A new polygalacturonase was found in a culture filtrate of *Aureobasidium pullulans*. The enzyme was purified and obtained in crystalline form with 10% recovery. The crystalline enzyme was a homogeneous protein by analyses by sedimentation and electrophoresis. The enzyme was most active around pH 4.5, and stable in the pH range of 4 to 6. Its molecular weight was 42,000 and its isoelectric point was pH 6.0. The enzyme was an endo-polygalacturonase, catalyzing the cleavage of glycosidic bonds of polygalacturonic acid at random.

The enzyme had less protopectinase activity than those of the endo-polygalacturonases that were isolated as protopectin-solubilizing enzymes from *Trichosporon penicillatum*, *Kluyveromycetes fragilis*, and *Galactomyces reessii*. Some characteristics were compared with the endo-polygalacturonases, which have potent protopectinase activity.

Previously, Sakai *et al.*\(^1\) isolated protopectin-solubilizing enzymes (protopectinases) and found that these enzymes have endo-polygalacturonase (endo-PG) activity.

Endo-PG is an enzyme that depolymerizes polygalacturonic acid (and/or pectin) and that is supposed to play a part in the first step of polygalacturonic acid assimilation in organisms. However, we found that some organisms producing endo-PG with protopectinase activity did not assimilate polygalacturonic acid. These suggested that there are two types of endo-PGs; one has protopectinase activity that helps degrade protopectin to result in degrading plant tissues, and the other degrades pectic substances to form galacturonic acid.

To confirm this possibility, an endo-PG produced by a pectin-assimilating microorganism was isolated and its properties were compared with the endo-PGs isolated as protopectin-solubilizing enzymes.

**MATERIALS AND METHODS**

Chemicals. CM-Sephadex C-50 and Sephadex G-75 were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Ampholine® was obtained from the LKB Co. (Bromma, Sweden). Standard proteins for the determination of the molecular weight and isoelectric point of the enzyme were from Sigma Chemicals (St. Louis, U.S.A.) and the Oriental Yeast Co. (Tokyo), respectively. All other chemicals were Wako Pure Chemical Industries (Osaka) certified reagent grade.

Microorganism and culture. *Aureobasidium pullulans* AY-037, isolated as a pectin-assimilating strain from an infected vegetable, was used throughout this study. The strain is deposited in the collection of the Fermentation Research Institute (Tsukuba, Ibaraki) with the strain number FERM P-6700. The microorganism was maintained on agar slants of a medium containing 0.5% pectin, 0.3% peptone, 0.05% yeast extract, 0.1% KH₂PO₄, and 0.05% NaCl, pH 6.5. For the production of endo-PG, the organism was cultured in a medium containing 0.5% peptin, 0.3% (NH₄)₂SO₄, 0.05% yeast extract, 0.05% MgSO₄·7H₂O, 0.1% KH₂PO₄, 0.05% CaCl₂·2H₂O, and 0.05% NaCl, pH 5.5, at 30°C for 30 hr on a shaker.

**Enzyme assay.** Protopectinase activity was determined

\(^1\) Studies on the Enzyme Produced by Yeasts. Part VII. For Part VI, see ref. 5.

* To whom correspondence should be addressed.

Abbreviations: endo-PG, endo-polygalacturonase; PE, pectinesterase.
by measuring the amount of pectic substance liberated from protopectin by the carbazole-sulfuric acid method, as follows: The reaction mixture was composed of 10 mg protopectin, 4 mmol acetate buffer, pH 5.0, and 0.5 ml of enzyme solution in a total volume of 2.5 ml, and the reaction was performed at 37°C for 30 min. One unit of enzyme activity was defined as the activity which liberated pectic substance corresponding to 1 μmol of D-galacturonic acid per ml reaction mixture, under the above conditions. Protopectin was prepared by the method of Sakai and Okushima.

Pectinesterase activity was determined as follows: Two milliliters of 1% apple pectin solution (10 mM acetate buffer, pH 4.0) containing 100 mM NaCl, 10 mM CaCl₂·2H₂O, and enzyme solution was incubated at 37°C for 30 min. The reaction was terminated by heating in a boiling water bath for 3 min, and carboxyl groups formed were determined by titration with 0.01 N NaOH using a titrator (Radiometer, Copenhagen, Denmark). One unit of enzyme activity was defined as a activity forming 1 μmol of carboxyl groups in the above conditions.

Pectic acid-liquefying activity (endo-PG activity) was determined as follows: To 6 ml of 0.5% pectic acid solution in McIlvaine buffer, pH 5.0, was added 1 ml of enzyme solution, and this mixture was incubated at 37°C for 5 min. The rate of viscosity reduction (A) was calculated by the equation $A = (T_a - T) / (T_a - T_0) \times 100$, where $T_a$ is the flow time (sec) of pectic acid solution added to the heat-inactivated enzyme, $T$ is the flow time (sec) of the reaction mixture, and $T_0$ is the flow time (sec) of water added to heat-inactivated enzyme. One unit of enzyme activity was defined as the activity reducing viscosity by 50% under the above conditions.

Pectic acid-saccharifying activity was determined by measuring the release of reducing groups according to the method of Jansen and MacDonnell.

Electrophoresis. Polyacrylamide gel electrophoresis was performed with 7% polyacrylamide gel according to the method described by Davis (at pH 4.0). SDS-polyacrylamide gel electrophoresis was performed as described by Weber and Osborn.

Isoelectric focusing. For determination of the pH value of the enzyme, disc gel isoelectric focusing was performed in 7.5% polyacrylamide gel containing 2% Ampholine (pH 3.5 to 10.0). Isoelectric focusing was performed at constant voltage (200 V) for 4 hr at 5°C. As anode and cathode solutions, 20 mM H₃PO₄ and 1 mM NaOH were used, respectively. After isoelectric focusing, the protein in the gel was stained with Coomassie Brilliant Blue G-250-trichloroacetic acid solution. The isoelectric point of the enzyme was determined on a graph of the relation between the mobility of marker proteins and their isoelectric points.

Determination of the molecular weight. For the determination of the molecular weight, the enzyme was subjected to electrophoresis by the method of Weber and Osborn using polyacrylamide gel containing SDS at a concentration of 0.1%.

Determination of amino acid composition. The amino acid composition of the enzyme was determined with a hydrolyzate of the enzyme on a Hitachi KLA-3B amino acid analyzer. For the determination of the amino acid composition, the enzyme was hydrolyzed for 24, 48, or 72 hr at 105°C with 6 N HCl in a sealed tube filled with nitrogen gas.

Determination of protein. Protein was determined by the method of Lowry et al. using bovine serum albumin as a standard.

RESULTS

Production of endo-PG

The endo-PG of the organism was produced in the culture fluid, accompanied with pectinesterase; it increased as the growth increased, and leveled off as the culture reached the stationary phase (Fig. 1).

Purification of endo-PG

The culture filtrate (40 liters) obtained from a 30-hr culture was concentrated to one-tenth of the original volume using an Amincon hollow fiber dialyzer (Lexington, U.S.A.), and it was used as the crude enzyme preparation.
Endo-polygalacturonase from A. pullulans

The concentrated culture filtrate was dialyzed thoroughly against 50 mM acetate buffer, pH 5.0, and then applied to a CM-Sephadex C-50 column (4.5 x 25 cm) equilibrated with 50 mM acetate buffer, pH 5.0. The column was washed with 300 ml of acetate buffer, pH 5.0, and then the enzyme was chromatographed with a linear gradient with 0 to 400 mM sodium chloride in 50 mM acetate buffer, pH 5.0, at a flow rate of 150 ml/hr. The endo-PG activity was recovered in a protein peak and the PE activity was also recovered in the peak.

The active fractions were pooled, concentrated to 3 ml, and applied to a Sephadex G-200 column (2.2 x 90 cm) equilibrated with 50 mM acetate buffer, pH 5.0. Chromatography was performed using the same buffer at a flow rate of 7.6 ml/hr. The endo-PG and PE activities were recovered in the same protein peak.

The active fractions were pooled and dialyzed against 50 mM acetate buffer, pH 5.0, and applied to a CM-Sephadex C-50 column (3 x 34 cm) in the same manner as mentioned above. As shown in Fig. 2, the endo-PG and PE activities were partially separated. The fractions containing predominantly endo-PG activity were pooled and concentrated in vacuo to 2 ml.

To the fraction was added solid ammonium sulfate until faint turbidity was observed. After standing overnight in a refrigerator, the enzyme formed parallelogram-shaped crystals (Fig. 3). The crystallization was repeated three times. The results of purification of the enzyme are summarized in Table I. This procedure resulted in 35-fold purification in the specific activity with about 11% recovery of the enzyme. Pectinesterase activity was not detected in the crystallized enzyme.
**Table I. Purification of Endo-Polygalacturonase from Culture Filtrate of Aureobasidium pullulans**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (x 10^4 units)</th>
<th>Specific activity (units/mg)</th>
<th>Relative purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>4,000</td>
<td>41.0</td>
<td>100</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>1st CM-Sephadex C-50 column</td>
<td>70.2</td>
<td>13.8</td>
<td>1,960</td>
<td>19.6</td>
<td>33.7</td>
</tr>
<tr>
<td>chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-200 column chromatography</td>
<td>54.8</td>
<td>14.5</td>
<td>2,640</td>
<td>26.4</td>
<td>35.4</td>
</tr>
<tr>
<td>2nd CM-Sephadex C-50 column</td>
<td>34.2</td>
<td>9.2</td>
<td>2,690</td>
<td>26.0</td>
<td>22.4</td>
</tr>
<tr>
<td>chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystallization</td>
<td>12.6</td>
<td>4.4</td>
<td>3,490</td>
<td>34.9</td>
<td>10.7</td>
</tr>
</tbody>
</table>

*Fig. 4. Polyacrylamide Gel Electrophoresis of Endo-polygalacturonase from *Aureobasidium pullulans*.

a: Polyacrylamide gel electrophoresis. A sample of crystalline enzyme (20 µg) was applied to a column and run at pH 4.0 for 2.5 hr at 5 mA per column. The direction of electrophoresis was from left (anode) to right (cathode).

b: SDS-polyacrylamide gel electrophoresis. Crystalline enzyme (20 µg) treated with sodium dodecyl sulfate (SDS) at 100°C for 5 min was applied on a 10% gel containing 0.1% SDS, and run at 7 mA per column for 5 hr. The direction of electrophoresis was from left (cathode) to right (anode).

**Homogeneity of the enzyme**

The enzyme preparation obtained was homogeneous as judged by polyacrylamide gel electrophoresis, both in the presence and absence of sodium dodecyl sulfate (SDS) (Fig. 4), and by sedimentation analysis (Fig. 5a).

**Physicochemical properties**

**Extinction coefficient.** The ultraviolet absorption spectrum of the enzyme was measured in distilled water. The $E_{1%}^{280}$ was determined to be 15.8.

**Molecular weight.** The molecular weight was determined to be 42,000 by SDS-polyacrylamide gel electrophoresis (Fig. 6).

**Sedimentation coefficient.** The apparent sedimentation velocities measured at various protein concentrations by analytical centrifugation were plotted against reciprocals of protein concentration (Fig. 5b). The corrected sedimentation coefficient, $s_{20,w}$, was calculated to be 6.2 S, using the values obtained in Figs. 5b and 5c, and 0.370 cm^3/g for the partial specific volume for the enzyme protein.

**Isoelectric point.** The isoelectric point of the enzyme was determined by isoelectric focusing in Ampholine-polyacrylamide gel. The relative mobility was plotted with marker proteins, and from the graph (Fig. 7), the isoelectric point was determined to be around pH 6.0.

**Amino acid composition.** The amino acid composition of the enzyme was determined with the hydrolyzates of the enzyme obtained at 24-, 48-, and 72-hr hydrolysis at 105°C, and the amino acid content was estimated by extrapolation of the amino acid content determined to zero time hydrolysis. The results are presented in Table II. The enzyme contains aspartic acid (asparagine), serine, threonine, glycine, and glutamic acid (glutamine) at relatively high levels, and a noticeable characteristic is the absence of methionine. The enzyme is composed of 455 amino acid residues, assuming that the molecular weight is 42,000.

**Enzyme properties**

**Pectic acid-degrading activity.** The enzyme catalyzed the hydrolysis of pectic acid and strongly decreased the viscosity while slightly...
Endo-polygalacturonase from *A. pullulans*

**Fig. 5.** Ultracentrifugal Analysis of Endo-polygalacturonase from *Aureobasidium pullulans*.

a: Sedimentation pattern. The crystalline enzyme (11 mg/ml) was centrifuged at 52,000 rpm at 20°C.
b: Plot of log x (distance from the center of rotor to sedimentation peak of enzyme protein) vs. sedimentation time. The enzyme was used at a concentration of 11 mg (△), 5 mg (▲), and 2.5 mg (●) per ml of 20 mM acetate buffer, pH 5.0, containing 200 mM NaCl.
c: Plot of $s_{20,w}$ vs. protein concentration for determination of sedimentation coefficient.

**Fig. 6.** Determination of Molecular Weight of Endo-polygalacturonase from *Aureobasidium pullulans* by SDS-polyacrylamide Gel Electrophoresis.

Standard marker proteins: a, α-lactalbumin (Mw 14,400); b, soybean trypsin inhibitor (Mw 20,100); c, carbonic anhydrase (Mw 30,000); d, ovalbumin (Mw 43,000); e, bovine serum albumin (Mw 67,000); f, phosphorylase b (Mw 94,000). The open circle indicates the migration position of the enzyme.

**Fig. 7.** Determination of Isoelectric Point of Endo-polygalacturonase from *Aureobasidium pullulans* by Isoelectric Focusing.

Conditions of electrofocusing are described in the text. pH marker: cytochrome c (horse heart muscle) derivatives (Oriental Yeast Co. Ltd., Tokyo), pH values are a, 4.1; b, 4.9; c, 6.4; d, 8.3; e, 9.7; f, 10.6. The open circle indicates the migration position of the enzyme.
Table II. Amino Acid Composition of Endo-Polygalacturonase from *Aureobasidium pullulans*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>μg/sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>μmol/mg</th>
<th>Number of residues⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td>Lysine</td>
<td>84.0</td>
<td>0.46</td>
<td>19.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>99.1</td>
<td>0.52</td>
<td>21.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>23.6</td>
<td>0.11</td>
<td>4.6</td>
</tr>
<tr>
<td>Tryptophan&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91.8</td>
<td>0.36</td>
<td>15.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>(Asparagine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>191.9</td>
<td>1.19</td>
<td>50.0</td>
</tr>
<tr>
<td>Serine</td>
<td>177.5</td>
<td>1.36</td>
<td>57.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>229.5</td>
<td>1.56</td>
<td>65.5</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>(Glutamine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.1</td>
<td>0.27</td>
<td>11.3</td>
</tr>
<tr>
<td>Proline</td>
<td>31.7</td>
<td>0.22</td>
<td>9.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>154.7</td>
<td>1.67</td>
<td>70.1</td>
</tr>
<tr>
<td>Alanine</td>
<td>81.6</td>
<td>0.74</td>
<td>31.1</td>
</tr>
<tr>
<td>Half-cystine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40.2</td>
<td>0.21</td>
<td>8.6</td>
</tr>
<tr>
<td>Valine</td>
<td>91.4</td>
<td>0.63</td>
<td>26.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>99.9</td>
<td>0.62</td>
<td>25.9</td>
</tr>
<tr>
<td>Leucine</td>
<td>68.6</td>
<td>0.42</td>
<td>17.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>31.2</td>
<td>0.16</td>
<td>6.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>61.7</td>
<td>0.30</td>
<td>12.7</td>
</tr>
<tr>
<td>Ammonia</td>
<td>40.2</td>
<td></td>
<td>21.9</td>
</tr>
<tr>
<td>Recovery:</td>
<td>132.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Crystalline enzyme (1.25 mg) was used for the analysis.
<sup>b</sup> Calculated based on a molecular weight of 42,000.
<sup>c</sup> Determined spectrophotometrically by the method of Edelhoch.¹²
<sup>d</sup> Determined by the method of Crestfield et al.¹²
<sup>e</sup> Not detected.

---

Fig. 8. Action of Endo-polygalacturonase from *Aureobasidium pullulans* on Pectic Acid.

Details of the experiment are described in the text. ●, viscosity decreased; ▲, reducing group formed.

increased the reducing groups of the pectic acid-containing reaction medium (Fig. 8). But it did not catalyze the transeliminative cleavage of pectic acid. From these observations, the enzyme was confirmed to be an endo-PG.

*Effects of pH on stability and activity of the enzyme.* The effects of pH on the stability of the enzyme was investigated by preincubating for 30 min at 37°C in various buffers of pH 3.0 to 9.0. The enzyme activity was not lost in the pH range of 4.0 to 6.0, but much of the activity was lost at alkaline pHs (Fig. 9a).

The effects of pH on the enzyme activity was investigated and the results are shown in Fig. 9b. The pH-activity profile shows maximum activity around pH 4.0 to 5.0.

*Effect of temperature on activity and stability of the enzyme.* The enzyme was treated for 30 min (at pH 5.0) at the temperatures indicated in Fig. 10a, and then the remaining activity was assayed. The enzyme was stable at
Endo-polygalacturonase from *A. pullulans*

Fig. 9. Effects of pH on Activity and Stability of Endo-polygalacturonase from *Aureobasidium pullulans*.

- **a**: pH-Stability. The enzyme (30 units/ml) in 20 mM McIlvaine buffer (M), 20 mM K$_2$HPO$_4$-Na$_2$HPO$_4$ buffer (P), or 50 mM Tris-HCl buffer (T) containing 50 μg/ml bovine serum albumin was incubated at 37°C for 30 min, and remaining activities were determined under the standard assay conditions.
- **b**: Optimum pH. Enzyme activity was assayed in McIlvaine buffer.

Fig. 10. Effects of Temperature on Enzyme Activity and Stability.

- **a**: Thermo-stability. The enzyme (30 units/ml) in 20 mM McIlvaine buffer, pH 5.0, containing 50 μg/ml bovine serum albumin was kept for 30 min at various temperatures (20 to 75°C), and the remaining activities were assayed under the standard assay conditions.
- **b**: Optimum temperature. The enzyme activities were assayed at various temperatures under the standard assay conditions.

### TABLE III. RELEASE OF PECTIC SUBSTANCE FROM VARIOUS PROTOPECTINS BY ENDO-POLYGALACTURONASES FROM VARIOUS STRAINS

<table>
<thead>
<tr>
<th>Origin of protopectin</th>
<th>Pectin released (g/10 g protopectin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aur. pullulans</em></td>
</tr>
<tr>
<td></td>
<td>yield (%) <em>a</em></td>
</tr>
<tr>
<td>Citrus unshiu (peel)</td>
<td>0.11</td>
</tr>
<tr>
<td>Burdock</td>
<td>0.20</td>
</tr>
<tr>
<td>Radish</td>
<td>0.15</td>
</tr>
<tr>
<td>Watermelon (peel)</td>
<td>0.11</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The reactions were performed in a solution containing 10 g protopectin and 100 units (as endo-polygalacturonase activity) enzyme in 0.02 M acetate buffer, pH 5.0, in a total volume of 20 ml at 37°C for 2 hr. The enzymes used were obtained by the methods described in the following references: *a*, ref. 2; *b*, ref. 5, *c*, ref. 4.

* Per cent of pectin liberated to whole pectic substance in the sample. Whole pectic substance was determined by the method described by Stoddart et al. 141

temperatures lower than 40°C, and lost most of its activity at 75°C.

The optimal temperature for enzyme activity was 50 to 60°C (Fig. 10b).

Protopectinase activity. Protopectinase activity was determined using protopectin from
various origins, and the results are presented in Table III, with that of other endo-PGs. The enzyme had a protopectinase activity, but the activity was very low compared with the other endo-PGs listed.

DISCUSSION

In this paper, a novel endo-PG from *Aureobasidium pullulans* is described. Recently, we isolated protopectin-solubilizing enzymes (protopectinases) from *Trichosporon penicillatum*, *Galactomyces reessii*, and *Kluyveromyces fragilis*, and investigated their properties.¹⁻⁵) These three enzymes had polygalacturonic acid-degrading activities besides protopectinase activities even though they had other different properties, and they were regarded as endo-PGs.

Endo-PG catalyzing the degrading of polygalacturonic acid (and/or pectin) seems to have a different biological role from a protopectinase which catalyzes the degradation of plant tissues (maceration activity).

Therefore, we assumed that protopectinase activity is not a common property of endo-PGs, and there are two types of endo-PGs at least; one is an enzyme having potent polygalacturonic acid-degrading activity but not (or less) protopectinase activity, and the other is an enzyme having potent protopectinase activity besides polygalacturonic acid-degrading activity.

The strain dealt with in this paper assimilated pectin as a sole carbon source and produced endo-PG, which is induced by pectin. This suggested that the endo-PG has the biological role of degrading pectin. The protopectinase activity of the present enzyme was very low; one order lower than those of the endo-PGs isolated as protopectinases, as shown in Table III, and the fact supports our assuming that there are two types of endo-PGs, classified by their protopectinase activities.

There are numerous reports concerning endo-PGs, but few have dealt with their protopectinase activity. Therefore, the characteristics of the present enzyme were compared with those of other endo-PGs isolated as protopectinases (Table IV). The present enzyme showed a lower affinity for protopectin (from *Citrus unshiu*) than the other three enzymes; the $K_m$ value for protopectin was one order higher than those of the other endo-PGs, whereas they showed almost the same affinity for polygalacturonic acid (Table V). The difference of affinity for protopectin seems to be one of the reasons that endo-PG show a different protopectinase activity.

### Table IV. Some Properties of Endo-Polygalacturonases from Various Strains

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>A. pullulans</th>
<th>T. penicillatum</th>
<th>G. reessii</th>
<th>K. fragilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By SDS-electrophoresis</td>
<td>42,000</td>
<td>40,000</td>
<td>40,000</td>
<td>40,000</td>
</tr>
<tr>
<td>By gel filtration</td>
<td>36,000</td>
<td>30,000</td>
<td>30,000</td>
<td>30,000</td>
</tr>
<tr>
<td>By sedimentation</td>
<td>—</td>
<td>29,300</td>
<td>26,800</td>
<td>32,800</td>
</tr>
<tr>
<td>$S_{20.w}$</td>
<td>6.20S</td>
<td>3.66S</td>
<td>3.77S</td>
<td>2.99S</td>
</tr>
<tr>
<td>$E_{280nm}$</td>
<td>15.8</td>
<td>9.2</td>
<td>1.0</td>
<td>11.9</td>
</tr>
<tr>
<td>Sugar content (%)</td>
<td>—</td>
<td>1.7</td>
<td>2.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Isoelectric point (pH)</td>
<td>5.9⁻6.0</td>
<td>7.6⁻7.8</td>
<td>8.4⁻8.5</td>
<td>5.9</td>
</tr>
<tr>
<td>Optimal pH</td>
<td>4.5⁻5.5</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Optimal temperature (°C)</td>
<td>50⁻60</td>
<td>50</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Specific activity (Units)</td>
<td>Polygalacturonase</td>
<td>3,492</td>
<td>24,900</td>
<td>16,219</td>
</tr>
<tr>
<td></td>
<td>Protopectinase</td>
<td>105</td>
<td>5,775</td>
<td>3,945</td>
</tr>
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</table>
TABLE V. AFFINITIES OF ENDO-POLYGALACTURONASES ON PROTOPECTIN AND POLYGALACTURONIC ACID

<table>
<thead>
<tr>
<th>Origin of enzyme</th>
<th>Km values</th>
<th>Ratio of activity protopeptinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protopeptin* (mg/ml)</td>
<td>Polygalacturonic acid (mg/ml)</td>
</tr>
<tr>
<td>A. pullulans</td>
<td>525</td>
<td>3.8</td>
</tr>
<tr>
<td>T. penicillatum</td>
<td>50</td>
<td>7.7</td>
</tr>
<tr>
<td>K. fragilis</td>
<td>90</td>
<td>6.6</td>
</tr>
<tr>
<td>G. reessii</td>
<td>30</td>
<td>9.0</td>
</tr>
</tbody>
</table>

The enzyme reactions were performed at pH 5.0 (at 37°C).

© Obtained from Citrus unshiu peel.

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 Protopectinase is the enzyme which attacks plant tissues and injures plants. Garibaldi and Bateman isolated three forms of pectate trans-eliminase from Erwinia chrysanthemi and found that two of them, which were basic proteins, macerated plant tissues, whereas the other, which was an acidic protein, did not.\(^1\) The facts seem to suggest that the damage of plant cell walls caused by pectolytic enzymes may be influenced by their basic properties. The endo-PGs isolated as protopectinases from T. penicillatum and G. reessii are basic proteins,\(^2\,4\) whereas that from K. fragilis is an acidic protein.\(^5\) However, the endo-PG from K. fragilis shows potent protopectinase activity whereas the present enzyme, an acidic protein, shows very low protopectinase activity. Thus, the protopectinase activity of endo-PG seems not to depend on the isoelectric point of the enzyme.

Differences were observed in their amino acid compositions (Fig. 11), though there is a similarity in lacking methionine. The present enzyme contains more threonine than the other PGs.

However, the relationship between amino acid compositions and the substrate specificities are not clear yet. The relationship between protopectinase activity and physicochemical properties of enzyme molecules are the attractive problems which remain to be resolved.

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