysis.](https://www.jbc.org/)

Fig. 3. Recoveries of valine and threonine from Peptide T24 by acid hydrolysis. Aliquots of Peptide T24 were hydrolyzed in 6 N HCl containing various concentrations of acetic acid. Hydrolysis was carried out at 105-106°C for 24 and 72 hours in sealed, evacuated tubes. Recoveries were calculated, taking the amount of phenylalanine recovered as 100%. ○, recoveries of valine after hydrolysis for 24 hours and C, for 72 hours; ▲, recoveries of threonine after hydrolysis for 24 hours and Δ, for 72 hours.
### Table XVI

**Structural studies on Peptide T25**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Thr-Gly (Thr, Trp, Asp) Ala-Tyr-Lys (NH₃) (PTH-⁻)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edman degradation</td>
<td>1.6 1.1 0.7 1.0 0.8 0.8 0.3</td>
</tr>
<tr>
<td>Step 1</td>
<td>0.9 1.1 N.D. 1.0 1.1 0.8 N.D. Thr</td>
</tr>
<tr>
<td>Step 2</td>
<td>0.9 0.4 N.D. 1.1 1.0 0.8 N.D. Gly</td>
</tr>
<tr>
<td>Carboxypeptidase A + B (pH 8) 4 hr</td>
<td>0 0 0 0 0 0.8 0.9</td>
</tr>
<tr>
<td>(pH 6) 17 hr</td>
<td>0 0.2 0.1 0.2 0.6 0.8 1.0</td>
</tr>
</tbody>
</table>

### Table XVII

**Amino acid sequences of tryptic peptides of T4 phage lysozyme**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Positions in protein</th>
<th>No. of residues</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1-8</td>
<td>8</td>
<td>Met-Asn-Ile-Phe-Glu-Met-Leu-Arg</td>
</tr>
<tr>
<td>T2</td>
<td>9-14</td>
<td>6</td>
<td>Ile-Asp-Glu-Gly-Leu-Arg</td>
</tr>
<tr>
<td>T3</td>
<td>15-16</td>
<td>2</td>
<td>Leu-Lys</td>
</tr>
<tr>
<td>T4</td>
<td>17-19</td>
<td>3</td>
<td>Ile-Tyr-Lys</td>
</tr>
<tr>
<td>T5</td>
<td>20-35</td>
<td>16</td>
<td>Asp-Thr-Glu-Gly-Tyr-Tyr-Thr-Ile-Gly-Ile-Gly-His-Leu-Thr-Lys</td>
</tr>
<tr>
<td>T5-1⁻</td>
<td>20-25</td>
<td>0</td>
<td>Asp-Thr-Glu-Gly-Tyr-Tyr</td>
</tr>
<tr>
<td>T5-2⁻</td>
<td>26-35</td>
<td>10</td>
<td>Thr-Ile-Gly-Ile-Gly-His-(Leu, Leu, Thr)-Lys</td>
</tr>
<tr>
<td>T4-5⁻</td>
<td>17-35</td>
<td>19</td>
<td>Thr-Ile-Tyr-Lys-(Asx, Thr, Glx, G1y, Tyr, Tyr, Thr, Ile, Ile, Glx, G1y, His)-(Leu, Leu, Thr)-Lys</td>
</tr>
<tr>
<td>T6</td>
<td>36-43</td>
<td>8</td>
<td>Ser-Pro-Ser-Leu-Asn-Ala-Ala-Lys</td>
</tr>
<tr>
<td>T7</td>
<td>44-45</td>
<td>5</td>
<td>Ser-Glu-Leu-Asp-Lys</td>
</tr>
<tr>
<td>T8</td>
<td>49-53</td>
<td>4</td>
<td>Ala-Ile-Gly-Arg</td>
</tr>
<tr>
<td>T9</td>
<td>53-60</td>
<td>8</td>
<td>Asn-Cys-Asn-Gly-Val-Ile-Thr-Lys</td>
</tr>
<tr>
<td>T10</td>
<td>61-65</td>
<td>5</td>
<td>Asp-Glu-Ala-Glu-Lys</td>
</tr>
<tr>
<td>T9-10ᵇ</td>
<td>66-69</td>
<td>13</td>
<td>Asn-Cys-Asn-Gly-Val-Ile-Thr-Lys-Asp-Glu-(Ala, Glu)-Lys</td>
</tr>
<tr>
<td>T11</td>
<td>66-77</td>
<td>11</td>
<td>Leu-Phe-Asn-Gln-Asp-Val-Asp-Ala-Ala-Val-Arg</td>
</tr>
<tr>
<td>T12</td>
<td>77-80</td>
<td>4</td>
<td>Gly-Ile-Leu-Arg</td>
</tr>
<tr>
<td>T13</td>
<td>81-83</td>
<td>3</td>
<td>Asn Ala Lys</td>
</tr>
<tr>
<td>T14</td>
<td>84-95</td>
<td>12</td>
<td>Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg</td>
</tr>
<tr>
<td>T15</td>
<td>96</td>
<td>1</td>
<td>Arg</td>
</tr>
<tr>
<td>T14-15ᵇ</td>
<td>84-96</td>
<td>13</td>
<td>Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-Arg-Arg</td>
</tr>
<tr>
<td>T16-1ᵇ</td>
<td>97-114</td>
<td>18</td>
<td>Cys-(Ala, Leu, Ile, Asx, Met, Val, Phe, Glx, Met, Gly, Glx, Thr, Thy, G1y, Val, Ala, G1y)-Phe</td>
</tr>
<tr>
<td>T16-1ᵇ⁻⁻</td>
<td>115-119</td>
<td>5</td>
<td>Thr-Asn-Ser-Leu-Arg</td>
</tr>
<tr>
<td>T17</td>
<td>130-134</td>
<td>5</td>
<td>Met-Leu-Gln-Gln-Lys</td>
</tr>
<tr>
<td>T18</td>
<td>135</td>
<td>1</td>
<td>Arg</td>
</tr>
<tr>
<td>T10</td>
<td>126-135</td>
<td>10</td>
<td>Trp-Asp-Glu-Ala-Ala-Val-Asn-Leu-Ala-Lys</td>
</tr>
<tr>
<td>T18-19ᵇ</td>
<td>125-135</td>
<td>11</td>
<td>Arg-Trp-Asp-Glu-Ala-Ala-Val-Asn-Leu-Ala-Lys</td>
</tr>
<tr>
<td>T20</td>
<td>136-137</td>
<td>2</td>
<td>Ser-Arg</td>
</tr>
<tr>
<td>T19</td>
<td>138-145</td>
<td>8</td>
<td>Trp-Tyr-Asn-Gln-Thr-Pro-Asn-Arg</td>
</tr>
<tr>
<td>T21</td>
<td>139-145</td>
<td>7</td>
<td>Tyr-Asn-Gln-Thr-Pro-Asn-Arg</td>
</tr>
<tr>
<td>T22</td>
<td>146-147</td>
<td>2</td>
<td>Ala-Lys</td>
</tr>
<tr>
<td>T23</td>
<td>148</td>
<td>1</td>
<td>Arg</td>
</tr>
<tr>
<td>T22-23ᵇ</td>
<td>146-148</td>
<td>3</td>
<td>Ala-Lys-Arg</td>
</tr>
<tr>
<td>T24</td>
<td>149-154</td>
<td>6</td>
<td>Val-Ile-Thr-Thr-Phe-Arg</td>
</tr>
<tr>
<td>T23-24ᵇ</td>
<td>148-154</td>
<td>7</td>
<td>Arg-Val-(Ile, Thr)-(Thr)-Phe-Arg</td>
</tr>
<tr>
<td>T25</td>
<td>155-162</td>
<td>8</td>
<td>Thr-Gly-Thr-Trp-Asp-Ala-Tyr-Lys</td>
</tr>
<tr>
<td>T26</td>
<td>163-164</td>
<td>2</td>
<td>Asn-Leu</td>
</tr>
</tbody>
</table>

* Peptides obtained by chymotryptic action in the trypsin preparation used.
* Overlapping peptides.
The molar recovery of valine and isoleucine was very low when acid hydrolysis was carried out under the usual conditions (6 N HCl, 105-106°, 24 hours). As was also discussed in the case of Peptide T9, some peptide bonds such as Ile-Ile are well known to be resistant to acid hydrolysis (30, 43, 44). In order to hydrolyze such bonds completely, 6 N HCl containing various concentrations of acetic acid was tried. Fig. 3 shows that the recoveries of valine in Peptide T24 increase with increasing concentrations of acetic acid. On the other hand, the recoveries of threonine decrease. When the sample was hydrolyzed for 72 hours in 6 N HCl containing 50% acetic acid (concentrated HCl-glacial acetic acid, 1:1), Val-Ile was almost completely hydrolyzed, although about 40% of the threonine was found to be decomposed. From these results, hydrolysis of the sample in 6 N HCl containing 50% acetic acid seems to be a useful method for peptides containing bonds which are resistant to acid hydrolysis. Under this condition, Ile-Ile was also hydrolyzed in good yield (10).

In the structural studies, the recovery of isoleucine after the first step of Edman degradation was increased remarkably under the usual conditions of acid hydrolysis, because the amino-terminal valine residue had been removed.

Dinitrophenylation of the peptide yielded DNP-valine.

The following two peptides.

**Edman degradation**

<table>
<thead>
<tr>
<th>Step</th>
<th>0</th>
<th>0.9</th>
<th>2.0</th>
<th>0.8</th>
<th>N.D.</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td>0</td>
<td>0</td>
<td>1.8</td>
<td>1.0</td>
<td>0.0</td>
<td>Ile</td>
</tr>
<tr>
<td>Step 3</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.6</td>
<td>Thr</td>
</tr>
<tr>
<td>Step 4</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>Thr</td>
</tr>
<tr>
<td>Step 5</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Phe</td>
</tr>
</tbody>
</table>

Carboxypeptidase A + B

|        | 0 | 0.1 | 0.8 | 0.9 | 0.8 |

This peptide was longer than Peptide T24 by 1 arginine residue at the NH2 terminus. Peptide T25 contains 13 residues of lysine and 13 arginine residues (1), which may indicate that 27 tryptic peptides should theoretically be obtained. However, Peptide T14 contained 1 lysine residue in addition to the COOH-terminal arginine residue. This lysine residue is resistant to tryptic digestion because a proline residue is adjacent.

In 23 nonoverlapping peptides, 13 lysine residues and 10 arginine residues were found, and 3 more arginine residues should be taken into account from the arginine content in the protein. Adding amino acid residues in the peptides and 3 free arginine residues gave a total of 164 amino acid residues for the lysozyme. Four overlapping peptides which contained each 1 extra arginine residue were isolated and they were T14-Arg (T14-15), Arg-T19 (T18-19), T22-Arg (T22-23), and Arg-T24 (T23-24). In order to take account of 3 free arginine residues, three out of four overlapping peptides mentioned above should be independent of each other, and the remaining one peptide should share its extra arginine residue with one of the other three. In the accompanying paper (19), it is shown that T22-23 and T23-24 share their extra arginine as T22 Arg T24, but not T22 Arg-T24. With the use of other overlapping peptides, such as T4-5, T9-10, T9-10-11, T14-15, T18-19, T22-23, and T23-24, T4 phage lysozyme can be assembled into 20 fragments, as follows: T1, T2, T3, T4-T5, T6, T7, T8, T9-T10-T11, T12, T13, T14-T15, T16, T17, T18-T19, T20, T21, T22-T23, T24, T25, T26.

In the accompanying papers (18-20), chymotryptic digestion, peptic digestion, dilute acid hydrolysis of T4 phage lysozyme, and the determination of the complete structure of the protein are presented.
Acknowledgments—The authors are grateful to Misses S. Kyo and N. Kuroda for their skilled technical assistance.

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