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Note

Serologic cross-reactivity of the D-galacto-D-mannans isolated from several pathogenic fungi against anti-*Hormodendrum pedrosoi* serum*

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We have recently reported the isolation and serological characterization of the antigenic D-galacto-D-mannans of three representative strains of the genus *Hormodendrum, i.e., H. pedrosoi, H. compactum*, and *H. dermatitidis*, which have been known to be the major causative agents of chromoblastomycosis[1]. Because galactomannans have been regarded as one of the most widely distributed polysaccharides among fungi[2], it was of interest to examine the cross-reactivity between the galactomannan of the genus *Hormodendrum* and those of the other pathogenic fungi. The present paper describes the nature of the cross-reactivity of five galactomannans produced by *Aspergillus fumigatus, Aspergillus niger, Trichophyton rubrum, Cladosporium werneckii*, and *H. pedrosoi* against anti-*H. pedrosoi* serum, by the use of quantitative precipitin and agar-gel double-diffusion reactions.

The existence of D-galactofuranose residues in *H. pedrosoi* galactomannan was ascertained by partial acid hydrolysis with 5mM sulfuric acid at 100° (see Fig. 1). About 40% of the reducing sugar was released within 5 h, and the monosaccharide components were analyzed by paper chromatography to detect galactose as the major reducing sugar. A significant decrease of D-galactose content in the acid-resistant core-moiety, isolated from the hydrolyzate as the nondialyzable fraction, is evident from the results shown in Table I. All five galactomannans, isolated from both homologous and heterologous sources, each gave one precipitin line in agar-gel, double-diffusion precipitin reactions against anti-*H. pedrosoi* serum, although a considerable difference was observed for the reactivity of each antigen (Fig. 2A). All precipitin lines produced by the heterologous polysaccharides showed spur formation with that of the homologous system. Two *Aspergillus* galactomannans behaved as weaker antigens, revealing only faint lines.

*Dedicated to Professor Michael Heidelberger in honor of his 87th birthday.

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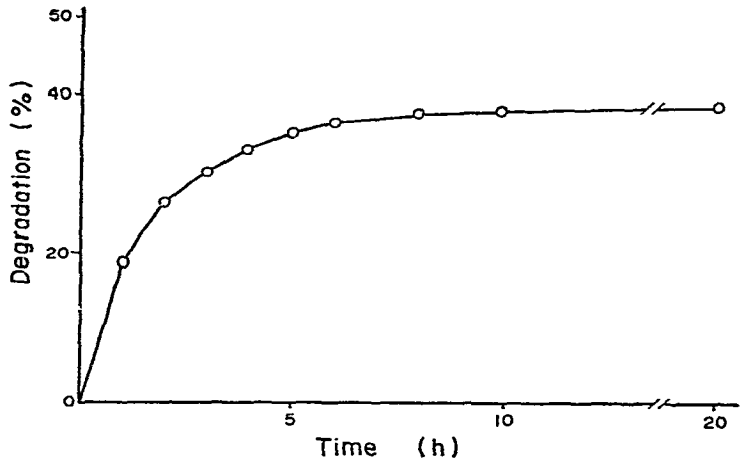

Fig. 1. Release of reducing sugar from *H. pedrosoi* galactomannan by acid hydrolysis with 5mM sulfuric acid at 100°.

TABLE I

COMPOSITION AND PROPERTIES OF FUNGAL GALACTOMANNANS AND ACID-RESISTANT CORE-MOIETY OF THE GALACTOMANNAN OF *H. pedrosoi*

Strain	*Carbohydrate*[a] (%)	*Nitrogen* (%)	*Phosphorus* (%)	$[\alpha]_D^{20}$,[b] (°)	*Ratio of mannose to galactose to glucose*
H. pedrosoi[1]	94.40	5.30[c]	0.6	−6.9	1:1.25:0.06[d]
A. fumigatus[5]	98.6	0.0	0.0	−2.0	1:1:0
A. niger[5]	98.7	0.0	0.0	+43.3	2.3:1:0
C. werneckii[6]	92.0	9.6[c]	1.8	−24	78:14
T. rubrum[7]	95.0	0.0	0.0	+45	2.45:1:(trace of glucose)
H. pedrosoi (acid-resistant core-moiety)	97.8	2.49	0.01[e]	+36.8	1:0.65:0.07[d]

[a]Determined by a modified Molisch method[3]. [b]In water, *c* 1.0. [c]As protein value, determined by Lowry–Folin method[4]. [d]A JEOL 3-C liquid chromatograph equipped with an anion-exchanger borate type column was used. [e]Determined by Ames–Dubin method[8].

The quantitative cross-precipitin curves shown by the five galactomannans against anti-*H. pedrosoi* serum (see Fig. 3) indicate that the homologous antigen precipitated the largest amounts of antibody nitrogen, whereas no heterologous polysaccharide gave an amount of nitrogen comparable with that of the homologous system. Both galactomannans from *C. werneckii* and *T. rubrum* revealed closely similar reactivities, although a considerable difference was found between the specific rotations of these polysaccharides. In addition, it was ascertained that no antibody nitrogen was precipitated by the acid-resistant core-moiety of *H. pedrosoi* galactomannan.

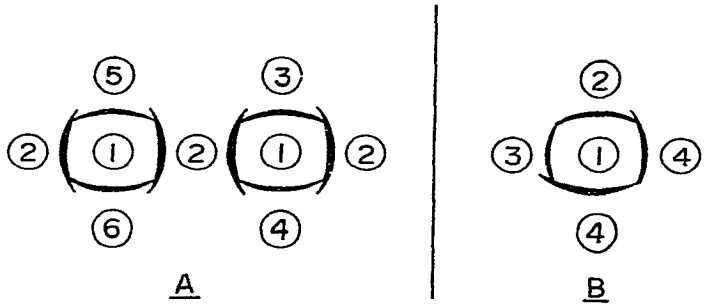

Fig. 2. Diagramatic representation of agar-gel double-diffusion patterns. *A*. Reaction between five fungal galactomannans and anti-*H. pedrosoi* serum: 1, Anti-*H. pedrosoi* serum; 2, *H. pedrosoi* galactomannan; 3, *C. werneckii* peptidogalactomannan; 4, *T. rubrum* galactomannan; 5, *A. fumigatus* galactomannan; and 6, *A. niger* galactomannan. *B*. Reaction of *H. pedrosoi* galactomannan and its acid-resistant core-moiety against anti-*Saccharomyces cerevisiae* serum: 1, Anti-*S. cerevisiae* serum; 2, *H. pedrosoi* galactomannan; 3, acid-resistant core-moiety of *H. pedrosoi* galactomannan; and 4, *S. cerevisiae* mannan. Each antigen was dissolved into saline at a concentration of 1 mg/ml.

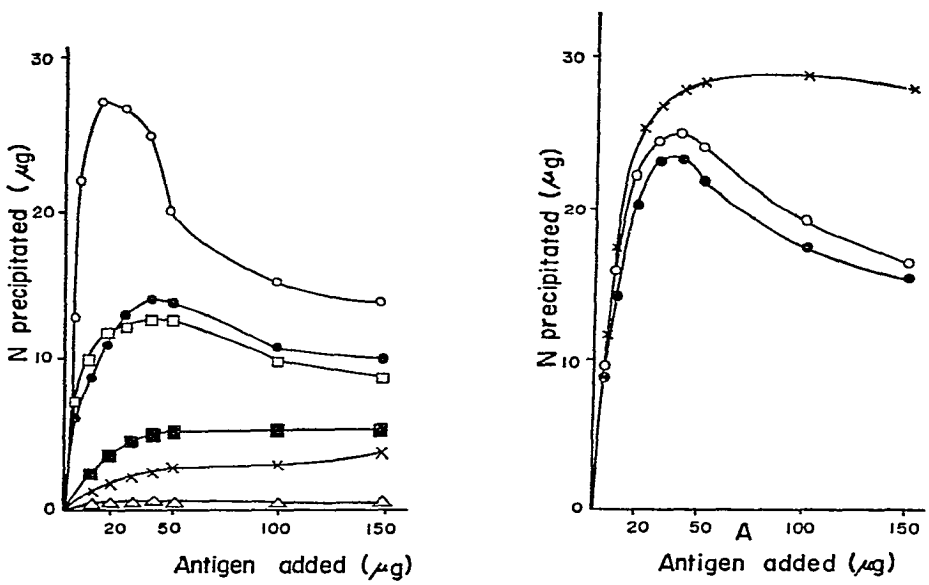

Fig. 3. Quantitative precipitin curves of five fungal galactomannans and the acid-resistant core-moiety of *H. pedrosoi* galactomannan against anti-*H. pedrosoi* serum. Each reaction mixture contained 100 μl of two-fold diluted antiserum and a total volume of 700 μl; ○, *H. pedrosoi* galactomannan; ●, *T. rubrum* galactomannan; □, *C. werneckii* peptidogalactomannan; ■, *A. fumigatus* galactomannan; ×, *A. niger* galactomannan; and △, acid-resistant core-moiety of *H. pedrosoi* galactomannan.

Fig. 4. Quantitative cross-precipitin curves of *H. pedrosoi* galactomannan and its acid-resistant core-moiety against anti-*Saccharomyces cerevisiae* serum. Each mixture contained 100 μl of antiserum and a total volume of 700 μl; ○, *H. pedrosoi* galactomannan; ●, acid-resistant core-moiety of *H. pedrosoi* galactomannan; and ×, *S. cerevisiae* mannan.

The galactomannan of *H. pedrosoi* and its acid-resistant core-moiety were also assayed for their cross-precipitin reaction against anti-*S. cerevisiae* serum (see Fig. 4). The precipitin activity of the core-moiety was not decreased to any extent when compared with that of the intact polysaccharide. When *H. pedrosoi* galactomannan and its core-moiety were assayed against the same antiserum, the precipitin lines formed were completely fused (see Fig. 2B).

On the basis of the just-described results, it is possible to suggest that *H. pedrosoi* galactomannan possesses 2 types of side-chains consisting of acid-labile D-galactofuranose residues and relatively acid-stable α-D-mannopyranose residues, and that the former side-chains are solely responsible for the immunological activity of *H. pedrosoi* galactomannan.

Although many fungi have been shown to involve galactomannans as their major polysaccharide constituent, relatively little concerning their cross-reactivity has been published. In a previous study[9], it was reported that the antisera of *Candida albicans*, a pathogenic yeast, and *S. cerevisiae* showed only borderline cross-reactivities against the galactomannan of *A. fumigatus*. More recently, Yokota *et al.*[10] investigated a series of cross-reactions between the antisera of *A. fumigatus* and *A. niger*, and the galactomannans of *A. fumigatus*, *A. niger*, *Penicillium chrysogenum*, *Trichophyton interdigitale*, and *Trichophyton granulosum*. The results showed that both antisera reacted strongly with the galactomannans of both *Aspergilli* and *P. chrysogenum*, but weakly with the galactomannans of both *Trichophyton*. These authors[10] also found that the acid-resistant core-moieties of *Aspergillus* galactomannans were unable to precipitate antibodies from the homologous antisera, although most of the cross-reactivity against anti-*S. cerevisiae* serum was retained.

The present results suggest that the antibody response to various fungal D-galacto-D-mannans predominantly depends on the D-galactofuranose residues, even though a considerable proportion of nonreducing, terminal residues of D-mannopyranose are present in the same molecule.

EXPERIMENTAL

Materials. — Mycelial galactomannans of *A. fumigatus* and *A. niger* were provided by Dr. M. Suzuki of this college. The method of preparation of both polysaccharides has been reported previously[5]. The specimen of extracellular galctomannan of *T. rubrum* was the same as that described previously[7]. The peptido-galactomannan of *C. werneckii*, Fr. B, was a kind gift of Dr. K. O. Lloyd[6]. The galactomannan of *H. pedrosoi* was the major extracellular galactomannan fraction designated previously[1] as Hp-FG-I-p.2. The components of these galactomannans are listed in Table I. All heterologous galactomannans used in the present study were found to contain a considerable proportion of acid-labile galactofuranosyl residues[5–7].

The preparation of anti-*H. pedrosoi* whole-cell serum was the same as that used in the preceding study[1]. It had a 1:1024 agglutination titer against the homologous cell suspension. Anti-*Saccharomyces cerevisiae* (baker's yeast) serum, which has been

shown to be specific for α-D-(1→3)- and α-D-(1→2)-linked mannopyranosyl residues[11,12], was a stock preparation of this laboratory.

Methods. — The quantitative precipitin reaction and agar-gel double-diffusion reaction were performed according to the procedures of Sunayama[13] and Ouchterlony[14], respectively.

The hydrolysis of galactomannan of *H. pedrosoi* (100 mg) was performed by dissolution in 5mM sulfuric acid, and heating the solution in a boiling water-bath. Aliquots (100 μl each) were withdrawn at regular (time) intervals, and the reducing power determined by the Somogyi–Nelson method[15]. After 5 h, a portion of hydrolyzate (7.5 ml) was collected, neutralized with sodium hydrogencarbonate solution, and dialyzed against distilled water. The retentate was concentrated under diminished pressure, and the nondialyzable, acid-resistant core-moiety of the galactomannan recovered by the addition of ethanol. After being deionized, the dialyzable fraction was examined by paper chromatography, which indicated galactose as the major reducing sugar.

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