Isolation and Purification of Anaphylactically Active Polysaccharide from Human Tubercle Bacilli

ICHIRO AZUMA, HAMAKO KIMURA, TOHRU NIINAKA, AND YUICHI YAMAMURA

The Third Department of Internal Medicine, School of Medicine, Osaka University, Osaka, Japan

Received for publication 6 October 1966

In an earlier paper (Y. Yamamura et al., Am. Rev. Respirat. Diseases 91:839, 1965; I. Azuma et al., Am. Rev. Respirat. Diseases, in press), we reported the isolation and purification of a polysaccharide from a culture filtrate of human tubercle bacilli Aoyama B strain. The polysaccharide was shown to have anaphylactic activity in guinea pigs which were sensitized by heat-killed tubercle bacilli. This polysaccharide was composed of arabinose and a small amount of galactose. This note describes the extraction and purification of polysaccharide from defatted cells of human tubercle bacilli Aoyama B strain. The polysaccharide was shown to have anaphylactic activity in sensitized guinea pigs.

Bacterial cells which were defatted by repeated extraction with ether-alcohol (1:1) and chloroform-methanol (1:1) were extracted with 1 N NaOH solution (100 g of cells per 2 liters of 1 N NaOH) at 70 C for 12 hr with stirring. The mixture was centrifuged at 10,000 rev/min for 60 min, and the supernatant fluid was neutralized with acetic acid. After centrifugation, the supernatant fluid was dialyzed against running water for 4 days and was concentrated to half volume. To the concentrate, 5 volumes of ethyl alcohol were added, and crude, precipitated polysaccharide was obtained by centrifugation and was designated “AB” fraction. The AB fraction was dissolved in a small amount of water and was separated into five fractions by fractional precipitation with ethyl alcohol. In the present experiments, two fractions, designated AB-66 and AB-80, were examined. AB-66 and AB-80 fractions were obtained by the addition of ethyl alcohol to a final concentration of 50 to 66% and 75 to 80%, respectively. Both AB-80 and AB-66 fractions contained a small amount of protein.

The purification of AB-80 and AB-66 fractions was carried out by the following procedure. After the repeated fractional precipitation with ethyl alcohol and acetone, the fractions were chromatographed on a column of ion-exchange resin (Dowex 50, H+ form) and were eluted with water or 0.2 M Na2HPO4 solution. The water eluant, which was designated AB-80A fraction, was further chromatographed on a column of diethylaminoethyl (DEAE) cellulose and was eluted with water, 0.2 M Na2HPO4, or 0.1 N NaOH solution. The eluants were recovered by the addition of ethyl alcohol and were designated AB-80Aa, AB-80Ab, and AB-80Ac, respectively. The AB-80Aa fraction, which was eluted with water, was loaded on columns of Sephadex G-75 and G-200 and eluted with 0.5 M NaCl solution. AB-80Aa and AB-66Aa fractions purified in this way did not contain protein or nucleic acid. The intradermal injection of AB-66Aa and AB-80Aa fractions in 10-μg doses did not elicit a tuberculin reaction in a tuberculous patient. Precipitation tests showed that AB-66Aa and AB-80Aa fractions reacted with rabbit antitubercle sera in dilutions as high as 1:512,000. The test for anaphylactic activity was examined in guinea pigs which were immunized with heat-killed tubercle bacilli in Freund adjuvant, by use of methods described previously (Y. Yamamura et al., Am. Rev. Respirat. Diseases 91:839, 1965). The strong anaphylactic activity in sensitized guinea pigs was found in AB-80Aa fraction.

The sugar components of AB-66Aa and AB-80Aa fractions were analyzed by gas-liquid chromatography. After methanalysis, trimethylsilyl derivatives of methyl glycosides were prepared by the methods of C. C. Sweeley et al. (J. Am. Chem. Soc. 85:2497, 1963) with some modifications. Gas-liquid chromatographic analysis of sugar derivatives was carried out by the methods of Sweeley et al. with the following as column packings: 5% of SE 52 on Shimadzu W, 60 to 80 mesh, and 15% of polyethylene glycol succinate on Chromosorb W, 60 to 80 mesh. As shown in Table 1, AB-80Aa was composed of arabinose and mannose, whereas AB-66Aa consisted chiefly of mannose with a small amount of arabinose. Details of chemical structure of AB-66Aa and AB-80Aa fractions are being investigated in our
laboratory. Polysaccharide fractions which were similar to AB-66Aa and AB-80Aa in chemical and immunological properties were also obtained from the cells of *Mycobacterium smegmatis*, *M. phlei*, *M. bovis* strain Ushi 10, and atypical mycobacteria, strain P. Further investigations on chemical and immunological properties of these and similar fractions will be reported later.

**Table 1. Chemical and immunological properties of polysaccharides obtained from Aoyama B strain of human tubercle bacillus**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>$[\alpha]_D^2$ (in water)</th>
<th>Elemental analysis</th>
<th>Sugar composition</th>
<th>Skin reaction$^a$</th>
<th>Active anaphylactic test$^b$</th>
<th>Precipitation reaction$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-80Aa</td>
<td>$+38.5$ (c = 1.019)</td>
<td>C, 39.71</td>
<td>Arabinose (6 parts)</td>
<td>Negative (at 10 µg)</td>
<td>$+++$ (at 0.6 mg)</td>
<td>Antigen titer, 1:512,000</td>
</tr>
<tr>
<td>AB-66Aa</td>
<td>$+74.2$ (c = 0.994)</td>
<td>C, 39.24</td>
<td>Mannose (6.5 parts)</td>
<td>Negative (at 10 µg)</td>
<td>$-$ (at 1 mg)</td>
<td>Antigen titer, 1:512,000</td>
</tr>
</tbody>
</table>

$^a$ Skin reaction was carried out in a tuberculous patient who reacted to old tuberculin.  

$^b$ Anaphylactic activity was examined in sensitized guinea pigs by methods described previously (Y. Yamamura et al., Am. Rev. Respirat. Diseases 91:839, 1965); $+++$, death as early as 5 min after the intravenous injection of antigen; $-$, no symptoms of anaphylaxis.  

$^c$ Rabbit antiserum was obtained by immunization with heat-killed tubercle bacilli in Freund adjuvant.