Note

Structures and Antitumor Activities of Polysaccharides Isolated from Mycelium of Volvariella volvacea

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During the course of our study on the relationship between structures and immunomodulating activities of fungal polysaccharides, we have isolated some β-(1→3)-β-glucans with different distribution of branches and the activities from various sources.1-4 As reported previously, we obtained mannanolactans, glycogen, and three different kinds of β-(1→3)-β-glucans by the fractional extraction from the fruiting body of an edible mushroom, Volvariella volvacea.4 Among them, an alkali-soluble glucan with O-6 substitutions (d.b.81 1/5) showed potent antitumor activities against Sarcoma 180 in ICR-mice, Meth A in Balb/c mice, and methylcholanthrene-induced fibrosarcoma in DBA mice. These potent immunomodulating activities of the β-glucan prompted us to study its fine structure and mechanism of the antitumor activity in detail.5

In this paper, we report the chemical structural feature and antitumor activities of the extracellular and cell-wall polysaccharides of the cultured mycelium of V. volvacea to compare their structural features and antitumor activities to those of polysaccharides isolated from the fruiting body.

V. volvacea (IFO 30010) was obtained from the Institute for Fermentation, Osaka, and maintained on malt-extract and glucose slants. For the cultivation, it was inoculated in liquid culture medium containing 0.3% malt extract, 0.3% yeast extract, 5.0% glucose, 0.05% K2HPO4, 0.05% KH2PO4, and 0.05% MgSO4·7H2O, and incubated at 28°C with shaking for 30 days. The cultured mycelium was collected by filtration on a glass-fiber, and the culture filtrate was dialyzed against distilled water for 2 days. The extracellular polysaccharide in the filtrate was precipitated by the addition of 3 volumes of ethanol and air-dried. The cell-wall polysaccharides were isolated from the mycelium by the fractional extraction as almost the same procedure as that for its fruiting body.4 After extraction with hot water (120°C, 20 min, twice), the residue was treated twice with 1 N NaOH for 2 hr at 25°C under a nitrogen atmosphere in the presence of sodium borohydride. The alkaline extract was neutralized with diluted acetic acid and dialyzed against distilled water for 2 days. The polysaccharide in the non-dialyzable solution was precipitated by the addition of 3 volumes of ethanol. Methylation analysis was done by Hakomori’s method.6 The monosaccharide compositions of the polysaccharide and the partially methanolysed glycans of fully methanolysed polysaccharide were identified by GLC-MS as their alditol acetates as described in our previous paper.4 Antitumor activities of the polysaccharide fractions were examined against Sarcoma 180 in ICR-JCL mice by intraperitoneal administration at a dose of 5 mg/kg for 10 days, starting one day after tumor implantation, as described previously.5

From 1 liter of liquid culture medium, 115.2 g of mycelium (in wet weight) and 1.35 g of extracellular polysaccharide of V. volvacea were obtained. The yield and carbohydrate components of each polysaccharide fraction are summarized in Table I. Carbohydrate compositions of water-soluble and extracellular polysaccharides were similar to that of water-soluble polysaccharide from the fruiting body.4 This fact indicates that these fractions contain mannanolactans like that of the fruiting body. In addition, the ratio of mannose to galactose in the extracellular polysaccharide (1.0:3.2) is similar to that of water-soluble polysaccharide (1.0:2.9) of the mycelium, which suggests that a part of cell-surface polysaccharide was liberated into the culture medium and isolated as an extracellular polysaccharide. The facts that acid hydrolysis of the alkali-soluble polysaccharide gave predominantly D-glucose and it showed absorption at 900 cm-1 suggest that this fraction is a β-D-glucan fraction similar to the same fraction isolated from its fruiting body.7

To compare the structural features of the polysaccharides from the cultured mycelium to those of the fruiting body, the polysaccharides were examined by methylation analysis. The methylated alkali-soluble glucan yielded 2,3,4,6-tetra-, 2,4,6-tri-, 2,3,4-tri-, 2,3,6-tri-, and 2,4-di-O-methyl-glucosyl in a molar ratio of 1.0, 1.8, 0.2, 0.9, and 1.0. These results suggested that the glucan has a branched structure, consisting of a backbone chain of (1→3)-linked glucosyl residues with one out of approximately three glucosyl residues substituted, mainly at the O-6 position. This structural feature is almost the same as that of the counterpart of the fruiting body. Most of the side chains are single α-glucosyl units, in addition a few branches appears to contain (1→6)- and (1→4)-linked oligosaccharide units. Concerning (1→4)-linked α-glucosyl residues, contamination of glycogen-type polysaccharide in this glucan fraction could be ruled out by the absence of 2,3-di-O-methyl-D-glucose in the methylated polysaccharide.

Methylated water-soluble and extracellular polysaccharide gave the same partially methylated glucoses, such as 2,3,4,6-tetra-O-methyl-D-glucose (mamnose), 2,3,6-tri-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-galactose, 2,3-di-O-methyl-D-glucose, and 3,4-di-O-methyl-D-galactose in the molar ratio of 2.0:5.8:0.8:13.0:0.5 and 3.0:5.6:0.3:2.6:1.4:1.6, respectively. These results and their positive iodine reaction suggested that these polysaccharide-fractions are composed of a glycogen-like glucan and a mannanolactan-like those of the fruiting-body of V. volvacea.4

Antitumor activities of the extracellular and alkali-soluble polysaccharides of the cultured mycelium of V. volvacea were examined using Sarcoma-180 ICR-mouse system. The results are shown in Table I. It is apparent that the alkali-soluble β-glucan fraction had high antitumor activity (inhibition ratio, 87.8%, ratio of complete regression, 2/5). On the other hand, the water-soluble and the extracellular polysaccharide did not inhibit tumor growth (inhibition ratio, 2.6% and 0.6%, respectively) under the same assay conditions.

In a series of our studies on the structural diversities of antitumor fungal β-glucans and their antitumor effects, we indicated that the activity is affected by the mode of distribution of glucosyl side chains attached to the (1→3)-β-linked backbone chains.5

Table I. Chemical Compositions and Antitumor Activities against Sarcoma 180 of the Mycelium and the Extracellular Polysaccharides

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (mg)</th>
<th>Carbohydrate component (%)</th>
<th>Antitumor activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Man Gal G1c</td>
<td>Tumor weight (g) Inhibition ratio (%)</td>
</tr>
<tr>
<td>Mycelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot water extract</td>
<td>50.3</td>
<td>5.4</td>
<td>15.8</td>
</tr>
<tr>
<td>Cold alkali extract</td>
<td>251.7</td>
<td>—</td>
<td>2.5</td>
</tr>
<tr>
<td>Extracellular polysaccharide</td>
<td>1349.5</td>
<td>9.0</td>
<td>28.8</td>
</tr>
</tbody>
</table>

a Dry weight from 1 liter of culture filtrate.

b A polysaccharide was intraperitoneally injected at a dose of 5 mg/kg for 10 days, starting after tumor implantation.

c Average tumor weight ± SD.

d Tumor weight of control group, 11.04 ± 1.60 g.
It has been established that the antitumor actions of (1→3)-β-D-glucans are related to their triple helix conformation.\textsuperscript{6,10} In addition to the triple strand conformation of the backbone chain, these results supported our findings that the antitumor-active substance is most likely branched (1→3)-β-D-glucan and the glucans having d.b. 1/3—5 appear to have the most potent activity.\textsuperscript{6}

References


