Purification and Characterization of an Immunologically Active Glycoprotein from *Aspergillus fumigatus*

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Many investigators have shown that immunological techniques are useful for the clinical diagnosis of human aspergillosis, and some investigators have described the preparation of antigens for skin and precipitation tests [S. B. Salvin, *Progr. Allergy* 7:213, 1963; J. Pepys and J. L. Longbottom, p. 813, in D. M. Weir (ed.), *Handbook of Experimental Immunology*, 1967; J. Pepys et al., *Am. Rev. Respirat. Diseases* 80:167, 1959; J. Biguet et al., *Ann. Inst. Pasteur*, 107:72, 1964; J. Biguet et al., *Rev. Immunologie* 31:317, 1967]. However, the chemical nature of these antigens has not been defined. In earlier papers (Azuma et al., *Am. Rev. Respirat. Diseases* 91:839, 1965; Azuma et al., *Japan. J. Med. Mycol.* 8:210, 1967; Azuma et al., *Mycopathol. Mycol. Acta*, in preparation), we reported the isolation of polysaccharide which was shown to have skin and serological activities in the sensitized rabbit and in human aspergillosis. The present note describes the isolation and characterization of potent antigenic glycoprotein for skin test and serological reaction from the mycelium and spore of *Aspergillus fumigatus*. The immunological role of this fraction was examined in the rabbit or guinea pig immunized with *A. fumigatus*. The mycelium and spore of *A. fumigatus* (IFO 5,840), which were defatted by repeated extraction with ethyl alcohol-ethyl ether (1:1), were extracted with 50% pyridine at 37°C for 24 hr with stirring. After concentration, the supernatant fluid was passed through a Seitz filter to remove the cell debris, and was concentrated to dryness under reduced pressure. The residue dissolved in water was dialyzed against running water for 48 hr and was concentrated under reduced pressure. The concentrate was dissolved in a small amount of water and fractionated into two fractions, designated APSK-33 and APSK-66, by fractional precipitation with acetone. APSK-33 and APSK-66 fractions were obtained by the addition of acetone to a final concentration of 0 to 33% and 33 to 66%, respectively. The purification of

APSK-33 and APSK-66 fractions was carried out by the following procedure. APSK-33 and APSK-66 fractions were chromatographed on a column of ion-exchange resin Dowex 50 (H⁺ form) and were eluted with water followed by a 0.2 M Na₂HPO₄ solution. The water eluate was further chromatographed on a column of diethylaminoethyl (DEAE) cellulose and was eluted with water followed by 0.2 M NaH₂PO₄. The water eluate was purified by gel filtration on a Sephadex G-50 column and was eluted with 0.5 M NaCl. By gel filtration on Sephadex G-50, a single peak was obtained by analysis for protein and carbohydrate which was excluded. APSK-33 fraction purified in this way did not contain protein as impurities, however, 1.01% of nitrogen determined by the micro-Dumas method was contained in purified APSK-66 fraction. The sugar components of APSK-33 and APSK-66 fractions were analyzed by gas-liquid chromatography by the methods described previously (Azuma et al., *J. Bacteriol.* 95:263, 1968). The molar ratio of sugar was calculated for the peak area of each sugar. As shown in Table 1, APSK-33 was composed of glucose, whereas APSK-66 consisted of galactose and mannose in a molar ratio of 1:1.5. These results suggested that APSK-33 was a pure polysaccharide, glucan, and APSK-66 was a glycoprotein containing galactomannan as carbohydrate moiety.

In the present experiments, male rabbits weighing approximately 3 kg each were immunized by the intramuscular injection of acetone-dried *A. fumigatus* with Freund's complete adjuvant for three times at 2-week intervals. The intradermal injection of APSK-66 fraction elicited the Arthus-type and delayed-type (tuberculin type) skin reactions in immunized rabbits (Table 1 and Fig. 1). A nonimmunized rabbit was used as control, and saline was also used as a negative control of antigen. In all control groups, negative results were obtained.

TABLE 1. Chemical and immunological properties of polysaccharides obtained from *Aspergillus fumigatus*

Fraction	Yield (%) ^a	Elementary analysis (%)	Sugar composition (molar-ratio)	Skin reaction (mm by mm)			Precipitation reaction ^d
				Immunized rabbit ^b		Control ^c	
				at 6 hr	at 24 hr		
Purified APSK-33	0.79	C, 42.95 H, 6.55 N, trace	Glucose	Negative	Negative	Negative	Negative
Purified APSK-66	0.29	C, 41.02 H, 6.85 N, 1.01	Galactose (1.0) Mannose (1.5)	14 × 15 14 × 12	16 × 17 17 × 15	Negative Negative	Antigen titer 1:1,024,000

^a Calculated for acetone-dried fungal cells.^b Skin reaction was carried out at 1-μg dose in rabbits immunized with *A. fumigatus* in Freund's complete adjuvant.^c A nonimmunized rabbit was used as control.^d Antigen titer was determined by the "ring test" with rabbit antiserum. FIG. 1. Skin reaction with APSK-66 (1 μg), papain-treated APSK-66 (10 μg), and periodate-oxidized APSK-66 (10 μg) in rabbit immunized with *Aspergillus fumigatus*.

The activity of delayed-type (tuberculin type) skin reaction was reduced when APSK-66 was treated with proteolytic enzyme, papain, Pronase, or trypsin. The Arthus-type skin reactivity was not affected by the treatment of papain as shown in Fig. 1. The Arthus-type skin reactivity of APSK-66 was lost completely by periodate oxidation, but the delayed-type (tuberculin type) skin reactivity was retained. Tests with immunized rabbits gave results similar to those obtained by skin tests of APSK-66 in guinea pigs immunized with *A. fumigatus*.

From the above results, it was concluded that the Arthus-type skin reactivity of APSK-66 was due to the carbohydrate (galactomannan) moiety and delayed-type (tuberculin type) skin reactivity was due to the protein moiety of APSK-66. APSK-66 fraction showed potent serological activity, precipitation, complement-fixation, and passive hemagglutination tests with rabbit antiserum. A passive cutaneous anaphylaxis test was also positive by APSK-66 in guinea pigs with guinea pig antiserum. By immunodiffusion analysis according to the methods of O. Ouchterlony (*Acta Pathol. Microbiol. Scand.* 25:186, 1948) with rabbit antiserum, a single line was shown with APSK-66 fraction. APSK-33 (glucan) fraction did not show any immunological activity in the present experiments.