Thermophilic Streptomyces Alkaline Proteinase

I. ISOLATION, CRYSTALLIZATION, AND PHYSICOCHEMICAL PROPERTIES

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SUMMARY

The isolation and crystallization of thermostable alkaline proteinases (A and B) from a thermophilic Streptomyces by sulfoethyl-Sepharose and diethyl-(2-hydroxypropyl)aminoethyl-Sepharose column chromatography are described. The purified preparations were essentially homogeneous by such criteria as sedimentation analysis in the ultracentrifuge and disc electrophoresis.

The physicochemical properties of the proteinase B are as follows: the molecular weight determined by gel filtration and sedimentation equilibrium, 21,500; the sedimentation coefficient, $s_{20,W} = 3.00$ S; the intrinsic viscosity, $[\eta] = 0.030$ dl per g; the isoelectric point, pH 9.5; the partial specific volume, $\bar{v} = 0.719$ ml per g; the nitrogen content, 16.3% $; E_{280}^\text{m} = 8.21$ cm at 280 nm. 18.2. The enzyme is composed of 210 residues of amino acid per molecule (Lys, His, Arg, Cys, Asp, Thr, Ser, Gln, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Trp, amide-N). The enzyme contains 1 cysteine residue, but no disulfide bond. The NH$_2$- and COOH-terminal residues were found to be tyrosines. Optical rotatory dispersion studies showed that the enzyme was a compact globular protein having 10 to 15% $\alpha$ helix. The properties of the A component were largely identical with those of the B component, except for the amide content.

A few attempts have been made to purify the proteolytic enzymes of thermophilic actinomycetes. The authors have characterized the proteinase of Streptomyces rectus by sulfoethyl-Sepharose and diethyl-(2-hydroxypropyl)aminoethyl-Sepharose chromatography. The purified preparations were essentially homogeneous by such criteria as sedimentation analysis in the ultracentrifuge and disc electrophoresis. In these respects, the proteolytic enzymes of thermophilic actinomycetes seem to have unique properties compared with those of other microbial alkaline proteinases. However, the detailed physicochemical properties of the former have never been investigated.

In our first report (1), the purification was conducted by Cm-Sepharose chromatography. The purified preparation was homogeneous as judged by ultracentrifugation, but further investigation has shown that the preparation contains some autolytic products and is contaminated with a slight activity of carboxypeptidase.

The present paper describes a largely revised purification, crystallization, and physicochemical properties of the enzyme.

EXPERIMENTAL PROCEDURE

Materials—The crude enzyme preparation prepared by ethanol precipitation of the culture filtrate after submerged fermentation of the thermophilic streptomycete S. rectus var. proteolyticus ATCC 21067 was obtained from Seishin Pharmaceutical Co., Ltd., Japan. Sephadex G-100, Cm-Sepharose C-50, SE-Sepharose C-50, and QAE-Sepharose A-50 were products of Pharmacia, Sweden. Diisopropyl phosphofluoridate was obtained from Sigma. N-Acetyltyrosine ethyl ester and benzoylcarbonyl-Gly-L-Leu were purchased from Protein Research Foundation, Osaka, Japan. Lysozyme (twice crystallized) and pepsin (twice crystallized) were obtained from Nutritional Biochemical Corporation. Trichloroacetic acid-activated enzyme was prepared according to the method of Tsuru et al. (4).

Protein Concentration—Protein concentration was determined from absorbance at 280 nm by the factor $E_{1%}^{280} = 18.2$.

Enzyme Assay—Proteolytic activity against casein was measured by the method previously described (5) and expressed as proteinase unit. Esterase activity was measured against N-acetyltirosine ethyl ester in a pH-stat (Radiometer model SER2/AUB1/TTT1 Autotitrator) with 0.05 N NaOH as titrant. The reaction solution was 4 ml in volume containing 0.01 M N-acetyltirosine ethyl ester, 0.1 M NaCl, and 5 mM borate. The reaction was carried out at 25$^\circ$ and pH 8.0. One esterase unit is defined as the enzyme activity which hydrolyzes 1 pmole N-acetyltyrosine ethyl ester per min. Carboxypeptidase activity against benzoylcarbonyl-Gly-L-Leu was determined as previously described (5).

Purification—The crude enzyme preparation (1 kg) was dissolved in 10 liters of 5 mM calcium acetate buffer, pH 5.6. Insoluble materials were removed by centrifugation. The supernatant was dialyzed against the same buffer. The enzyme was adsorbed on Cm-Sepharose at pH 5.6 in 0.01 M sodium acetate
buffer and eluted batchwise three times with 0.5 M sodium acetate buffer, pH 5.6. The eluates were collected, diazloyzed against 5 mM calcium acetate buffer, pH 5.6, and lyophilized.

A 2.0-g sample of the lyophilized preparation was dissolved in 100 ml of 0.01 M borate buffer, pH 9.3, and diazloyzed against the same buffer at 0° overnight. The small amount of insoluble material that formed were centrifuged and discarded. The supernatant solution was passed through a QAE-Sephadex column (5 × 80 cm) equilibrated with 0.01 M borate buffer, pH 9.3, at a flow rate of 20 ml per hour. The active fractions were collected.

The eluate from QAE-Sephadex was adjusted to pH 5.6 with N HCl and diazloyzed against 0.025 M calcium acetate buffer, pH 5.6. The enzyme was adsorbed on a SE-Sephadex column (3 × 60 cm) equilibrated with the same buffer. Elution was performed with an exponential gradient from 0.025 M to 0.1 M calcium ion concentration in the aforementioned buffer. The first active component was designated as proteinase A, and the second as proteinase B. Each fraction was rechromatographed in the same condition using a column (1.5 × 40 cm). The active fraction was diazloyzed against 5 mM calcium acetate buffer, pH 5.6, and lyophilized.

Crystallization—The purified enzyme was dissolved in 0.01 M calcium acetate buffer, pH 5.6, containing 2.0 M NaCl, to 2% protein concentration. After standing at 4° overnight, the crystals that were formed were collected by centrifugation. For activity assay, the crystals were suspended in 0.01 M calcium acetate buffer, pH 5.6, and the suspension was diazloyzed against the same buffer to complete dissolution.

Sedimentation Analyses—Sedimentation velocity analyses were performed with a Hitachi model UCA-1 ultracentrifuge at 55,430 rpm at 20°. Sedimentation equilibrium analyses were performed according to the method of Yphantis (6) with a Hitachi Tiselius electrophoresis apparatus model HTD-1 equipped with the schlieren cylindrical lens system over a pH range of 8 to 11. All the buffers used were borate-HCl or borate-Na₂CO₃ with an ionic strength of 0.15. The specific conductance of the enzyme solution was determined with a cell of 50 mm path length. The data were treated against 5 mM calcium acetate buffer, pH 5.6, and lyophilized.

Ultraviolet Absorption Spectrum—The spectrum was determined with a Hitachi model EPS-2 recording spectrophotometer.

Optical Rotatory Dispersion—Measurements of optical rotatory dispersion were made with a Jasco ORD/UV-5 recording spectropolarimeter at room temperature. For the measurements in the region of 300 to 600 nm, 0.8 to 1.0% protein solutions were used with a cell of 50 mm path length. The data were treated by the method of Yang and Doty (9) to determine the dispersion constant, \( \alpha \). The Moffitt-Yang equation (10) was also applied to determine the parameters, \( a_0 \) and \( b_0 \), employing a value of 212 nm for \( \lambda_c \). The far ultraviolet optical rotatory dispersion measurements were made in a 1 mm path length quartz cell with 0.01 to 0.1% protein solutions. Measurements below 210 nm were made under a nitrogen gas stream. Results were expressed as reduced mean residue rotations, [\( \theta \)] [\( r \)] . The dispersion of the refractive index of the solvent was assumed to be that for pure water. The mean residue weight (101.8) was calculated from the amino acid composition and molecular weight.

Amino Acid Analyses—The enzyme proteins inactivated with trichloroacetic acid were hydrolyzed with glass-distilled 6 N HCl in sealed, evacuated glass tubes at 110° at 1° for 24, 48, and 72 hours. Amino acid analyses were performed with a Hitachi model 034 automatic amino acid analyzer, according to the procedure of Moore and Stein (11). An independent determination of cysteine as cysteic acid was made by the method of Moore (12). The content of cysteine was determined by the spectrophotometric method of Goodwin and Morton (13). The amide NH, was estimated by the method of Rees (14). Free sulphydryl content was measured spectrophotometrically with p-chloromercuribenzoate by the method of Boyer (15).

NH₂-terminal Amino Acid Determination—The analysis was made by the method of Sanger (16). The trichloroacetic acid-inactivated enzyme was dinitrophenylated as described by Fraenkel-Conrat et al. (17). The DNP-protein was hydrolyzed in sealed, evacuated tubes for 16 hours at 105° with 6 N HCl. The ether-soluble DNP-amino acids were identified by two-dimensional thin layer chromatography on Silica Gel G film (18). The identity of DNP-amino acids was verified through regeneration of the free amino acids by heating with concentrated ammonia (19) followed by conventional amino acid chromatography.

COOH-terminal Amino Acid Analyses—Weighed portions of the trichloroacetic acid-inactivated enzyme were dissolved in 0.1 M phosphate buffer, pH 8.0, containing 0.056 M sodium lauryl sulfate, as described by Buhler (20), and incubated with carboxypeptidase A at 25° with a substrate to enzyme ratio of 50:1 (mole/mole). Samples were removed periodically and the reaction was stopped by addition of Dowex 50-X8 (H⁺ form). The amino acids adsorbed on the resin were eluted with 3 N NH₄OH and determined quantitatively in the Hitachi amino acid analyzer.

Nitrogen Analyses—The micro-Kjeldahl method was used for the determination of the nitrogen content of the enzyme.

RESULTS

Purification

The results of purification are summarized in Table I. The specific activities increased about 3-fold and the yield was 37% in total. The specific activities of proteinases A and B were the same for both casein and N-acetyltirosine ethyl ester. Contaminating carboxypeptidase was completely removed by QAE-Sephadex chromatography. The use of calcium acetate buffer in SE-Sephadex chromatography prevented autolysis completely, and a single peak was obtained in rechromatography (Fig. 1).

The crystals were obtained in high yield and were thin octagonal plates for both proteinases. However, in the following experiments, lyophilized preparations after rechromatography were used, since the crystals were markedly insoluble and showed no increase in specific activity.
The homogeneity of the purified preparations were examined in disc polyacrylamide gel electrophoresis (Fig. 2) and sedimentation analysis (Fig. 3). Both methods demonstrated the homogeneity of the enzymes.

**Physical Properties**

* Sedimentation Coefficient—$s_{20, w}$ was plotted as a function of protein concentration (0.3 to 1.2%) in 0.1 M NaCl buffered at pH 5.6 with 5 mM calcium acetate. No dependence of concentration was observed for either proteinase. The extrapolation to zero protein concentration yielded a value of 3.00 S for both of the enzymes.

### Table I

Results of purification of thermophilic *Streptomyces* alkaline proteinases

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Volume</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>P.U.$^a$</td>
<td>P.U./mg protein</td>
<td>%</td>
</tr>
<tr>
<td>1. Extraction</td>
<td>1000</td>
<td>3258</td>
<td>423</td>
<td>100</td>
</tr>
<tr>
<td>2. Batch treatment with Cm-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. QAE-Sephadex chromatography</td>
<td>540</td>
<td>2417</td>
<td>1007</td>
<td>74.2</td>
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<tr>
<td>4. SE-Sephadex chromatography</td>
<td>620</td>
<td>1557</td>
<td>1102</td>
<td>47.8</td>
</tr>
<tr>
<td>Proteinase A</td>
<td>155</td>
<td>642</td>
<td>1142</td>
<td>19.7</td>
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<tr>
<td>Proteinase B</td>
<td>165</td>
<td>860</td>
<td>1140</td>
<td>26.4</td>
</tr>
<tr>
<td>5. Rechromatography</td>
<td>154</td>
<td>573</td>
<td>1198</td>
<td>17.6</td>
</tr>
<tr>
<td>Proteinase A</td>
<td>158</td>
<td>834</td>
<td>1201</td>
<td>25.5</td>
</tr>
<tr>
<td>Proteinase B</td>
<td>160</td>
<td>1198</td>
<td>1201</td>
<td>22.7</td>
</tr>
<tr>
<td>6. Crystallization</td>
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<tr>
<td>Proteinase A</td>
<td>50</td>
<td>486</td>
<td>1006</td>
<td>14.9</td>
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<tr>
<td>Proteinase B</td>
<td>50</td>
<td>739</td>
<td>1160</td>
<td>22.7</td>
</tr>
</tbody>
</table>

* Proteinase units.

**Fig. 1 (left).** Rechromatography of SE-Sephadex Fractions A and B. Each effluent of the first SE-Sephadex chromatography was diluted against 0.025 M calcium acetate buffer, pH 5.6. A part of the solution (A, 10 ml; B, 20 ml) was applied to a column (1.5 X 40 cm) of SE-Sephadex C-50 in the same buffer. Elution was performed at $4^\circ$ by an exponential gradient from 0.025 M to 0.1 M calcium acetate buffer, pH 5.6. The flow rate was 10 ml per hour. One fraction was 5 ml per tube. ●, absorbance at 280 nm; ○, proteinase activity against casein.

**Fig. 2 (right).** Disc electrophoretic patterns of purified proteinases A and B of thermophilic *Streptomyces* in polyacrylamide gels at pH 4.3. The electrophoresis was conducted at room temperature for 60 min at 5 ma per tube, and the protein was stained with aniline black. Arrows indicate the position of sample applied (each 80 µg). 1, proteinase A; 2, proteinase B; 3, mixture of A and B.

**Fig. 3.** Sedimentation patterns of purified proteinases A and B of thermophilic *Streptomyces* at 55,430 rpm. A, proteinase A; B, proteinase B in 5 mM calcium acetate buffer, pH 5.6, containing 0.1 M NaCl. Protein concentration was 1.2%. Photographs were taken at the indicated times after the maximum speed had been attained. Bar angle, 70 degrees. Temperature, $20^\circ$. 

$^{a} s_{20, w}$.
Intrinsic Viscosity—The reduced viscosity, \( \eta_r/c \), was plotted as a function of protein concentration (0.4 to 1.5%) in 1 mM calcium acetate buffer, pH 5.6, where \( \eta_r \) is the specific viscosity and \( c \) is the protein concentration. No dependence on concentration was observed for either proteinase. A value of 0.030 dl per g for intrinsic viscosity was obtained for both enzymes.

Isoelectric Point—Moving boundary electrophoresis was performed with 0.7% protein solution of proteinase A or B inactivated with diisopropyl phosphofluoridate to prevent autolysis. The mobilities were determined using the descending boundary. As shown in Fig. 4, the isoelectric points of proteinases A and B were found at pH 9.35 and pH 9.50, respectively.

Molecular Weight—The molecular weight was determined by two methods. One is the gel filtration method of Andrews (8). As shown in Fig. 5, a straight line was obtained between \( V_e \) (elution volume) and log molecular weight of standard proteins. The proteinases A and B were eluted in the same position corresponding to a molecular weight of 21,500.

Another determination was performed by the sedimentation equilibrium method of Yphantis (6). The apparent molecular weight as a function of protein concentration is plotted in Fig. 6. The extrapolation to zero concentration yielded a value of 21,500 for both enzymes.

Ultraviolet Absorption Spectrum—The spectrum was recorded with a 0.027% solution of proteinase B in 0.02 M calcium acetate buffer, pH 5.6. The absorption maximum was found to be at 280 nm and the minimum at 250 nm. The curve represented a typical absorption pattern of protein, the ratio of the absorbance at 280 nm to that at 260 nm being 2.22. The \( \epsilon_{280} \) value at 280 nm was 18.2. A slight shoulder at around 290 nm was noted which is possibly due to a contribution to the absorption pattern from tryptophan residues. The spectrum of proteinase A was the same as that of proteinase B.

Optical Rotatory Dispersion—The optical rotatory dispersion parameters are given in Table II. The helix contents calculated from the values of \( b_0 \) and \( [\alpha]_{233} \) were 15 and 9%, respectively. Therefore, it is reasonable to consider that the helix content is about 10 to 15%. No significant difference was evident between proteinases A and B on the basis of optical rotatory dispersion data.

The ultraviolet optical rotatory dispersion curve of proteinase B is shown in Fig. 7. The curve is typical for a polypeptide

![Fig. 4. Electrophoretic mobility of thermophilic Streptomyces alkaline proteinases A and B as a function of pH.](image1)

![Fig. 5. Estimation of molecular weights of thermophilic Streptomyces proteinases A and B by Sephadex G-100 gel filtration.](image2)

![Fig. 6. Molecular weights of thermophilic Streptomyces proteinases A and B as a function of protein concentration.](image3)

![Table II](image4)
Fig. 7. Ultraviolet ORD spectra of thermophilic Streptomyces proteinase B. Measurements were made with 0.01 to 0.1% solutions of proteinase B in 0.1 mM or 20 mM calcium acetate buffer, pH 5.6, at room temperature.

Amino Acid Composition—The results of amino acid analyses are given in Table III. The values are expressed as number of residues per molecule, assuming a molecular weight of 21,500. The results indicate that proteinase B consists of 210 amino acid residues per molecule and contains one cysteine residue. p-Chloromercuribenzoate titration showed 0.7 to 0.8 residue of free sulfhydryl group per molecule. No difference was detected between proteinases A and B, except the amide content. The A fraction contained 24 moles of amide-NH₂ per molecule, while the B fraction contained 25 moles.

Nitrogen Content—Nitrogen analyses by the micro-Kjeldahl method yielded a value of 16.0% for proteinase A and 16.3% for proteinase B.

NH₂-terminal Amino Acid—Two-dimensional silica gel thin layer chromatography of hydrolysates of DNP-protein showed that di-DNP-tyrosine or di-DNP-lysine was the only detectable DNP-amino acid present. For the identification, the DNP-amino acid obtained was hydrolyzed by ammonium hydroxide and the amino acid regenerated was identified by two-dimensional thin layer chromatography. The results showed that tyrosine was the only detectable amino acid. Table IV summarizes the results of the NH₂-terminal analyses. The yield was about 30%. The data indicate that 1 mole of tyrosine is present as the NH₂-terminal residue in both proteinases.

COOH-terminal Amino Acid—Among the amino acids released from proteinase B by the action of carboxypeptidase A, tyrosine was the first to appear and alanine was the second. On 3-hour incubation, 0.99 pmole of tyrosine, 0.88 pmole of alanine, and 0.55 pmole of valine were released from 1.0 pmole of enzyme. Other minor components were glutamic acid (0.23 pmole), isoleucine (0.21 pmole), and serine (0.14 pmole). The result indicates that tyrosine is the COOH-terminal amino acid and alanine the penultimate residue. The experiment with proteinase A showed the same result.

### Table III

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Number of residues per molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.8</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>0.9</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>26.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>16.9</td>
</tr>
<tr>
<td>Serine</td>
<td>21.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>10.0</td>
</tr>
<tr>
<td>Proline</td>
<td>9.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>24.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>32.5</td>
</tr>
<tr>
<td>Valine</td>
<td>16.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>10.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>11.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.7</td>
</tr>
<tr>
<td>Ammonia</td>
<td>28.4</td>
</tr>
</tbody>
</table>

Total: 210

* Based on a molecular weight of 21,500.
* Average values from the 24-, 48-, and 72-hour hydrolysates in duplicate.
* Measured as cysteic acid after performic acid oxidation.
* Values extrapolated to zero time.
* Average value from 72-hour hydrolysates.
* Determined by spectrophotometric method (14).
* Values obtained by the Conway method.

**Table IV**

<table>
<thead>
<tr>
<th>DNP-protein</th>
<th>Di-DNP-Tyr found*</th>
<th>NH₂-terminal residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase A</td>
<td>0.031</td>
<td>1.0</td>
</tr>
<tr>
<td>Proteinase B</td>
<td>0.033</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Average values of duplicate determinations.
amidation occurred in proteins. Robinson et al. (22) reported recently that spontaneous de-

difference would lead to a difference in the isoelectric point. The proteinase A might have been formed by deamidation of filtration method, but it is larger than those of

essential difference was seen in the amide content. Proteinase B the previous study showed that the pH optima, the stability, and the sensitivity to inhibitors were identical. The only es-
tion coefficient, viscosity, optical rotatory dispersion, amino

volume was calculated from the amino acid composition ac-

ting to the method of Cohn and Edsall (21).

using 0.02 m phosphate buffer, pH 5.8. Under these conditions, it was impossible to obtain a chromatographically homogeneous preparation, since autolysis occurred during the operation of chromatography. Moreover, the carboxypeptidase which was abundantly present in the culture filtrate (5) could not be re-
moved. The present method overcomes these difficulties. Autolysis is completely prevented by the use of calcium acetate in SE-Sephadex chromatography and the carboxypeptidase is successfully removed by QAE-Sephadex at pH 9.3. However, the present and earlier methods achieved an increase in specific activity to almost the same extent.

The thermophilic Streptomyces alkaline proteinase has been shown to be a protein molecule that exists as a single poly-

peptide chain with no S-S bond. The enzyme contains 210 amino acid residues with tyrosines as the NH*- and COOH-
terminal amino acids. The physicochemical properties of the proteinase B are summarized in Table V. The partial specific volume was calculated from the amino acid composition ac-
cording to the method of Cohn and Edsall (21). The proteinases A and B are remarkably homologous with one another. No significant difference could be detected be-
tween the two in such criteria as molecular weight, sedimentation coefficient, viscosity, optical rotatory dispersion, amino acid composition, terminal amino acid, and specific activity. The previous study showed that the pH optima, the stability, and the sensitivity to inhibitors were identical. The only es-
tional difference was seen in the amide content. Proteinase B contained 1 mol more amide-NH2 than proteinase A. The difference would lead to a difference in the isoelectric point. The proteinase A might have been formed by deamidation of asparaginyl or glutaminyl residues of the proteinase B. In fact, Robinson et al. (22) reported recently that spontaneous de-
amidation occurred in proteins.

The molecular weight of thermophilic Streptomyces proteinase was determined to be 21,500 by the sedimentation equilibrium method and the gel filtration method. The value resembles that of T. fusca which Desai and Dhala (3) determined by gel filtration method, but it is larger than those of Streptomyces griseus alkaline proteinases (23).

A characteristic feature of the amino acid composition of thermophilic Streptomyces alkaline proteinase is the presence of 1 cysteine residue which is reactive to p-chloromercuribenzoate.

Those of S. griseus contain disulfide bonds, but no cysteines (24, 25). Aspergillus alkaline proteinases contain no cystine or cysteine (26, 27). It is interesting that the proteinases of two other genera of thermophilic actinomycetes are also inhibited by p-chloromercuribenzoate (3, 28). As pointed out by Ovcharov and Kononovol (28), the fact may indicate different structures at the active sites of proteinases from mesophilic and thermo-

philic microorganisms.

Other unique characteristics of the thermophilic Streptomyces proteinase are its NH2- and COOH-terminal amino acids. Un-
til now, proteinases with tyrosines as NH2- or COOH-terminal amino acids are unknown, except α- and γ-chymotrypsin pos-
sessing a COOH terminal tyrosine, which is formed during the activation process of chymotrypsinogen (29). The NH2- and COOH-terminal residues of subtilisins are alanine and glutamine, respectively (30, 31). Most of the Aspergillus alkaline proteinases have glycine and alanine (32, 33). The NH2-terminal residue of S. griseus proteinase is isoleucine (25).

The optical rotatory dispersion studies have shown that the thermophilic Streptomyces proteinase is a compact globular protein with α-helical structure. The content of α helix was found to be about 10 to 15%, which was similar to that in Aspergillus sojae alkaline proteinase (34). The thermostability of the thermophilic Streptomyces proteinase would probably be caused by the rigid conformation of the molecule. The relation of thermostability to structure of this proteinase will be reported in a succeeding paper of this series.

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