Purification and Some Properties of β-1,3-Glucanase from \textit{Streptomyces} sp.¹

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β-1,3-Glucanases are widely distributed among fungi, bacteria, and higher plants. Consequently, studies on them have been extensive. There are some reports¹⁻⁵ on the studies of \textit{Streptomyces} β-1,3-glucanase, whose purpose was to lyse the cell walls of yeast and fungi.

In previous papers,⁶,⁷ we have reported two types of enzyme systems from species of \textit{Streptomyces} that were isolated from soil using curdlan as a carbon source. One enzyme system hydrolyzed curdlan to glucose and laminarinbiose, and another degraded curdlan to mainly produce glucose and gentiobiose. We have also reported the mechanism of formation of gentiobiose from curdlan by the latter enzyme system,⁸ finding that two kinds of enzymes, β-1,3-glucanase and β-glucosidase, took part in the formation of gentiobiose from curdlan.

This paper describes the purification and some properties of β-1,3-glucanase produced extracellularly by \textit{Streptomyces} sp., and actions of the glucanase on laminari-oligosaccharides and their alcohols.

MATERIALS AND METHODS

Chemicals. Curdlan (β-1,3-glucan) was purchased from Wako Pure Chemical Industries (Japan). Sophorose was prepared from a partial acid hydrolysate of stevioside. Stevioside was purchased from Sanyo-Kokusaku Pulp Co., Ltd. Laminarinbiose (G₂) and gentiobiose were prepared from hydrolysates of curdlan by the β-1,3-glucanase system produced by \textit{Streptomyces} sp. K27-4 and \textit{Streptomyces} sp. W19-1, respectively.⁶,⁷ Laminari-oligosaccharides (G₃ ~ G₆) were prepared from a partial hydrolysate of curdlan by the purified β-1,3-glucanase of \textit{Streptomyces} sp. W19-1. All other chemicals were obtained commercially and were of analytical grade.

Preparation of the crude enzyme. \textit{Streptomyces} sp. W19-1 was used in the preparation of the crude enzyme by submerged culture following the procedure described in our previous paper.⁷ After cultivation, the culture broth was filtered through filter paper (Tōyō Roshi No. 2, Tōyō Roshi Co., Ltd.) and the filtrate was used as an enzyme source in this study.

¹ Studies on the β-1,3-Glucanase System of \textit{Streptomyces}. Part IV.
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Enzyme assay. The β-1,3-glucanase activity was measured as described in our previous paper. One unit of the glucanase activity was defined as the amount of the enzyme that released reducing sugar equivalent to 1 μmol of glucose per min from curdlan under the optimum conditions.

Measurement of sugar and protein. Sugar and protein were measured by the methods of Somogyi and Lowry, respectively, as in our previous paper.

Gel electrophoresis. Polyacrylamide disc-gel electrophoresis was done at pH 8.3 by the method of Davis. After electrophoresis, protein in the gel was fixed with 12.5% trichloroacetic acid for 30 min, and stained with 0.05% Coomassie Brilliant Blue R-250 (Sigma Chemical Co., U.S.A.). SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis was done in 0.1% SDS-0.1 M sodium phosphate buffer solution, pH 7.2, at 6 mA per gel by the method of Weber and Osborn.

Isoelectric focusing. Isoelectric focusing was done by the method of Vesterberg and Svensson in a 110 ml column using Servalyt (Serva Feinbiochemica, West Germany). The amount of protein in the electro-focusing run was about 15 mg. Carrier Servalyts (pH 3~5: pH 2~4=4:1) were used in a sucrose gradient and a voltage of 800 V was applied for 48 hr at 4°C.

Thin-layer chromatography. Thin-layer chromatography was done on a plate of Merck TLC plate silica gel 60 with a solvent system of chloroform-methanol-water (90:65:15, v/v). The sugars were detected by heating at 140°C for 5 min after spraying with 50% sulfuric acid.

Hydrogenation of laminari-oligosaccharides. Laminari-oligosaccharides (G2~G6) were hydrogenated into the corresponding sugar alcohols (G2~G6) by sodium borohydride.

RESULTS

I. Enzyme purification

The culture filtrate from Streptomyces sp. W19-1 has two activities, a β-1,3-glucanase and a β-glucosidase. They were separated by hydroxyapatite column chromatography after ammonium sulfate fractionation as described in our previous paper. The unadsorbed fraction contained β-1,3-glucanase, and the adsorbed part had β-glucosidase activity.

The β-1,3-glucanase-rich fraction was dialyzed against 0.02 M phosphate buffer solution (pH 6.0). The dialyzate was put on a DE-52 column (25 × 300 mm) equilibrated with the same buffer solution. After the column was washed with 1 liter of the same buffer solution, it was eluted with a linear gradient of NaCl (0~0.2 M). A β-1,3-glucanase-rich fraction (No. 121~138) was obtained (Fig. 1). The fraction was concentrated using a membrane of UK-10 (Tōyō Roshi Co., Ltd.) for further purification.

A column (40×900 mm) of Biogel P-100 was equilibrated with 0.02 M phosphate buffer solution (pH 6.0) containing 0.1 M NaCl. The concentrated glucanase solution was put on the column and eluted with the same buffer solution.
TABLE 1. SUMMARY OF PURIFICATION OF $\beta$-1,3-Glucanase FROM Streptomyces sp.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Purification (-fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>2,873</td>
<td>8,041</td>
<td>2.8</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>80% saturated (NH$_4$)$_2$SO$_4$ fractionation</td>
<td>1,110</td>
<td>6,846</td>
<td>6.2</td>
<td>2.2</td>
<td>85.1</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>293.8</td>
<td>5,923</td>
<td>20.2</td>
<td>7.2</td>
<td>73.7</td>
</tr>
<tr>
<td>DE-52</td>
<td>58.7</td>
<td>3,576</td>
<td>60.9</td>
<td>21.8</td>
<td>44.5</td>
</tr>
<tr>
<td>Biogel P-100</td>
<td>28.4</td>
<td>2,949</td>
<td>103.7</td>
<td>37.0</td>
<td>36.7</td>
</tr>
<tr>
<td>Isoelectric focusing</td>
<td>25.2</td>
<td>2,767</td>
<td>109.8</td>
<td>39.2</td>
<td>34.4</td>
</tr>
</tbody>
</table>

Fig. 3. Electrophoresis of Purified $\beta$-1,3-Glucanase in SDS-Polyacrylamide Gel.

The purified enzyme solution (about 100 $\mu$g) treated with SDS at 100°C for 5 min was put on a 7.5% gel containing 0.1% SDS, and run at 6 mA per column.

solution (data not shown). The resultant glucanase-rich fraction (No. 51 ~ 58) was concentrated by ultrafiltration using the same apparatus. After dialysis, the glucanase was put on a 110 ml column for isoelectric focusing. Figure 2 shows the isoelectric focusing profile of *Streptomyces* $\beta$-1,3-glucanase. The steps for enzyme purification are summarized in Table I. The purified sample gave a single band on polyacrylamide disc gel electrophoresis and on SDS-polyacrylamide gel electrophoresis (Fig. 3).

II. Properties of the purified enzyme

Physicochemical properties. *Streptomyces* $\beta$-1,3-glucanase was in the protein peak with an isoelectric point of pH 3.7 (Fig. 2). The molecular weight of the purified enzyme was 36,000 by SDS-polyacrylamide gel electrophoresis using molecular markers of BDH Co. as standards. By gel filtration on Biogel P-100, however, we got a molecular weight of approximately 30,000 by the method of Andrews.

Enzymatic properties. *Streptomyces* $\beta$-1,3-glucanase had the highest activity at pH 5.5 (Fig. 4A) and was stable at pHs between 3.5 and 6.5 (Fig. 4C). The optimum temperature

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**Fig. 4. Effects of pH and Temperature on Activity and Stability of *Streptomyces* $\beta$-1,3-Glucanase.**

A: Effects of pH on glucanase activity. The enzyme reaction was done at various pHs (2~8) and 55°C for 30 min. McIlvaine buffer system was used. B: Effects of temperature on glucanase activity. The enzyme reaction was done at various temperatures from 35 to 70°C for 30 min at pH 5.5. C: Effects of pH on stability. The enzyme solution was maintained at 30°C for 1 hr in various pHs (2~8), and the remaining activity was assayed after adjustment to pH 5.5. D: Effects of temperature on stability. The enzyme solution was preincubated at various temperatures from 35 to 70°C under pH 5.5 for 30 min, and the remaining activity was assayed.
for the enzyme reaction was about 55°C (Fig. 4B). The enzyme was stable up to 50°C (Fig. 4D).

The effects of Ca\(^{2+}\) on enzyme stability were also tested. The enzyme activity was completely lost by incubation at 55°C for 4 hr without Ca\(^{2+}\) (Fig. 5). However, the enzyme was stabilized by the addition of 1 mM Ca\(^{2+}\).

Effects of various compounds on the enzyme. Hg\(^{2+}\), N-bromosuccinimide, and \(p\)-chloromercuribenzoate inhibited the enzyme, but EDTA did not affect it (Table II).

Action of the enzyme on laminari-oligosaccharides. To 0.5 ml of 1.0% solution of each laminari-oligosaccharides was added 0.5 ml of the purified enzyme solution (pH 5.5). After the beginning of incubation at 55°C, samples at the reaction were taken, heated at 100°C for 10 min, and then subjected to thin-layer chromatography. Laminaritriose (G\(_3\)) was hydrolyzed to glucose (G\(_1\)) and laminaribiose (G\(_2\)) (Fig. 6). Laminaritetraose (G\(_4\)), laminarpentaose (G\(_5\)) and laminarihaxose (G\(_6\)) were finally hydrolyzed to G\(_1\), G\(_2\) and a small amount of G\(_3\). However, small amounts of unknown oligosaccharide located under the spot of G\(_4\) were observed during the reaction with these oligosaccharides. Therefore, this indicated that the \(\beta\)-1,3-glucanase from Streptomyces \(\beta\)-1,3-Glucanase

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>BaCl(_2)</td>
<td>96</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>126</td>
</tr>
<tr>
<td>CdCl(_2)</td>
<td>97</td>
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<tr>
<td>CoCl(_2)</td>
<td>118</td>
</tr>
<tr>
<td>CuCl(_2)</td>
<td>100</td>
</tr>
<tr>
<td>FeCl(_2)</td>
<td>86</td>
</tr>
<tr>
<td>FeCl(_3)</td>
<td>104</td>
</tr>
<tr>
<td>HgCl(_2)</td>
<td>3</td>
</tr>
<tr>
<td>NiCl(_2)</td>
<td>102</td>
</tr>
<tr>
<td>PbCl(_2)</td>
<td>98</td>
</tr>
<tr>
<td>ZnCl(_2)</td>
<td>96</td>
</tr>
<tr>
<td>MnCl(_2)</td>
<td>100</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate</td>
<td>98</td>
</tr>
<tr>
<td>N-Bromosuccinimide</td>
<td>1</td>
</tr>
<tr>
<td>EDTA</td>
<td>100</td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>95</td>
</tr>
<tr>
<td>(p)-Chloromercuribenzoate (PCMB)</td>
<td>25</td>
</tr>
</tbody>
</table>

![Fig. 5. Effects of CaCl\(_2\) on Stability of Streptomyces \(\beta\)-1,3-Glucanase.](image)

The enzyme solution with and without CaCl\(_2\) was incubated at 55°C. At certain intervals the enzyme solution was withdrawn and the residual activity was assayed. O—O, with 1 mM CaCl\(_2\); •—•, none.

![Fig. 6. Action of Streptomyces \(\beta\)-1,3-Glucanase on Laminari-oligosaccharides.](image)

A, authentic glucose (G\(_1\)), laminaribiose (G\(_2\)), laminaritriose (G\(_3\)), laminaritetraose (G\(_4\)), laminarpentaose (G\(_5\)) and laminarihaxose (G\(_6\)) from top to bottom.
**β-1,3-Glucanase from Streptomyces**

**DISCUSSION**

β-1,3-Glucanase is widely distributed and many workers have studied the β-1,3-glucanases. Almost all of them studied the lysis of yeasts and fungi, and their substrate was yeast glucan or laminarin. To induce β-1,3-glucanases from microorganisms, yeast glucan or laminarin was usually used. In our studies, we have used curdlan, which is mainly or exclusively composed of β-1,3-glicosidic linkages, as both a substrate and a carbon source.

The molecular weight of the purified enzyme was 36,000 by SDS-polyacrylamide gel electrophoresis. This value was higher than the 30,000 obtained by molecular-sieve chromatography; the enzyme seemed to have some affinity for the gel. This enzyme was not a metal enzyme, but was stabilized by Ca$^{2+}$ as well as the Bacillus circulans enzyme.

Tabata et al. reported a lytic β-1,3-glucanase from Streptomyces that degraded yeast glucan and laminarin to produce G$_2$ and G$_3$. Bielecki et al. reported that Streptomyces lytic β-1,3-glucanase hydrolyzed laminari-oligosaccharides (DP ≤ 5). Beyer et al. found three types of β-1,3-glucanase from Streptomyces sp. ATCC 11238, and one of them yielded smaller oligosaccharides from laminarin. The β-1,3-glucanase in a culture filtrate from Streptomyces sp. K27 also ultimately hydrolyzed curdlan to G$_1$ and G$_2$. The glucanase of Streptomyces sp. W19-1 also produced G$_1$ and G$_2$ from curdlan as Streptomyces sp. K27 did, and G$_1$ and G$_2$ from G$_3$ ~ G$_6$. Therefore, it seems that these enzyme preparations belong to a smaller-oligosaccharides-producing type.

However, almost no detailed studies on the transglucosylation, including disproportionation, of β-glucanases have been done, and the transfer action of β-1,3-glucanase from Streptomyces has not been reported previously. The β-1,3-glucanase from Streptomyces sp. W19-1 indicates that the hydrolysis of G$_3$ proceeded via transglucosylation, because without transglucosylation, G$_3$ would theoretically be hydrolyzed to G$_1$ and G$_2$ in a molar ratio of 1:1. But the amount of glucose was less than that of G$_2$ when the enzyme acted on G$_3$ (Fig. 6). Furthermore, some unknown oligosaccharides were observed during the hydrolysis of G$_3$ ~ G$_6$ (Fig. 6). The glucanase also indicated that...
the degradation of $G_3^{\text{H}}$ to $G_6^{\text{H}}$ proceeded via disproportionation (Fig. 7). On the other hand, the mode of transfer by $\beta$-transglucosylase of Sclerotinia libertiana has been investigated.\textsuperscript{17) The mode, however, is different from that of Streptomyces sp. W19-1, because Sclerotinia enzyme\textsuperscript{17) synthesizes a water-insoluble glucan having an average degree of polymerization of 14 in the presence of cellotetraose. Streptomyces $\beta$-1,3-glucanase produced finally $G_1$ and $G_2$ with any given oligosaccharide such as $G_3$ to $G_6$. But both enzyme preparations are similar in the mode of action on sugar alcohols, because of the disproportionation.

The mode of action of the $\beta$-1,3-glucanase from Streptomyces sp. W19-1 on some polysaccharides, such as laminarin and lichenan, will be further investigated.

REFERENCES