

Immunological Studies on Dermatophytes

II. Serological Reactivities of Mannans Prepared from Galactomannans I and II of *Microsporium quinckeanum*, *Trichophyton granulosum*, *Trichophyton interdigitale*, *Trichophyton rubrum*, and *Trichophyton schoenleinii*

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The contribution of terminal galactofuranose residues to the antigenic specificity and to cross-reactivity of galactomannans isolated from five species of dermatophytes, *Microsporium quinckeanum*, *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, and *T. schoenleinii*, was investigated. Galactofuranose units were removed from galactomannans I and galactomannans II by mild acid hydrolysis. The resulting mannans were tested for serological reactivity with rabbit antiserum to *M. quinckeanum* by qualitative precipitation in gel and by quantitative complement-fixation analyses. Our results showed that, with this antiserum, the galactofuranose residues contributed greatly to the antigenic specificity and to cross-reactivity of the galactomannans II, but these residues were less significant as antigenic determinants in the galactomannans I. We have shown that mannans isolated from three *Candida* species reacted with rabbit antiserum to *M. quinckeanum*.

Rabbit antiserum to autoclaved mycelium of a dermatophyte, *Microsporium quinckeanum*, is comprised of antibodies which react with neutral polysaccharides isolated from this species, as well as with those isolated from four additional dermatophyte species (7). This investigation was carried out to establish the role of D-galactofuranoside nonreducing end groups in the immunological specificities of the galactomannans I and II isolated from these species, namely, *M. quinckeanum*, *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, and *T. schoenleinii*. We also studied mannans isolated from three species of *Candida*.

MATERIALS AND METHODS

The isolation of the polysaccharides and the elucidation of their gross chemical structures have been investigated (1-6). Figure 1 illustrates the preparation of the mannans from the galactomannans by partial acid hydrolysis (6).

Mannans isolated from *Candida albicans* serotype B, *C. stellatoidea*, and *C. tropicalis* were obtained from H. F. Hasenclever, National Institutes of Health, Bethesda, Md. Their isolation and gross chemical structures have been reported (8).

Preparation of the antiserum to *M. quinckeanum* used in these analyses and the immunochemical tests performed were described previously (7).

RESULTS

Reactions of mannans prepared from galactomannans I and II with rabbit antiserum to M. quinckeanum. The reactions of mannans I and mannans II with rabbit antiserum to *M. quinckeanum* were examined by gel diffusion analyses. Each of the mannans I gave precipitin bands that appeared identical with the band obtained with galactomannan I of *M. quinckeanum* (Fig. 2). The bands obtained with the mannans II seemed identical with each other, but these bands differed in intensity—*T. rubrum* mannan II was the weakest (Fig. 3). The galactomannan II of *M. quinckeanum* showed spur formation with the mannans II. This indicated that some antigenic determinants which were on the galactomannans II were no longer present in the mannans II prepared from them.

Quantitative analysis of the serological reactivities of mannans I and mannans II by complement fixation with whole rabbit antiserum to M. quinckeanum. As given in Table 1, the reactivities of the mannans I were quite similar to those of the galactomannans I (7). The mannan I from *T. schoenleinii* was the least reactive. The mannan I of *M. quinckeanum* was even more reactive than the parent galactomannan I. However, mannans II were less reactive than their

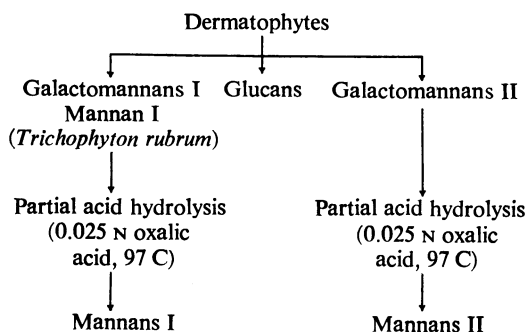


FIG. 1. Preparation of the mannans.

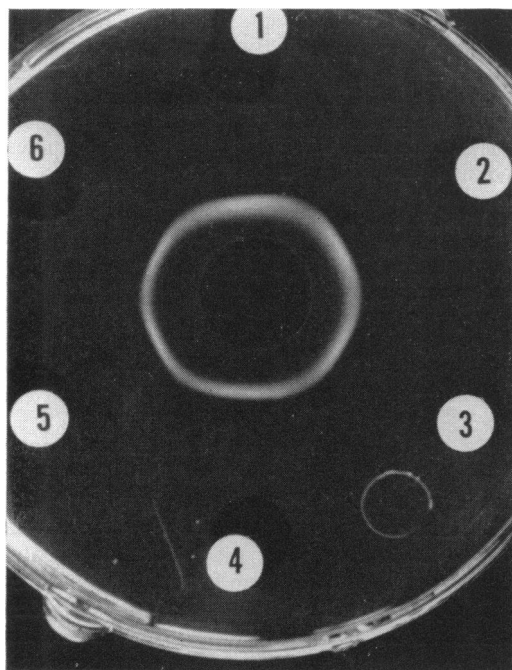


FIG. 2. Immunodiffusion analysis of mannans I. Center well: Rabbit antiserum to *Microsporium quinckeanum*. Periphery: galactomannan I, *M. quinckeanum* (1); mannan I, *M. quinckeanum* (2); mannan I, *Trichophyton granulosum* (3); mannan I, *T. interdigitale* (4); mannan I, *T. rubrum* (5); and mannan I, *T. schoenleinii* (6). Concentration of polysaccharides is 100 $\mu\text{g/ml}$.

parent galactomannans II; *T. rubrum* mannan II was the least reactive (7).

Complement-fixation analysis of the serological reactivities of mannans I with antiserum absorbed with galactomannan II and mannans II with antiserum absorbed with galactomannan I of *M. quinckeanum*. Table 1 summarizes the relative

reactivities of these polysaccharides. The galactomannan I of *M. quinckeanum* reacted well with antiserum absorbed with galactomannan II. However, the reactivities of the mannans I with antiserum absorbed with galactomannan II were decreased, except for the mannan isolated from *T. rubrum*. The mannan I from *T. schoenleinii* was the least reactive. The relative reactivities of these polysaccharides with this absorbed antiserum did not differ significantly from those obtained with whole rabbit antiserum.

The reactivities of the mannans II were diminished when antiserum absorbed with galactomannan I was used. Larger amounts of each were required for fixation of complement. Compared with the other four mannans II, the relative reactivity of the homologous mannan II varied considerably from that measured with whole antiserum. A cross-reaction between mannans II and antibody specific for galactomannan I or for mannan I of *M. quinckeanum* seemed possible.

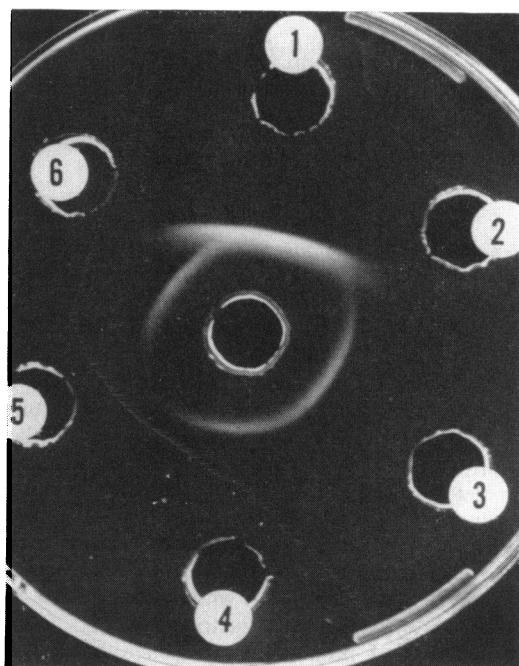


FIG. 3. Immunodiffusion analysis of mannans II. Center well: rabbit antiserum to *Microsporium quinckeanum*. Periphery: galactomannan II, *M. quinckeanum*, 100 $\mu\text{g/ml}$ (1); mannan II, *M. quinckeanum* (2); mannan II, *Trichophyton granulosum* (3); mannan II, *T. interdigitale* (4); mannan II, *T. rubrum* (5); and mannan II, *T. schoenleinii* (6). Concentration of polysaccharides is 200 $\mu\text{g/ml}$.

TABLE 1. Complement-fixation analysis of serological reactivities of mannans I and mannans II with whole rabbit antiserum to *Microsporium quinckeanum*, antiserum absorbed with galactomannan I, and antiserum absorbed with galactomannan II^a

Source	Mannan I (μ g)			Mannan II (μ g)		
	Whole antiserum ^b	Absorbed antiserum		Whole antiserum ^e	Absorbed antiserum	
		With galactomannan II ^c	With galactomannan I ^d		With galactomannan I ^d	With galactomannan II ^f
<i>M. quinckeanum</i>001	0.055	0.607	0.290	0.640	.160
<i>Trichophyton granulorum</i>003	0.045	1.00	0.021	1.500	.063
<i>T. interdigitale</i>003	0.030	0.750	0.010	0.810	.063
<i>T. rubrum</i>001 ^g	0.005 ^g	0.030 ^g	2.00	2.00 ^h	.700
<i>T. schoenleinii</i>011	2.00 ^h	0.030	0.003	0.160	.063
<i>M. quinckeanum</i> ⁱ002	0.008	0.550	0.002	0.006	.620

^a Results are given as the amount of antigen necessary to fix 50% of the guinea pig complement in the system as determined from the reaction curves. The change in optical density at 541 m μ , as the difference between the antiserum control and the reaction mixture, was used to measure the amount of guinea pig complement fixed, which is proportional to the amount of antibody present.

^b Antiserum (0.2 ml) diluted 1:400 for analysis.

^c Antiserum (0.2 ml) absorbed with galactomannan II diluted 1:200 for analysis.

^d Antiserum (0.2 ml) absorbed with galactomannan I diluted 1:100 for analysis.

^e Antiserum (0.2 ml) diluted 1:800.

^f Antiserum (0.2 ml) absorbed with galactomannan II diluted 1:100.

^g A mannan I was isolated from *T. rubrum*.

^h No complement fixation.

ⁱ In these tests, reactivities of galactomannans I and II, rather than mannans I and II, were determined.

Complement-fixation analysis of the serological reactivities of mannans I with antiserum absorbed with galactomannan I and mannans II with antiserum absorbed with galactomannan II of *M. quinckeanum*. Although precipitating antibody to the galactomannan I was no longer detectable after absorption (Table 1), the galactomannan I of *M. quinckeanum* still reacted with antiserum absorbed with galactomannan I, owing to the sensitivity of the complement-fixation analysis in determining small amounts of residual antibody to this polysaccharide. However, larger quantities of the mannans I were required for reactivity with this galactomannan I-absorbed serum than for reactivity with whole serum or serum absorbed with galactomannan II. Only the mannan I prepared from galactomannan I of *T. schoenleinii* reacted more strongly with this galactomannan I-absorbed serum than with antiserum absorbed with galactomannan II. This mannan I may be cross-reacting with antibody to galactomannan II of *M. quinckeanum*.

The antiserum absorbed with galactomannan II of *M. quinckeanum* still contained residual antibody to galactomannan II (Table 1). Each of the mannans II, except that from *T. rubrum*, exhibited a stronger reaction than galactomannan

II of *M. quinckeanum* with this galactomannan II-absorbed antiserum, and each reacted more strongly than with antiserum absorbed with galactomannan I. These analyses confirmed a cross-reaction between the mannans II and antibody to galactomannan I of *M. quinckeanum*.

Analysis of mannans isolated from *Candida* species as antigens reacting with rabbit antiserum to *M. quinckeanum*. Although reactions with this antiserum were not detectable by precipitation in gel, the serological cross-reactivities of these polysaccharides with antiserum to *M. quinckeanum* could be measured by complement-fixation analyses (Table 2). Each of these mannans also reacted with this antiserum following absorption with either galactomannan I or galactomannan II of *M. quinckeanum*.

DISCUSSION

The serological reactivities of the mannans I prepared by mild acid hydrolysis of the galactomannans I showed that the galactofuranoside units contributed little to the cross-reactivity of galactomannans I with antiserum to *M. quinckeanum*. Galactomannan I of *T. schoenleinii* was apparently an exception; removal of the galactofuranoside units decreased its

reactivity. In contrast, the galactofuranoside units contributed greatly to the specificity and cross-reactivities of the galactomannans II. These results are not surprising because of the relative amounts of galactofuranoside units in these two groups of polysaccharides. The galactomannans II contained considerably more galactofuranoside units (23 to 29%); these units measured only 0 to 18% in the galactomannans I (3, 4). Antibodies to galactomannans II should therefore have a greater proportion of active sites directed toward galactofuranoside groups. In the galactomannans I, apparently the structural feature responsible for the serological reactivities is the linear sequence of 1→6 linked mannopyranose units that predominate in this group of polysaccharides.

Absorption of antibodies to galactomannan II of *M. quinckeanum* from this antiserum did not affect the serological reactivities of the galactomannans I nor did absorption of antibodies to galactomannans I influence the reactivities of the galactomannans II (7). However, in our investigation, reactivities of mannans II were decreased with antiserum absorbed with galactomannan I, and the reactivity of *M. quinckeanum* mannan II,

relative to the others, was not the same as with the whole antiserum (Table 1). These results can be explained in terms of the structures of the polysaccharides. It is known (3, 4) that the galactomannans II could contain some monosaccharide units joined as shown in the partial structure in Fig. 4. Removal of the galactofuranoside groups apparently would expose additional linear 1 → 6 linked D-mannopyranose units. An increase in the number of those units in the galactomannans II would account for the cross-reactivity with antibody to galactomannans I because a linear chain of 1 → 6 linked D-mannopyranose units is the predominant structural feature of the galactomannans I. It has been postulated (7) that a variation in the sequence of 1 → 2 and 1 → 6 linkages in the linear portions of the galactomannans II could be responsible for the variations in serological reactivities within that group. The exposure of additional 1 → 6 linkages by removal of galactofuranoside groups from the galactomannans II would alter the sequences of 1 → 2, 1 → 6 linkages and could thus explain the different relative reactivities of the mannans II, as compared to the galactomannans II (7).

Also, the reactivities of the mannans I were diminished when antiserum absorbed with galactomannan II was used (Table 1); this occurrence indicated a cross-reaction with antibodies to galactomannans II. Therefore, it is likely that removal of galactofuranoside units from the galactomannans I exposed a structural feature that also was present in the galactomannans II. This structural feature could well be a linear 1 → 2 linkage. Unlike the galactomannans II, the galactomannans I contained no linear 1 → 2 linked D-mannopyranose. But 1 → 2 linkages were involved in the promotion of branch points in the galactomannans I, and if galactofuranoside units formed one or more of the branches, then removal would expose some linear 1 → 2 linkages. The mannans I cross-

TABLE 2. Complement-fixation analysis of mannans from *Candida* species as antigens reacting with rabbit antiserum to *Microsporium quinckeanum*^a

Source of polysaccharide	Amount of mannan (μg)
<i>C. albicans</i>	2.00
<i>C. stellatoidea</i>	1.58
<i>C. tropicalis</i>	0.258
<i>M. quinckeanum</i>	
Mannan I ^b	<0.005

^a Reactivity given as amount of mannan necessary for 50% fixation of complement in system; for analyses, 0.2 ml of whole antiserum diluted to 1:100 was used.

^b Mannan II was not included in the analysis.

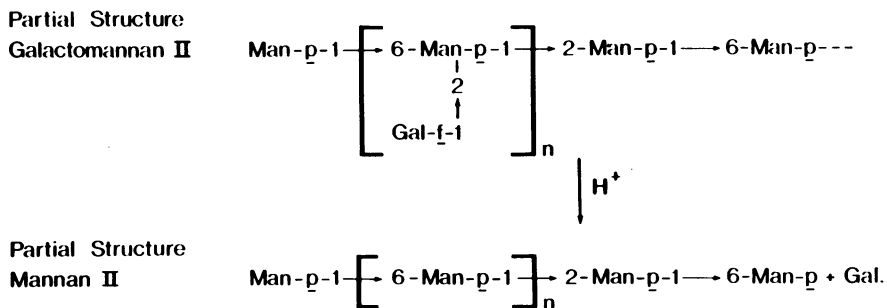


FIG. 4. Exposure of linear 1 → 6 linkages by removal of galactofuranoside units from galactomannan II.

reacted with antibody to galactomannan II of *M. quinckeanum* to a lesser extent than did the mannans II with antibody to galactomannan I because the galactomannans I contained fewer galactofuranoside units than did the galactomannans II. Thus, removal of the galactofuranoside units from galactomannans I would expose fewer new linear linkages than would be revealed by a similar process in the galactomannans II.

The mannans from three *Candida* species demonstrated cross-reactivity with the antiserum used in this study. Those mannans should therefore have some structural features in common with the galactomannans of the dermatophytes. The predominant structural feature of the *Candida* mannans is a linear chain of 1 → 2 linked α-D-mannopyranose units (8). Also, such units are present in the galactomannans II of the dermatophytes; this occurrence is probably an explanation for the observed cross-reactivity.

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