Multiple Forms of the Lytic Glucanase of Flavobacterium dormitator var. glucanolyticae and the Properties of the Main Component Enzyme

Hiroyuki MORI, Shinpei YAMAMOTO and Susumu NAGASAKI
Faculty of Agriculture, Kochi University, Nankoku, Kochi
Received November 22, 1976

The lytic $\beta$-1,3-glucanases on yeast cell wall so far isolated from culture media of microorganisms are classified according to their action patterns into three types; endo $\beta$-1,3-glucanase of smaller oligosaccharide-producing type, endo $\beta$-1,3-glucanase of larger oligosaccharide-producing type, and exo $\beta$-1,3-glucanase. However, except Arthrobacter YCWD-3 microorganism producing two of these enzymes and above has not been reported.

In the previous papers from this laboratory, we have reported about the purification and properties of lytic $\beta$-1,3-glucanase produced extra-cellularly by Flavobacterium dormitator var. glucanolyticae. The enzyme (termed glucanase II in this report) was found to attack $\beta$-1,3-glucans in an endo manner with glucose, laminaribiose, and laminaritriose as end products, and therefore classified into smaller oligosaccharide-producing type and exo $\beta$-1,3-glucanase. However, except Arthrobacter YCWD-3 microorganism producing two of these enzymes and above has not been reported.

In the previous paper from this laboratory, we have reported about the purification and properties of lytic $\beta$-1,3-glucanase produced extra-cellularly by Flavobacterium dormitator var. glucanolyticae. The enzyme (termed glucanase II in this report) was found to attack $\beta$-1,3-glucans in an endo manner with glucose, laminaribiose, and laminaritriose as end products, and therefore classified into smaller oligosaccharide-producing type. On further investigation on the lytic enzyme system of this organism for yeast cell wall, a lytic $\beta$-1,3-glucanase of larger oligosaccharide-producing type has been separated in homogeneous state. This paper describes the occurrence in F. dormitator of two lytic $\beta$-1,3-glucanases of different types and the properties of the lytic $\beta$-1,3-glucanase of larger oligosaccharide-producing type.

The purification procedure of the enzyme is given in Table I. As is shown in Fig. 1, the lytic activity applied onto a SP-Sephadex C-50 column was divided into five fractions, glucanase I ~ IV regions and the breakthrough region, according to their different affinities for the ion exchanger. Glucanase IV was further purified by rechromatography on the same column. From Table I it is obvious that glucanase IV is a major component of the enzyme system responsible for the lysis of yeast cells by this organism, and that the purification procedure is comparatively simple. Lytic $\beta$-1,3-glucanase of bacterial origin had been purified by Kitamura and Yamamoto, Doi et al., Rombouts and Phaff, and Bacon et al., from Arthrobacter luteus, Arthrobacter YCWD-3, Bacillus circulans WL-12, and Cytophaga johnsonii, respectively. The yields of these enzyme preparations were very low for B. circulans WL-12, 7% for A. luteus, 21% for A. YCWD-3 (in this case the enzyme preparation contained six isozymes), and 9% for C. johnsonii. Therefore, F. dormitator seems to be favorable for a source of lytic enzyme.

The purified enzyme accounted for 43% of the lytic activity presented in the concentrated culture filtrate, gave a single band on an analytical disc electrophoresis using 7% polyacrylamide gel, and sedimented as a single symmetrical peak on ultracentrifugation ($s_{20,w} = 4.05$). Purity of the enzyme was also supported by the result of specificity test mentioned below. The immunodiffusion test shown in Fig. 1 demonstrates the structural distinction between glucanase II and glucanase IV. The absorption spectrum of the enzyme showed a maximum at 280 nm, and there was no significant absorption in the visible light range. The molecular weight was determined to be 33,000 and 32,700 by BioGel P-100 gel filtration and by sedimentation equilibrium, respectively.

The enzyme was specific for $\beta$-1,3-glucans such as pachyman (1.69 I.U.), yeast glucan (0.58 I.U.) and laminarin (0.20 I.U.). The hydrolysis product of laminarin by the enzyme was predominantly pentose as shown in Fig. 2. Xylan, amyllose, dextrin, pullulan, chitin, inulin, luteose, cellulose and yeast mannan were not attacked by the enzyme. Osmotically
FIG. 1. Typical Elution Pattern of the Lytic Enzymes on SP-Sephadex C-50 Column Chromatography and Immunodiffusion Pattern in Agar of Reactions of Glucanase II (II) and Glucanase IV (IV) with Glucanase IV Antiserum (A).

The crude enzyme solution shown in Table I was applied to a SP-Sephadex C-50 column (5 × 48 cm) previously equilibrated with 0.01 M K-phosphate buffer, pH 5.0, and eluted as indicated in the figure. ○—○, lytic activity; ●–●, β,1,3-glucanase; dotted line, OD at 280 nm. For further experimental methods see reference 1).

TABLE I. SUMMARY OF THE PURIFICATION PROCEDURES OF THE LYtic ENZYME

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Lytic activity</th>
<th>Yield based on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>11,000</td>
<td>147,347</td>
<td>59,400</td>
<td>100 (%)</td>
</tr>
<tr>
<td>Concentrated&lt;sup&gt;a&lt;/sup&gt; and dialysed culture filtrate</td>
<td>185</td>
<td>8,530</td>
<td>18,084</td>
<td>30 100 (%)</td>
</tr>
<tr>
<td>SP-Sephadex C-50 chromatography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakthrough</td>
<td>370</td>
<td>6,005</td>
<td>4,070</td>
<td>7 23 (%)</td>
</tr>
<tr>
<td>Glucanase I</td>
<td>280</td>
<td>103</td>
<td>1,090</td>
<td>2 6.6 (%)</td>
</tr>
<tr>
<td>Glucanase II</td>
<td>480</td>
<td>86</td>
<td>2,471</td>
<td>4 13.2 (%)</td>
</tr>
<tr>
<td>Glucanase III</td>
<td>275</td>
<td>38.5</td>
<td>2,926</td>
<td>5 16.5 (%)</td>
</tr>
<tr>
<td>Glucanase IV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>490</td>
<td>89</td>
<td>7,595</td>
<td>13 43 (%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ammonium sulfate 20–80%.

<sup>b</sup> Purified by rechromatography on the same column.

Sensitive cells were produced by the enzyme from the log-phase cells of; Saccharomyces cerevisiae, S. cerevisiae LK2G12, S. cerevisiae var. ellipsoideus, S. carlsbergensis, Hansenula capsulata, Debaryomyces globosus, Saccharo-

FIG. 2. Gel Filtration Diagram of Enzyme Digested Laminarin.

Reaction mixture (2 ml) containing 5 mg/ml of laminarin and 20 μg/ml of enzyme was incubated at 40°C for 24 hr and then applied on to a BioGel P-2 (—400 mesh) column (1.6×185 cm). Flow rate was 1 drop/30 sec. Carbohydrate was measured by the phenol-sulfuric acid method. Dotted line shows calculated molar concentration.
mycodes ludwigii, Candida mycoderma, C. kru-sei, C. macedoniensis, Wickerhamia fluorescens, and Pichia satunus. While, from Torulopsis famata, Cryptococcus neoformans, Tricosporon behrendii, Torula rubra var. α, Rhodotorula marina, and R. lactosa osmotically sensitive cell was not produced. Evidently, cells in the stationary phase of growth were less sensitive to the action of the enzyme than those in the logarithmic phase of growth.

REFERENCES