Polysaccharides of *Mycobacterium bovis* Ushi 10, *Mycobacterium smegmatis*, *Mycobacterium phlei*, and Atypical *Mycobacterium* P1

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Received for publication 3 April 1970

Four kinds of polysaccharides, glucan, mannan, arabinomannan, and arabinogalactan, were purified from the defatted cells of four strains of mycobacteria. By the chemical and immunological analyses, we concluded that arabinomannan and arabinogalactan are common serologically active polysaccharides in mycobacteria.

In previous papers (1, 2), we reported the purification and properties of polysaccharides from defatted cells of human tubercle bacillus strain Aoyama B and culture filtrate of strain H37Rv. These polysaccharides were composed of four kinds of polysaccharides, glucan, mannan, arabinomannan, and arabinogalactan. Arabinomannan and arabinogalactan showed potential serological and anaphylactic activities in rabbits and guinea pigs sensitized with heat-killed tubercle bacilli. More recently, Birnbaum and Affronti (6) described the presence of polysaccharide I (an arabinogalactan) in four strains of mycobacteria, *Mycobacterium tuberculosis* H37Ra, *M. kansasii*, and the Batty and Gause strains. This note describes the fractionation and immunological properties of polysaccharides from defatted cells of *M. bovis* Ushi 10, *M. smegmatis*, *M. phlei*, and the atypical *Mycobacterium* P1 strain.

As an example of the extraction and purification of polysaccharides from these four strains, the fractionation procedure of polysaccharides from the defatted cells of *M. bovis* Ushi 10 is described. The acetone-dried cells of *M. bovis* Ushi 10, which were cultured in Sauton's synthetic media for 7 weeks, were defatted by the repeated extraction with ether-ethyl alcohol (1:1), chloroform, and chloroform-methyl alcohol (2:1). After defatting, a 350-g amount of defatted cell of *M. bovis* Ushi 10 was then extracted with a 1 N NaOH solution (100 g of defatted cells/1,000 ml of 1 N NaOH solution) at 65°C for 24 hr with stirring under nitrogen stream. The mixture was centrifuged at 10,000 rev/min for 60 min. The supernatant fluid was neutralized with acetic acid, and the precipitate which formed was removed by the centrifugation. The supernatant fluid was then dialyzed against running water for 3 days and concentrated to 0.2 volume. By the addition of 10 volumes of ethyl alcohol to the concentrate, 7.2 g of crude polysaccharide was obtained.

This crude polysaccharide was dissolved in a small amount of water and was fractionated into seven fractions by the fractional precipitation with ethyl alcohol. As shown in a previous paper (1), each fraction was further purified by the following procedures. For example, the MB-80 fraction, which was obtained by the addition of ethyl alcohol to a final concentration of 75 to 80%, was chromatographed on a column of ion-exchange resin Dowex 50 (H+ form) and was eluted with water followed by 0.2 M NaH2PO4 solution. The water eluate was further chromatographed on a diethylaminoethyl-cellulose column, and the column was eluted with water followed by 0.2 M Na2HPO4 solution. The water eluate was gel-filtrated on Sephadex G-50 and G-200 column, and then the purified MB-80 fraction was obtained by the addition of ethyl alcohol to a final concentration of 75 to 80%.

From the results of chemical analyses and the determination of optical rotation on the purified seven subfractions of *M. bovis* Ushi 10, it was shown that four kinds of polysaccharides, glucan, mannan, arabinomannan, and arabinogalactan, were contained as main constituents of the polysaccharides. Similar results were obtained by chemical and physical analyses of purified fractions from *M. smegmatis*, *M. phlei*, and the atypical *Mycobacterium* P1 strain.

The arabinomannans and arabinogalactans obtained from four strains of mycobacteria showed potent antigen activity in precipitation, complement-fixation, and passive hemagglutination tests with rabbit antisera against heat-killed ho-
### Table 1. Polysaccharides purified from defatted cells of mycobacteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Polysaccharides</th>
<th>Sugar compositions (molar ratio)</th>
<th>Precipitation test</th>
<th>Complement-fixation test</th>
<th>Passive hemagglutination test</th>
<th>Systemic anaphylaxis in guinea pigs/1-mg sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycobacterium bovis Ushi 10</strong></td>
<td>Arabinogalactan</td>
<td>Arabinose (2.7) Galactose (1.0)</td>
<td>1:1,024,000</td>
<td>Ag 0.012 µg (Ab 1:640)</td>
<td>1:20,480</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Arabinomannan</td>
<td>Arabinose (1.1) Mannose (1.0)</td>
<td>1:512,000</td>
<td>Ag 0.024 µg (Ab 1:640)</td>
<td>1:10,240</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Mannan</td>
<td>Mannose (5.6) Arabinose (1.0)</td>
<td>1:32,000</td>
<td>Not examined</td>
<td>1:160</td>
<td>Not examined</td>
</tr>
<tr>
<td></td>
<td>Glucan</td>
<td>Glucose</td>
<td>1:6,000</td>
<td>Not examined</td>
<td>1:40</td>
<td>Not examined</td>
</tr>
<tr>
<td><strong>M. smegmatis</strong></td>
<td>Arabinogalactan</td>
<td>Arabinose (2.6) Galactose (1.0)</td>
<td>1:1,024,000</td>
<td>Ag 0.012 µg (Ab 1:640)</td>
<td>1:10,240</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Arabinomannan</td>
<td>Arabinose (4.3) Mannose (1.0)</td>
<td>1:512,000</td>
<td>Ag 0.012 µg (Ab 1:640)</td>
<td>1:5,120</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Mannan</td>
<td>Mannose (3.1) Arabinose (1.0)</td>
<td>1:16,000</td>
<td>Not examined</td>
<td>1:320</td>
<td>Not examined</td>
</tr>
<tr>
<td></td>
<td>Glucan</td>
<td>Glucose</td>
<td>1:6,000</td>
<td>Not examined</td>
<td>1:40</td>
<td>Not examined</td>
</tr>
<tr>
<td><strong>M. phlei</strong></td>
<td>Arabinogalactan</td>
<td>Arabinose (2.6) Galactose (1.0)</td>
<td>1:2,048,000</td>
<td>Ag 0.006 µg (Ab 1:1,280)</td>
<td>1:20,480</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Arabinomannan</td>
<td>Arabinose (2.8) Mannose (1.0)</td>
<td>1:2,048,000</td>
<td>Ag 0.006 µg (Ab 1:1,280)</td>
<td>1:10,240</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Mannan</td>
<td>Mannose</td>
<td>1:16,000</td>
<td>Not examined</td>
<td>1:80</td>
<td>Not examined</td>
</tr>
<tr>
<td></td>
<td>Glucan</td>
<td>Glucose</td>
<td>1:16,000</td>
<td>Not examined</td>
<td>1:40</td>
<td>Not examined</td>
</tr>
<tr>
<td><strong>Atypical Mycobacterium P1</strong></td>
<td>Arabinogalactan</td>
<td>Arabinose (2.5) Galactose (1.0)</td>
<td>1:512,000</td>
<td>Ag 0.024 µg (Ab 1:640)</td>
<td>1:10,240</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Arabinomannan</td>
<td>Arabinose (1.2) Mannose (1.0)</td>
<td>1:256,000</td>
<td>Ag 0.024 µg (Ab 1:640)</td>
<td>1:5,120</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Mannan</td>
<td>Mannose (8.5) Arabinose (1.0)</td>
<td>1:64,000</td>
<td>Not examined</td>
<td>1:160</td>
<td>Not examined</td>
</tr>
<tr>
<td></td>
<td>Glucan</td>
<td>Glucose</td>
<td>1:64,000</td>
<td>Not examined</td>
<td>1:80</td>
<td>Not examined</td>
</tr>
</tbody>
</table>

*Titers are determined by the "ring test" with rabbit antisera against homologous heat-killed mycobacterial cells.

Results are expressed as micrograms of antigen necessary for 50% lysis, determined by the method of Kabat and Mayer (7) with rabbit antisera against homologous mycobacterial cells.

Titers are expressed as the highest dilution of rabbit antisera which gave a positive 2+ reaction by the method of Stavitsky (9).

Systemic anaphylaxis activity was examined in guinea pigs sensitized by heat-killed homologous mycobacterial cells by the method previously described (1).

Symbol ++++, indicates death as early as 5 min after the intravenous injection of antigens.
mologous and heterologous mycobacterial cells (Table 1). On the other hand, mannans and glucans are less active in serological tests. Immuno-diffusion analysis in Ouchterlony's method showed the production of bands of identity between arabinogalactans from M. bovis Ush 10, M. smegmatis, M. phlei, atypical Mycobacterium P1 purified in the present paper, and M. tuberculosis Aoyama B purified in previous work (1) by using rabbit antisera against M. bovis Ush 10. Similarly, these arabinogalactans gave the bands of identity with antisera from M. smegmatis, M. phlei, atypical Mycobacterium P1, and M. tuberculosis Aoyama B strains. However, the precipitin lines of arabinogalactans formed a spur with those of arabinomannans by using rabbit antisera against heat-killed mycobacteria cells as shown in a previous paper (1). These results suggest that the determinant groups of arabinogalactan and arabinomannan are not identical.

The systemic anaphylactic activity of these polysaccharides was examined in guinea pigs sensitized with heat-killed M. tuberculosis Aoyama B by the method described previously (1). Arabinomannans and arabinogalactans showed potent systemic anaphylactic activity (Table 1). With the passive cutaneous anaphylaxis test, marked bluing was induced by the arabinomannans and arabinogalactans in guinea pigs by using guinea pig antisera against homologous and heterologous heat-killed mycobacterial cells.

The Arthus-type skin reaction was shown by the intracutaneous injection of arabinomannans and arabinogalactans obtained from four strains of mycobacteria into guinea pigs sensitized with heat-killed homologous or heterologous mycobacterial cells. These polysaccharides could not inhibit the migration of peritoneal exudate cells from guinea pigs sensitized previously.

More recently, we extended these studies to Corynebacterium diphtheriae and Nocardia asteroides (unpublished data). Two kinds of serologically active polysaccharides, arabinogalactan and arabinogalactomannan, were obtained. With precipitation tests, these two polysaccharides showed cross-reactions with rabbit antisera against those mycobacteria examined above. Arabinomannans and arabinogalactans obtained from the mycobacterial cells, described in this and the previous papers (1, 2), also showed a precipitation reaction with rabbit antisera against C. diphtheriae and N. asteroides. We are now working in depth on the cross-reactivity of arabinogalactans purified from mycobacteria, nocardia, and corynebacteria. From above results, we conclude that four kinds of polysaccharides, glucan, mannan, arabinomannan, and arabinogalactan, are common constituents of mycobacterial polysaccharides, and that arabinomannan and arabinogalactan are common serologically active polysaccharide antigens of mycobacteria, nocardia and corynebacteria. On the cellular distribution of these polysaccharides, it has been reported that arabinogalactan was present in cell walls (4, 8) and wax D (3) as mycolic acid-arabinogalactan-mucoprotein complexes, and the hypothetical structure of mycolic acid-arabinogalactan moiety of cell walls and wax D was proposed (5). Arabinomannan was contained mainly in cytoplasm of mycobacteria (unpublished data).

The detailed chemical structure and immunological specificity of arabinomannans and arabinogalactans are now being investigated in our laboratory.

LITERATURE CITED