Primary Structure of Streptococcal Proteinase

I. ISOLATION, COMPOSITION, AND AMINO ACID SEQUENCES OF THE TRYP TIC AND CHYMOTRYPTIC PEPTIDES OF CYAN OGEN BROMIDE FRAGMENTS 1 TO 4*

(Received for publication, August 6, 1975)

ALEXANDER A. KORT, JEAN R. WYSOCKI, AND T.-Y. LIU†

From the Division of Bacterial Products, Bureau of Biologies, Food and Drug Administration, Bethesda, Maryland 20014; the Biology Department, Brookhaven National Laboratory, Upton, New York 11973; and the Biochemistry of Microbial Structure Section, National Institute of Child Health and Human Development, Bethesda, Maryland 20014

Tryptic and chymotryptic peptides were isolated and characterized from cyanogen bromide fragments 1 to 4 of streptococcal proteinase and subjected to sequence analysis by the Edman degradation, carboxy-peptidase digestion, and hydrolytic regeneration of the amino acid residues from the phenylthiocarbamyl derivatives. The results, together with the sequence data of the cyanogen bromide fragment 5 reported in the accompanying papers, provide the structural formula of streptococcal proteinase.

Streptococcal proteinase is a sulfhydryl enzyme, first isolated by Elliott (1), which is elaborated by group A streptococci. It is excreted into culture fluid as a zymogen, later transformed into an active enzyme by proteolysis followed by reduction (2, 3). Both zymogen and enzyme have been isolated in crystalline form (4). Good yields of homogeneous enzyme have been obtained from zymogen by treatment with trypsin (5).

Earlier experiments (6, 7) showed that the proteinase is a single polypeptide chain with a molecular weight of about 32,000. It contains only 1 half-cystine residue/mol, and thus offers unique advantages for the study of the role of -SH group in proteolysis. This paper and the two succeeding papers (8, 9) summarize our work on the structural formula of the enzyme.

EXPERIMENTAL PROCEDURE

Materials

Zymogen was crystallized from streptococcal culture filtrates by treatment with ammonium sulfate (4) and converted to proteinase by digestion with trypsin (5). The CB1 peptides, CB1, CB2, CB3, and CB4, were prepared and purified as described in the accompanying paper (9). Trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated), a-chymotrypsin, and carboxypeptidases A and B (both diisopropylphosphorofluoridate-treated) were from Worthington. Aminopeptidase M and carboxypeptidase C were obtained from Rohm and Haas, Darmstadt, Germany. Thermolysin was obtained from Daiwa Kasei K. K., Osaka, Japan. Nagarse was purchased from Enzyme Development Corp., N. Y.

* Supported in part by the United States Atomic Energy Commission.
† Present address, Division of Bacterial Products, Bureau of Biologies, Food and Drug Administration, Bethesda, Maryland 20014.

The sulfonated polystyrene resin Dowex 50-X2 was AG 50W-X2 (200 to 400 mesh) from Bio-Rad and UR-30 from Beckman. The anion exchange resin Dowex 1-X2 was AG 1-X2 (200 to 400 mesh) from Bio-Rad. Trifluoroacetic acid and phenylisothiocyanate (Sequenal grade) were obtained from Pierce Chemical Co. N-Ethylmorpholine was redistilled; pyridine was redistilled over ninhydrin. All other chemicals were reagent grade.

Table 1

<table>
<thead>
<tr>
<th>Hydrolysis of phenylthiohydantoins with 4 N methanesulfonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
<tr>
<td>as Glycine</td>
</tr>
<tr>
<td>Aspartic Acid</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>as Homoserine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>+allo-iso-</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Phevlalanine</td>
</tr>
</tbody>
</table>

TABLE I

Hydrolysis of phenylthiohydantoins with 4 N methanesulfonic acid

Amino Acid Recovered

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Recovery at 22 hr</th>
<th>Recovery at 48 hr</th>
<th>Recovery at 72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>88.3</td>
<td>87.6</td>
<td>88.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>93.4</td>
<td>90.4</td>
<td>91.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>63.0</td>
<td>63.5</td>
<td>64.3</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>8.5</td>
<td>9.94</td>
<td>11.5</td>
</tr>
<tr>
<td>as Glycine</td>
<td>34.4</td>
<td>34.4</td>
<td>34.4</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>91.2</td>
<td>90.3</td>
<td>94.3</td>
</tr>
<tr>
<td>Serine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>86.3</td>
<td>82.9</td>
<td>89.1</td>
</tr>
<tr>
<td>Proline</td>
<td>89.3</td>
<td>91.9</td>
<td>90.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>90.0</td>
<td>94.4</td>
<td>90.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>90.2</td>
<td>95.2</td>
<td>90.4</td>
</tr>
<tr>
<td>Valine</td>
<td>84.4</td>
<td>96.9</td>
<td>92.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>52.3</td>
<td>35.6</td>
<td>21.9</td>
</tr>
<tr>
<td>as Homoserine</td>
<td>37.1</td>
<td>37.1</td>
<td>37.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>83.2</td>
<td>91.5</td>
<td>85.4</td>
</tr>
<tr>
<td>+allo-iso-</td>
<td>82.4</td>
<td>88.2</td>
<td>87.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>82.5</td>
<td>88.9</td>
<td>90.8</td>
</tr>
<tr>
<td>Phevlalanine</td>
<td>81.9</td>
<td>87.8</td>
<td>84.4</td>
</tr>
</tbody>
</table>

1941
Primary Structure of Streptococcal Proteinase

TABLE II
Summary of isolation of tryptic peptides from CB1 and CB2

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Gel filtration</th>
<th>Dowex 50-X2</th>
<th>Dowex 1-X2</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1-T1</td>
<td>1A</td>
<td>IV</td>
<td>1D</td>
<td>1</td>
</tr>
<tr>
<td>CB1-T2</td>
<td>1A</td>
<td>IV</td>
<td>1D</td>
<td>2</td>
</tr>
<tr>
<td>CB1-T3</td>
<td>1A</td>
<td>II</td>
<td>1B</td>
<td>4</td>
</tr>
<tr>
<td>CB1-T3a</td>
<td>1A</td>
<td>III</td>
<td>1C</td>
<td>2</td>
</tr>
<tr>
<td>CB1-T4</td>
<td>1A</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB2-T1</td>
<td>2A</td>
<td>I</td>
<td>2D</td>
<td>1</td>
</tr>
<tr>
<td>CB2-T2</td>
<td>2A</td>
<td>I</td>
<td>2D</td>
<td>2</td>
</tr>
<tr>
<td>CB2-T3</td>
<td>2A</td>
<td>II</td>
<td>2B</td>
<td>2</td>
</tr>
<tr>
<td>CB2-T4</td>
<td>2A</td>
<td>III</td>
<td>2C</td>
<td>1</td>
</tr>
<tr>
<td>CB2-T5</td>
<td>2A</td>
<td>III</td>
<td>2C</td>
<td>2</td>
</tr>
<tr>
<td>CB2-T6</td>
<td>2A</td>
<td>II</td>
<td>2B</td>
<td>1</td>
</tr>
<tr>
<td>CB2-T7</td>
<td>2A</td>
<td>IV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE III
Summary of isolation of chymotryptic peptides from CB1 and CB2

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Gel filtration</th>
<th>Dowex 50-X2</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1-C1</td>
<td>3A</td>
<td>II</td>
<td>3C</td>
</tr>
<tr>
<td>CB1-C2</td>
<td>3A</td>
<td>III</td>
<td>3D</td>
</tr>
<tr>
<td>CB1-C3</td>
<td>3A</td>
<td>I</td>
<td>3B</td>
</tr>
<tr>
<td>CB1-C4</td>
<td>3A</td>
<td>I</td>
<td>3B</td>
</tr>
<tr>
<td>CB1-C4a</td>
<td>3A</td>
<td>I</td>
<td>3B</td>
</tr>
<tr>
<td>CB1-C5</td>
<td>3A</td>
<td>I</td>
<td>3D</td>
</tr>
<tr>
<td>CB1-C5a</td>
<td>3A</td>
<td>II</td>
<td>3C</td>
</tr>
<tr>
<td>CB1-C5b</td>
<td>3A</td>
<td>II</td>
<td>3C</td>
</tr>
<tr>
<td>CB1-C6</td>
<td>3A</td>
<td>IV</td>
<td>5E</td>
</tr>
<tr>
<td>CB2-C1</td>
<td>4A</td>
<td>IV</td>
<td>4E</td>
</tr>
<tr>
<td>CB2-C2</td>
<td>4A</td>
<td>I</td>
<td>4B</td>
</tr>
<tr>
<td>CB2-C2a</td>
<td>4A</td>
<td>II</td>
<td>4C</td>
</tr>
<tr>
<td>CB2-C2b</td>
<td>4A</td>
<td>IV</td>
<td>4E</td>
</tr>
<tr>
<td>CB2-C3</td>
<td>4A</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>CB2-C4</td>
<td>4A</td>
<td>III</td>
<td>4D</td>
</tr>
<tr>
<td>CB2-C5</td>
<td>4A</td>
<td>II</td>
<td>4C</td>
</tr>
<tr>
<td>CB2-C5a</td>
<td>4A</td>
<td>II</td>
<td>4C</td>
</tr>
<tr>
<td>CB2-C6</td>
<td>4A</td>
<td>II</td>
<td>4C</td>
</tr>
<tr>
<td>CB2-C7</td>
<td>4A</td>
<td>IV</td>
<td>4E</td>
</tr>
<tr>
<td>CB2-C7a</td>
<td>4A</td>
<td>III</td>
<td>4D</td>
</tr>
<tr>
<td>CB2-C7b</td>
<td>4A</td>
<td>VI</td>
<td></td>
</tr>
<tr>
<td>CB2-C8</td>
<td>4A</td>
<td>II</td>
<td>4C</td>
</tr>
<tr>
<td>CB2-C9</td>
<td>4A</td>
<td>IV</td>
<td>4E</td>
</tr>
</tbody>
</table>

Fig. 1. Separation of CB1 tryptic peptides. A, gel filtration of a tryptic digest of 3.02 μmol of CB1 peptide on Sephadex G-25 (fine). Column, 1.6 × 375 cm; eluent, 10% acetic acid; flow rate, 18 ml/hour. Fractions of 3.0 ml were collected and 0.1 ml aliquots of each fraction were analyzed with ninhydrin after alkaline hydrolysis. B to D, chromatography of the tryptic fractions II to IV on AG 50W-X2. A column (0.9 × 40 cm) was developed with a linear gradient of 0.2 M pyridine acetate, pH 5.0 (2.5 M in acetic acid), 250 ml each, at 37°. Fractions of 3.0 ml were collected, and 0.1 ml of each fraction was hydrolyzed in alkaline and analyzed with ninhydrin. Bars and numbers show pooled fractions.

Methods

Digestion of CB1 and CB2 Peptides with Proteolytic Enzymes—Peptides were dissolved in 0.2 M ammonium formate buffer, pH 8.0, to a concentration of 1.0 to 1.5 μmol/ml. Trypsin, dissolved in 1 mM formic acid (1 mg/ml) was added to 2% (w/w), and digestion was allowed to proceed for 4 hours for CB1, and 8 hours for CB2 at 37°. The reaction mixture was acidified to pH 2.0 by the addition of concentrated formic acid (88%), and lyophilized. The lyophilized powders were dissolved in 10% acetic acid and chromatographed on Sephadex G-25 columns.

Chymotryptic digestion of CB1 and CB2 was performed in 0.1 M ammonium bicarbonate buffer, pH 7.95, at a concentration of 1.0 to 1.5 μmol/ml of peptides and at an enzyme/substrate weight ratio of 1/50. Digestion was carried out at 25° for 4 hours for both CB1 and CB2. The reaction mixtures were acidified with formic acid to pH 2.0 and lyophilized. For chromatography on Sephadex G-25 columns, the lyophilized powders were dissolved in 10% acetic acid.

Isolation of Peptides—In most cases, the peptides isolated by the combination of gel filtration at room temperature and ion exchange chromatography at the indicated temperature were pure enough for sequence studies, as judged by amino acid analyses and paper electrophoresis. When necessary, further purification was achieved by electrophoresis, which was carried out at 60 to 75 volts/cm in tanks (Savant) containing Varsol as coolant. Three buffer systems were used: pH 4.5 (pyridine/acetic acid/water, 25/100/75), pH 3.5 (pyridine/
Primary Structure of Streptococcal Proteinase

Fig. 2. Separation of CB2 tryptic peptides. A, gel filtration of a tryptic digest of 2.5 pmol of CB2 on Sephadex G-25 (fine). Column, 1.6 x 375 cm; eluent, 10% acetic acid; flow rate, 18 ml/hour. Fractions of 3.0 ml were collected and 0.1-ml aliquots of each fraction were analyzed with ninhydrin after alkaline hydrolysis. B and C, chromatography of the tryptic fractions II and III on AG 50W-X2. Condition of elution was similar to Fig. 1B. D, chromatography of tryptic fraction I on Dowex 1-X2. A column (0.9 x 25 cm) was developed at 37° with a 3% pyridine (v/v), pH 9.18. Fractions of 3.0 ml were collected and 0.1 ml of each fraction was hydrolyzed in alkali and analyzed with ninhydrin. Bars and numbers show pooled fractions. E, chromatography of 1.1 pmol of chymotryptic digest of CB1-T5 on AG 50W-X2. Condition of elution was similar to Fig. 1B.

Fig. 3. Separation of CB1 chymotryptic peptides. A, gel filtration of a chymotryptic digest of 4.2 pmol of CB1 on Sephadex G-25 (fine). Column, 1.6 x 375 cm; eluent, 10% acetic acid; flow rate, 18 ml/hour. Fractions of 3.0 ml were collected, and 0.1-ml aliquots of each fraction were analyzed with ninhydrin after alkaline hydrolysis. B to E, chromatography of the chymotryptic fractions I to IV on AG 50W-X2. Condition of elution was identical to Fig. 1B.

Amino Acid Analysis—Peptides were hydrolyzed in 500 µl (or 50 µl) of 4 M methanesulfonic acid containing 0.2% tryptamine in evacuated, sealed tubes at 115° for 22 hours (10) unless otherwise stated. The hydrolysate was neutralized with 0.4 ml (or 40 µl) of 4.0 N NaOH at 0° and brought to 3.0 (or 0.3) ml with the pH 2.2 buffer of the amino acid analyzer. The chromatography was performed according to Spackman et al. (11) with an accelerated system (12). The amount of sample used ranged from 10 to 50 nmol for the Beckman 120 C analyzer and 0.1 to 1.0 nmol for the Beckman 121 M analyzer. The yields of peptides were based upon the amino acid analyses.

The values for serine, threonine, tyrosine, and tryptophan are not corrected for destruction during hydrolysis, unless so stated. Homoserine was determined as described (13). In most instances, asparagine and glutamine were determined by comparison of amino acid analyses of acid and enzymic digests of peptides. In some instances, serine, asparagine, and glutamine were completely separated on the column of a Beckman 121 M analyzer using pH 2.87 buffer (0.16 M sodium citrate) at 32°.

We are grateful to Eduardo Oliveira of our laboratory for the suggestion of this chromatographic system for the separation of serine, asparagine, and glutamine.
Sequences of Peptides—The proteinase and purified peptides derived from it were subjected to sequence analysis using the Edman degradation in its manual or automated form and exopeptidase digestion. The data which substantiated the sequence conclusions drawn are entirely contained in the miniprint supplement.  

The primary structural analysis of the proteinase was accomplished by sequence analysis of unknown regions within tryptic peptides and ordering of the tryptic peptides through the identification of overlapping peptides. The ability to perform an extended Edman degradation of the proteinase molecule with a sequenator was of considerable use in aligning the tryptic peptides of CB1.

Edman Degradation—The purified peptides were sequenced by the subtractive Edman degradation procedure as described by Gray (14). Positive Edman determinations were performed essentially as described by Edman and Begg (15). The PTH-derivative released at each step was also identified as the amino acid after hydrolysis with 4 M methanesulfonic acid at 150°C for 22 hours (Table I).

Digestion of Peptides with Amino Peptidases and Carboxypeptidases—Peptides (1 to 20 nmol) were subjected to further digestion with thermostable or chymotrypsin. Usually, 100 to 200 nmol of peptides dissolved in 500 μl of enzyme solution (0.2 to 0.5 mg/ml of 0.1 M NH₄HCO₃) were incubated at 37°C for 3 to 6 hours. The degree of digestion was monitored by the results of paper electrophoresis.

Hydrolysis of Peptides with Amino Peptidases and Carboxypeptidases—Peptides (1 to 20 nmol) were hydrolyzed with 10 to 20 μg of either amino peptidase M or carboxypeptidases (A, B, or both) in 100 μl of 0.1 M NH₄HCO₃ at 37°C or room temperature. Carboxypeptidase C was used to remove proline and acidic residues in 0.1 M ammonium acetate at pH 5.0. The hydrolysates were lyophilized, dissolved in pH 2.2 citrate buffer, and applied directly to the amino acid analyzer.

RESULTS AND DISCUSSION

Isolation of Tryptic and Chymotryptic Peptides—By the combination of the procedures summarized in Tables II and III, and Figs. 1 through 4, a series of tryptic and chymotryptic peptides from CB1 and CB2 were isolated, which, together with CB3, CB4, and CB5 of the accompanying communica...
Primary Structure of Streptococcal Proteinase

CHART II

Amino acid sequence of CB2

CHART III

Sequence of CB3

Ala-Ile-Ser-Glu-Leu-Hse

CHART IV

Sequence of CB4

Ala-Asp-Val-Gly-Ile-Ser-Val-Asp-Hse

provided the necessary sequence information and overlaps for aligning the tryptic peptides of CB1 and CB2.

Sequences of CB1 to CB4—The sequences of CB1 to CB4 are shown in Charts I to IV. The documentation for the determination of the sequence of these peptides are in the miniprint supplement.

Hydrolysis of Peptides and PTH-Derivatives with Methanesulfonic Acid—The use of methanesulfonic acid in lieu of HCl for the hydrolysis of peptides offers the advantage that tryptophan is not destroyed during the hydrolysis. This procedure was used to great advantage for the regeneration of amino acid from the PTH derivatives (Table I) and in the routine hydrolysis of tryptic and chymotryptic peptides in the present study. The isolation, characterization, and sequencing of the tryptophan-containing peptides CB2-T4, CB2-C7, CB2-C7a, and CB2-C7b depends on the successful application of this technique.

Acknowledgments—The samples of zymogen used in this study and the accompanying manuscripts were prepared by Dr. S. Elliott in the laboratory of Drs. M. McCarty and R. C. Lancefield at the Rockefeller University. We offer our sincere thanks for their cooperation throughout the course of this research. We thank Drs. Francis J. Morgan and Robert E. Canfield of Columbia University, New York, for assisting us in the sequencer analysis, and to Mr. N. Alonzo for supervising the amino acid analyses. We are particularly grateful to Ms. J. R. Wysocki for her expert technical assistance.

REFERENCES

Primary Structure of Streptococcal Proteinase 1947

...
Primary structure of streptococcal proteinase. I Isolation, composition, and amino acid sequences of the tryptic and chymotryptic peptides of cyanogen bromide fragments 1 to 4.
A A Kortt, R J Wysocki and T Y Liu


Access the most updated version of this article at [http://www.jbc.org/content/251/7/1941](http://www.jbc.org/content/251/7/1941)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/251/7/1941.full.html#ref-list-1](http://www.jbc.org/content/251/7/1941.full.html#ref-list-1)