

Isolation and Purification of Anaphylactically Active Polysaccharide from Human Tubercle Bacilli

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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 NaH₂PO₄, 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- μ g 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 methanolysis, 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 Shimalite 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 P₁. 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*

Fraction	$[\alpha]_D^{25}$ (in water)	Elemental analysis	Sugar composition	Skin reaction ^a	Active ana- phylactic test ^b	Precipitation reaction ^c
AB-80Aa	+38.5 (<i>c</i> = 1.019)	% C, 39.71 H, 6.43 N, 0	Arabinose (6 parts) Mannose (4 parts)	Negative (at 10 μg)	+++ (at 0.6 mg)	Antigen titer, 1:512,000
AB-66Aa	+74.2 (<i>c</i> = 0.994)	C, 39.24 H, 6.72 N, 0	Mannose (6.5 parts) Arabinose (1 part)	Negative (at 10 μg)	— (at 1 mg)	Antigen titer, 1:512,000

^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.