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Evidence for Adhesin Activity in the Acid-Stable Moiety of the Phosphomannoprotein Cell Wall Complex of *Candida albicans*

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Previously, we showed that *Candida albicans* hydrophilic yeast cells adhere specifically to mouse splenic marginal-zone macrophages. The adhesins are part of the yeast cell wall phosphomannoprotein complex, and one adhesin site, which reacts with the monoclonal antibody 10G, was identified as a β-1,2-linked tetramannose in the acid-labile portion of the complex. We report here that the acid-stable part of the complex, which has not been reported previously to have adhesin activity, is in large part responsible for yeast cell binding to the splenic marginal zone. The phosphomannoprotein complex, termed Fr.II, was isolated from *C. albicans* serotype B yeast cells by β-mercaptoethanol extraction and concanavalin A-agarose affinity chromatography. Fr.II is devoid of the serotype A-specific antigen factor 6, which functions in yeast cell attachment to epithelial cells. The acid-stable part of Fr.II (i.e., Fr.IIS) was obtained by mild acid hydrolysis and size exclusion fractionation. Fr.IIS was further fractionated into four fractions, Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4, by concanavalin A-agarose column chromatography and elution with a linear gradient of α-methyl-D-mannopyranoside. Adhesin activity of these fractions was determined by their ability to block yeast cell binding to the splenic marginal zone. Fr.IIS1 and Fr.IIS2 yielded more material and stronger adhesin activity than either Fr.IIS3 or Fr.IIS4. Only Fr.IIS1 did not react with antibodies (anti-factor 5 and monoclonal antibody 10G) specific for the acid-labile β-1,2-linked oligosaccharides. Fr.IIS1-coated latex beads attached specifically to the marginal zone in a pattern identical to that of yeast cell binding. Furthermore, Fr.IIS1-latex bead attachment was inhibited by soluble Fr.II or Fr.IIS. Initial chemical analyses indicate that the adhesin site on Fr.IIS1 is a carbohydrate because adhesin activity was destroyed by periodate oxidation but not by proteinase K digestion.

A variety of characteristics of *Candida albicans* have been reported as possible virulence factors (7, 9, 15, 32). The initial step in the pathogenesis of candidiasis is adherence of the fungus to host tissue (5, 23). We have used an ex vivo assay to study mechanisms of adherence of *C. albicans* to mouse spleen and lymph node tissues (10, 14, 16, 22) and described that cell wall mannans are responsible for binding of hydrophilic yeast-form cells to mouse macrophages of the splenic marginal zone and of lymph node tissue (21). When *C. albicans* cells were injected intravenously, many yeast cells accumulated near or within the marginal zone of the spleen (10, 22). To help gain an understanding of mechanisms by which *C. albicans* disseminates (4), it is important to define the adhesin molecule(s) of *C. albicans* responsible for the ex vivo and in vivo adherence of yeast cells to splenic tissue.

Many adhesins of *C. albicans* that play a role in attachment of the fungus to various host tissues or to inanimate surfaces have been characterized (3, 5, 12, 13, 20, 24, 27, 28, 35), and the cell wall mannoprotein has received the most attention. The protein portion of the mannoprotein appears to contain an adhesin site for yeast attachment to epithelial cells (29, 37) and is responsible for hydrophobic interactions (15, 17). Others have found that the carbohydrate portion is important in yeast cell adherence to host epithelial cells and fibrinogen (6, 30, 31, 34), and we found it to be involved in adherence to splenic marginal-zone macrophages (22, 28).

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We showed that hydrophilic *C. albicans* yeast cells adhere specifically to macrophages located in the splenic marginal zone and in the subcapsular and medullary sinuses of peripheral lymph node tissue of mice (10, 14, 21). The specific adherence can be demonstrated for the spleen and lymph node tissues by an ex vivo assay and by in vivo analysis for splenic tissue. We also showed that the mannan portion of the phosphomannoprotein, designated Fr.II, isolated from yeast-form *C. albicans* cells by β-mercaptoethanol (2-ME) extraction and concanavalin (ConA)-agarose affinity chromatography, contained the adhesins responsible for attachment to macrophages in both types of tissues (21).

It was recently reported that the factor 6 antigen of serotype A strains of *C. albicans* plays a role in fungal adherence to epithelial cells (30, 31). Chaffin et al. (8) compared the adherence properties of a cell surface *C. albicans* mutant with those of wild-type *C. albicans* and suggested the importance of cell wall mannan, especially β-1,2-linked oligomannosyl residues. In accordance with these findings, Li and Cutler (28) identified one adhesin site on the acid-labile portion of the mannoprotein as a β-1,2-linked tetramannosyl residue. However, in both of the latter studies, the results indicated that additional adhesins were on the mannoprotein.

To define other adhesin sites in the phosphomannoprotein complex involved in adherence of *C. albicans* to the marginal zone of the mouse spleen, we isolated an acid-stable mannoprotein fraction from a serotype B strain of *C. albicans* and examined its adhesin activity. We report that the acid-stable mannoprotein fraction shows strong adhesin activity but is devoid of epitopes for factors 5 (36) and 6 (26) or for antigen

10G (28), which contains the β-1,2-linked oligomannosyl adhesin site.

MATERIALS AND METHODS

Organisms and culture conditions. *C. albicans* TCH32 (serotype A), NUM1385 (serotype A), and A9 (serotype B) were used. Strain A9 was used extensively in previous studies (10, 21, 22, 32). Strain TCH32 was isolated from a patient with candida vaginitis, and its characteristics were described previously (2). Stock cultures of both strains were maintained in 50% glycerol at −80°C. Yeast cells were spread onto GYEP (2% glucose, 0.3% yeast extract, 1% peptone) agar plates and cultured for 48 h at 37°C, and yeast cells from a single colony were inoculated into GYEP broth and cultured overnight at 37°C under aeration by reciprocal shaking at 100 strokes per min. The cells were inoculated into fresh GYEP broth and cultured for 24 h at 37°C under shaking conditions. For ex vivo adherence assays, the stationary-phase yeast cells which had a hydrophilic surface (16, 21, 32) were washed four times in cold distilled water and the cell number was adjusted to 1.5×10^8/ml in the Dulbecco modified Eagle medium (DMEM) described below. The hydrophilic stationary-phase yeast cells were also used for isolation of the adhesin.

Acid-stable fraction of the phosphomannoprotein complex. The *C. albicans* phosphomannoprotein fraction and Fr.II eluted with α-methyl-D-mannopyranoside from a ConA-agarose column were obtained as described previously (21). Briefly, *C. albicans* yeast-form cells at the stationary phase of growth in GYEP broth were treated for 30 min at 20 to 23°C with 0.3 M 2-ME in 0.1 M EDTA (pH 9.0) to solubilize the adhesin molecules. After the supernatant material was centrifuged, it was dialyzed against distilled water, lyophilized, and referred to as the 2-ME extract. The 2-ME extract was applied to a ConA-agarose column, and bound materials were eluted with 0.5 M α-methyl-D-mannopyranoside. The eluted fractions were pooled, dialyzed against distilled water for 48 h, lyophilized, and designated Fr.II.

For preparation of the acid-stable part of Fr.II, Fr.II (100 mg) was dissolved in 10 ml of 10 mM HCl and heated for 60 min at 100°C (25, 28). The solution was cooled to room temperature, neutralized with 100 mM NaOH, and concentrated in vacuo to ca. 1 ml. The sample was applied to a Toyopearl HW 40S (Toyo Soda Manufacturing Company Ltd., Tokyo, Japan) column (2.5 by 95 cm) and eluted with distilled water at a flow rate of 15 ml/h (2.5 ml per fraction). Each fraction was monitored for carbohydrate content (11), and fractions corresponding to each carbohydrate peak were pooled. The void volume was collected separately, referred to as the acid-stable fraction, and designated Fr.IIS.

Fr.IIS was further fractionated by affinity chromatography on a ConA-agarose (Honen Co., Tokyo, Japan) column. Fr.IIS (40 mg) was dissolved in 10 ml of 20 mM phosphate buffer (pH 7.2) containing 0.15 N NaCl (PBS) and applied to the column (2 by 10 cm). The column was washed with PBS until the A_{220} and A_{280} of the eluate fractions were negligible. ConA-bound materials were eluted with a linear gradient of 0 to 0.5 M α-methyl mannopyranoside at a flow rate of 20 ml/h (5 ml per fraction). Fractions corresponding to a total of four peaks determined by A_{220} and A_{280} were pooled, and each was rechromatographed under the same conditions, dialyzed against distilled water, and lyophilized. The pools were referred to as Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4, and each was tested for adhesin activity as indicated below.

Antibodies. Antisera specific for factors 5 and 6 of *C. albicans* were obtained from a commercial source (Candida Check; Iatron Laboratories Inc., Tokyo, Japan). Factor 5 corresponds to a β-1,2-linked oligomannosyl residue of both serotype A and B strains of *C. albicans* (36), and factor 6 corresponds to serotype A-specific β-1,2-linked mannopyranose units in the acid-stable part of the cell wall phosphomannoprotein (26).

Mouse immunoglobulin M (IgM) monoclonal antibody (MAb) 10G is specific for a mannan determinant on the cell wall surface of *C. albicans*. MAb 10G reacts with the acid-labile part of the phosphomannoprotein complex and is specific for a β-1,2-linked tetramannosyl residue (28).

An anti-*C. albicans* polyclonal rabbit serum obtained previously from animals immunized against *C. albicans* NUM1385 (serotype A) (19) was also used as a positive control for mannan fractions in the dot blot analysis.

Sodium periodate and proteinase K treatments. For sodium periodate treatment, 50 μl of either Fr.II or Fr.IIS was treated with 50 μl of various concentrations of sodium periodate as described previously (21). For proteinase treatment, Fr.IIS and Fr.IIS1 were digested with 10 to 100 μg of proteinase K (E. Merck, Darmstadt, Germany) per ml under the same conditions as those described previously (21). The treated and control samples (i.e., samples treated with buffer only) were tested for adhesin activity and used in Western blot (immunoblot) analysis.

Immunoblotting. The existence of β-1,2-linked mannosyl residues in the phosphomannoprotein fractions and in each peak separated by a size exclusion column of mild-acid-treated Fr.II was examined by a dot blot or a competitive inhibition dot blot test. Three microliters of phosphomannoprotein sample solution (5 mg/ml) was placed onto each of several locations on nitrocellulose sheets (Cellulosenitrat type E BA 85; Schleicher & Schuell, Dassel, Germany) and dried for 60 min at 37°C. The sheets were blocked by soaking in 3% skim milk in 20 mM Tris-buffered saline (TBS) for 30 min. Each sheet was reacted with one of the following antisera for 2 h at 30°C: polyclonal rabbit anti-*C. albicans* serum (1:500), factor 5 (1:200), factor 6 (1:200), or MAb 10G (1:500). After the sheets were washed in the buffer, they were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG serum (1:1,000; Bio-Rad Laboratories, Inc., Hercules, Calif.) for antigen factors 5 and 6 or in goat anti-mouse IgM serum (1:1,000; Jackson Immunoresearch Laboratories, Inc.) for antigen 10G for 2 h at 30°C.

For determination of the release of acid-labile oligomannosyl residues from Fr.II, a competitive inhibition dot blot test was done. Five-microliter volumes of various concentrations of Fr.II were placed onto nitrocellulose sheets, dried, and blocked with skim milk. Each sheet was incubated for 2 h at 30°C with MAb 10G (1:500) in the presence of 50 μg of fractions per ml eluted from the HW 40S size exclusion column or with anti-factor 5 polyclonal antibody (1:200) in the presence of a mixture of the fractions containing the acid-labile components (Fr.IIL1 to Fr.IIL7; each at 50 μg/ml). The sheets were washed and incubated with horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgM antibodies for 2 h at 30°C. All antibodies were diluted in 1% skim milk–TBS. After the second incubation, all sheets were washed in TBS and incubated in a mixture of 50 ml of TBS plus 30 μl of 30% H_2O_2 plus 10 ml of methanol containing 30 mg of 4-chloro-1-naphthol.

In dot blot analysis, the reactivity of fractionated samples with various antibodies was measured as dot intensity by a DVS 3000 image analysis system and a CCD camera (Hamamatsu Photonics K. K., Hamamatsu City, Japan).

Adhesin-coated latex beads. Coating of glycan adhesins onto latex beads was done as described before (21, 28). Evidence that the latex beads became coated was obtained by observing

agglutination of the beads in the presence of the anti-*C. albicans* rabbit polyclonal serum, whereas bovine serum albumin (BSA)-coated control beads did not agglutinate. BSA coating of control beads was confirmed by agglutination of the coated beads in the presence of anti-BSA rabbit serum, which was obtained from rabbits immunized against BSA emulsified in Freund complete adjuvant.

Ex vivo adherence assay. The ex vivo adherence stationary method described previously (21, 22, 33) was used throughout the work.

For binding of latex beads coated with adhesin to splenic tissue, 100 μl of 1.5×10^8 coated beads per ml was allowed to interact with the cryosections of spleen for 40 min instead of 15 min as described for the yeast cells. After the tissue was incubated, it was fixed and washed gently, and sections were embedded in 30% glycerin in PBS for manual counting of adherent beads by bright-field microscopy (33).

Solubilized adhesin activity. The presence of adhesin activity in various fractions was tested by the ex vivo adherence inhibition assay described previously (21). Briefly, splenic tissue cryosections were pretreated with test fractions by overlaying the sections with solubilized adhesin fractions in DMEM (Sigma Chemical Co., St. Louis, Mo.) containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; GIBCO, Grand Island, N.Y.) and 5% fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah; adjusted to pH 7.4) for 15 min at 5 to 8°C. After pretreatment, yeast cells or adhesin-coated latex beads were added to the sections. If adhesins are present in the solubilized materials, pretreatment of tissue should block the attachment of the yeast cells or coated beads to the marginal zone.

Measurement of carbohydrate, protein, and phosphate. Total amounts of carbohydrate, protein, and phosphate in each fraction were determined by the phenol-sulfuric acid method (11) with D-mannose as the standard, by the Bio-Rad protein assay system using BSA as the standard, and by the method of Ames and Dubin (1) using KH_2PO_4 as the standard, respectively.

RESULTS

Fractionation of the acid-stable part of the mannoprotein. Mannoproteins with adhesin activity were isolated from *C. albicans* A9 (a serotype B strain) by 2-ME extraction and fractionation by ConA-agarose column chromatography. The resultant adhesin fraction eluted from the ConA column with α-methyl-D-mannopyranoside was referred to as Fr.II as described previously (21). Fr.II hydrolyzed with HCl and fractionated through the size exclusion column yielded seven oligosaccharide acid-labile peaks retained by the column and a large acid-stable fraction eluted in the void volume (Fig. 1, Fr.IIS). These fractions are referred to as Fr.IIL1, Fr.IIL2, Fr.IIL3, Fr.IIL4, Fr.IIL5, Fr.IIL6, Fr.IIL7, and Fr.IIS, respectively. We tested the acid-labile peaks for β-1,2-linked oligomannosyl residues by the competitive binding dot blot test. Fr.IIL1 and Fr.IIL2 did not inhibit the reactivity of MAb 10G (which is specific for β-1,2-linked oligomannosyl residues) to Fr.II, whereas the remaining fractions were inhibitory (not shown). The reaction of Fr.II with anti-factor 5 antibody (which also is specific for β-1,2-linked oligomannosyl residues) was inhibited by a mixture of Fr.IIL1 through Fr.IIL7 (not shown). These results indicated that the acid-labile β-1,2-linked oligomannosyl residues were released from Fr.II by mild acid hydrolysis, and the acid-stable part was defined as the void volume fraction (Fr.IIS) from the size exclusion column.

Application of Fr.IIS to a ConA-agarose column and elution

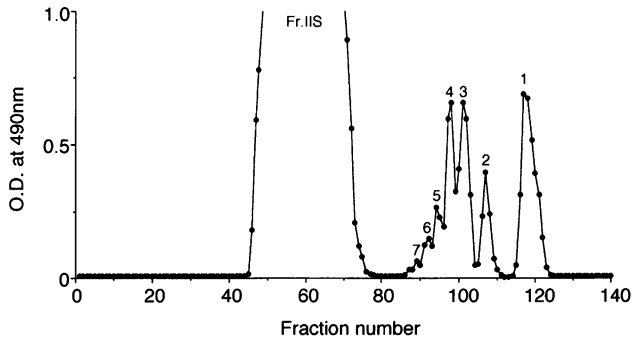


FIG. 1. Size exclusion chromatographic profile of the phosphomannoprotein (Fr.II) acid hydrolysate. Fr.II was acid hydrolyzed, and the fractions were separated on a Toyopearl HW 40S size exclusion column and monitored for carbohydrate content by the phenol-sulfuric acid colorimetric assay. Seven fractions (Fr.IIL1, Fr.IIL2, Fr.IIL3, Fr.IIL4, Fr.IIL5, Fr.IIL6, and Fr.IIL7) of the acid-labile oligomannosyl residues were separated on the column, and the acid-stable part of Fr.II was eluted in the void volume and referred to as Fr.IIS. Each fraction was concentrated in vacuo, appropriately rehydrated, and tested for adhesin activity. O.D., optical density.

with a linear gradient of α-methyl mannopyranoside yielded four peaks designated Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4 (Fig. 2). The dry weight yields of Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4 from Fr.IIS (40 mg) were 16, 10, 6, and 4 mg, respectively.

The ratios of respective carbohydrate, protein, and phosphorus concentrations for each fraction (in milligrams [dry weight]) were as follows: Fr.II, 94.6:2.5:1.2; Fr.IIS, 91.6:5.8:1.3; Fr.IIS1, 92.7:4.7:1.3; Fr.IIS2, 93.1:4.0:1.3; Fr.IIS3, 93.3:3.9:1.3; and Fr.IIS4, not determined. The adhesin activity and characterization of these fractions were determined as indicated below.

Immunoblotting of phosphomannoprotein fractions. Dot blot analysis of the mannoprotein fractions showed that factor

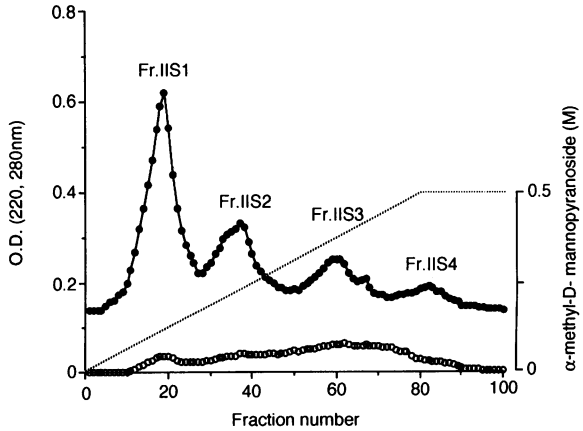


FIG. 2. Fractionation of Fr.IIS by ConA-agarose column chromatography. Fr.IIS was applied to a ConA-agarose column, and fractions were eluted with a linear gradient of α-methyl mannopyranoside. The A_{220} (closed circles) and A_{280} (open circles) of each fraction were monitored. Four peaks were determined and referred to as Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4, respectively. All fractions showed adhesin activity by the ex vivo adherence assay. Fr.IIS1 did not contain detectable amounts of acid-labile β-1,2-linked oligomannosyl residues. O.D., optical density.

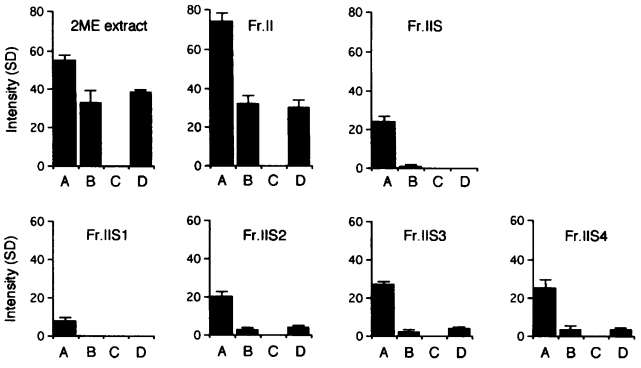


FIG. 3. Reactivity of serotype B *C. albicans* phosphomannoprotein fractions with various antibodies as determined by the dot blot test. Three microliters of phosphomannoprotein fractions was placed on nitrocellulose sheets, and the sheets were incubated with polyclonal antibody anti-*C. albicans* serum, factor 5 and factor 6, and MAb 10G. After the sheets were incubated with the secondary antibody (horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgM) and substrate reactivity had occurred, the intensity of each dot was measured by an image analysis system. Anti-factor 6 antibody reacted only with extracts from a serotype A strain (TCH32; not shown) and did not react with fractions from strain A9 (serotype B). The letters on each *x* axis stand for anti-*C. albicans* (strain 1385) polyclonal rabbit serum (A), anti-factor 5 polyclonal rabbit serum (B), anti-factor 6 polyclonal rabbit serum (C), and MAb 10G (D). Error bars indicate standard deviations.

6 could be detected in extracts from *C. albicans* TCH32 (serotype A; not shown) but was not detectable in any fractions from the *C. albicans* A9 strain (Fig. 3), which is a serotype B strain and should not produce factor 6. Fr.IIS, obtained as a result of mild acid hydrolysis of Fr.II, had only slight reactivity with anti-factor 5 and essentially no reactivity with MAb 10G compared with Fr.II (Fig. 3), indicating possible slight contamination with acid-labile β-1,2-oligomannosyl components. When the four ConA-agarose affinity fractions of Fr.IIS were tested against the antibodies, Fr.IIS1 did not react with anti-factor 5 antiserum or with MAb 10G, indicating undetectable amounts of acid-labile components. However, a slight reaction was observed in the other three fractions (Fr.IIS2, Fr.IIS3, and Fr.IIS4; Fig. 3).

The reactivity profile of the rabbit anti-*C. albicans* (strain NUM1385, serotype A) differed from those of the other antibodies. The anti-strain NUM1385 reacted with all fractions; however, the anti-strain NUM1385 showed the least amount of reactivity against Fr.IIS1 (Fig. 3). These data indicate that Fr.IIS1 is relatively free of acid-labile components from the phosphomannoprotein complex and contains less antigen that cross-reacts with antibodies raised against the serotype A strain.

Adhesin activity of the acid-stable part of the phosphomannoprotein. Fr.II, Fr.IIS, Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4 were tested for their ability to inhibit adherence of *C. albicans* yeast cells to the marginal zone of mouse splenic tissue (Table 1; Fig. 4). Tissue sections pretreated with Fr.II blocked adherence of yeast cells, as expected (21), at all pretreatment concentrations tested (e.g., when tissues were pretreated with Fr.II at 1 μg/ml, an average of 14.5 yeast cells per field adhered to tissue compared with 52.5 yeast cells per field for tissues pretreated with the diluent DMEM; Table 1). The ability of the soluble material to block yeast adherence is a criterion used to conclude that the soluble material has adhesin activity. By this same criterion, Fr.IIS obtained from Fr.II by mild acid

TABLE 1. Adhesin activity of the acid-stable fraction (Fr.IIS) of the phosphomannoprotein complex (Fr.II) of *C. albicans*[a]

Pretreatment with[b]:	Avg no. of adherent yeast cells/field (SE) at concn of:				
	0[c]	0.1	1.0	10.0	100.0
Fr.II		24.7 (5.6)	14.5 (3.7)	7.4 (2.6)	1.7 (0.8)
Fr.IIS		21.5 (4.8)	9.0 (4.4)	3.0 (1.5)	1.8 (0.8)
DMEM	52.5 (7.3)				

[a] Cryosections of spleen were pretreated with various concentrations of Fr.II or Fr.IIS, the sections were overlaid with *C. albicans* yeast cells, and the number of yeast cells that adhered to the marginal zone were determined.

[b] Fr.II is the phosphomannoprotein complex obtained by 2-ME extraction and fractionation by ConA-affinity chromatography. Fr.IIS is the acid-stable fraction from Fr.II. DMEM is described in Materials and Methods. DMEM alone represents the control for maximum binding without inhibition.

[c] Concentration of each fraction (in micrograms per milliliter of DMEM) used to pretreat the tissue cryosections.

hydrolysis also showed strong adhesin activity, albeit less than that of Fr.IIS. Activity was also found associated with Fr.IIS1, Fr.IIS2, Fr.IIS3, and Fr.IIS4, but Fr.IIS1 and Fr.IIS2 gave stronger activity than Fr.IIS3 and Fr.IIS4 (Fig. 4).

To obtain more-direct evidence of adhesin activity associated with Fr.IIS1, latex beads coated with this fraction bound the splenic marginal zone in a pattern essentially identical to the binding distribution of whole yeast cells (Fig. 5). Furthermore, the adherence of the coated beads was inhibited in the presence of soluble Fr.IIS or Fr.IIS1 (data not shown).

A preliminary investigation into the chemical nature of the Fr.IIS and Fr.IIS1 adhesin fractions showed them most likely to be carbohydrates because treatment with various concentrations of sodium periodate reduced their ability to block yeast cell adherence to the splenic marginal zone (Fig. 6), whereas proteinase K treatment did not affect their adhesin activity (data not shown).

DISCUSSION

The *C. albicans* cell wall phosphomannoprotein complex is composed of acid-stable and phosphate-bound acid-labile moieties. Mild acid hydrolysis breaks the phosphate bond of the

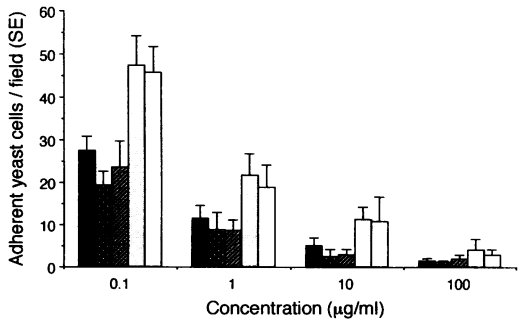


FIG. 4. Inhibitory activity of phosphomannoprotein fractions obtained from Fr.IIS (■) by affinity chromatography on a ConA-agarose column. Various concentrations of Fr.IIS1 (▨), Fr.IIS2 (▩), Fr.IIS3 (▩), and Fr.IIS4 (□) were added to splenic tissue before the addition of yeast cells, and their adhesin activities were tested as described in Materials and Methods. The results are expressed as the mean numbers of adherent yeast cells per field with standard errors (bars). Fr.IIS1 and Fr.IIS2 gave stronger adhesin activity than Fr.IIS3 and Fr.IIS4. Control groups pretreated with DMEM gave only an average of 57.8 yeast cells per field (data not shown).

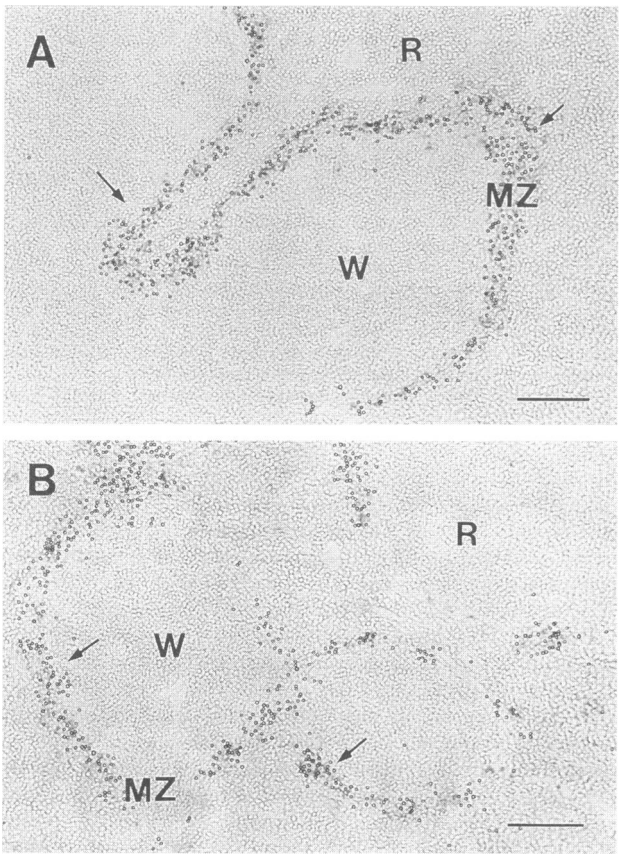


FIG. 5. Attachment of the adhesin-coated latex beads to the marginal zone of mouse splenic tissue. Fr.II (A) and Fr.IIS1 (B) were coated onto latex beads, and the beads were allowed to interact with cryosections of splenic tissue. In both cases, the coated latex beads attached specifically to the marginal zone (arrows). The binding of the coated beads was inhibited by soluble Fr.IIS and Fr.S1, but BSA-coated control beads did not bind to the marginal zone of the spleen (not shown). Abbreviations: MZ, marginal zone; W, white pulp; R, red pulp. Bar, 100 μm.

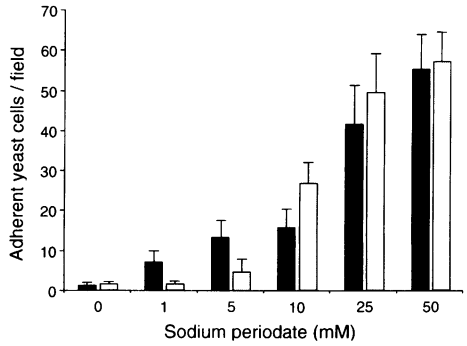


FIG. 6. Effect of sodium periodate on adhesin activity of the phosphomannoprotein complex. Fr.II (■) and Fr.IIS1 (□) antigens were treated with 1 to 50 mM sodium periodate, and then their adhesin activities were tested. Results are expressed as the mean numbers of adherent yeast cells per field with standard errors (bars). Sodium periodate treatment destroyed the adhesin activity of the fractions as evidenced by restoration of yeast cell binding.

phosphomannoprotein, resulting in release of β-1,2-linked oligomannosyl residues (Fig. 7) (25, 36).

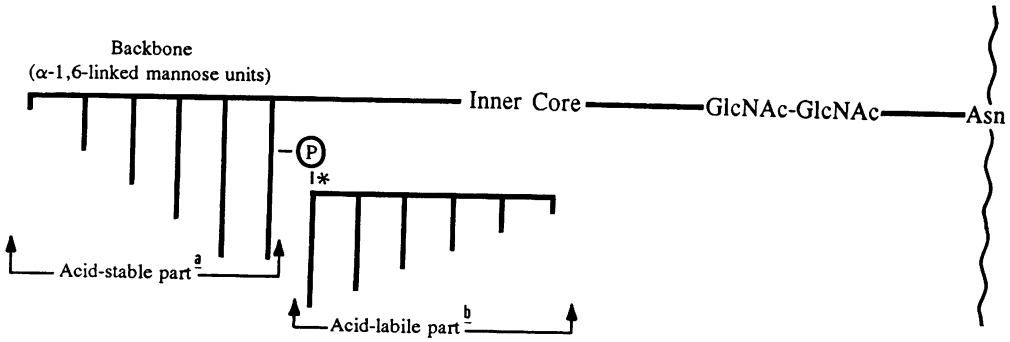
In addition to adhesin activity of the acid-labile portion of the phosphomannoprotein complex (28), in the present study we showed that the acid-stable moiety of the complex also contains an adhesin site(s) that is responsible for adherence of *C. albicans* yeast cells to the mouse splenic marginal zone. When Fr.II was treated with 10 mM HCl at 100°C and fractionated on a size exclusion column, the results from dot blot competitive binding tests showed that the hydrolysis caused the release of the majority of acid-labile oligosaccharide residues from Fr.II, and the acid-stable part of mannoprotein eluted in the void volume as expected (Fig. 1). However, Fr.IIS gave slight reactivity with anti-factor 5 antiserum, indicating a small amount of contamination of Fr.IIS with acid-labile oligosaccharides. Separation of Fr.IIS into four fractions by ConA-agarose chromatography yielded one fraction, Fr.IIS1, that showed no reactivity with either anti-factor 5 antiserum or MAb 10G (Fig. 3). Fr.IIS1 was thus pursued for further analysis since it lacked the β-1,2-linked oligomannosyl residues.

Antigen factor 6 has been reported to be associated with β-linked oligomannosyl residues in the acid-stable part of the phosphomannoprotein of *C. albicans*. Factor 6 appears to function as an adhesin moiety but is produced only by serotype A strains (30). In accordance with the reported strain specificity of factor 6, anti-factor 6 did not react with fractions obtained from the serotype B strain used in our studies but was reactive with a serotype A strain (TCH32) of *C. albicans* (Fig. 3). These results show that antigen 6 is not involved in the attachment of serotype B *C. albicans* cells to the marginal zone of splenic tissue. Factor 6 may have more importance in attachment of *C. albicans* to other types of host cells, since Miyakawa et al. reported that factor 6 plays a role in the attachment of serotype A strains to epithelial cells (31). Although we have not ruled out a role for factor 6, we have not been able to detect a difference between serotype A and B strains with regard to their ability to adhere to splenic marginal-zone macrophages (10, 22).

The β-1,2-linked oligomannosyl residues located in the acid-labile part of the phosphomannoprotein of serotype B strains (Fig. 7) appear to function in the specific attachment of *C. albicans* yeast cells to the marginal zone of the mouse spleen. Chaffin et al. (8) reported that a *C. albicans* mutant lacking β-1,2-linked oligosaccharides showed less attachment to the marginal zone than the normal wild-type strain. Li and Cutler (28) identified the epitope for MAb 10G to be a β-1,2-linked tetramannosyl residue in the acid-labile part of the phosphomannoprotein complex, and the epitope functions as an adhesin site on the complex. However, evidence that there is an additional adhesin site(s) in the phosphomannoprotein complex was also presented in both articles.

Our studies support the existence of at least one adhesin site in the acid-stable portion of the phosphomannoprotein complex. As expected, not all of the adhesin activity could be attributed to the acid-stable fraction Fr.IIS (Table 1), but adhesin activity of Fr.IIS was actually higher than adhesin activity detected in the acid-labile fractions (data not shown). Fr.IIS, which did not contain either factor 5 or 10G antigens, showed strong adhesin activity. Finally, latex beads coated with Fr.IIS1 showed binding specificity for the splenic marginal zone. These results indicate that at least two types of splenic marginal-zone macrophage receptors are involved in the attachment of *C. albicans* yeast cells.

The chemical nature of *C. albicans* adhesins associated with the phosphomannoprotein complex has been the subject of investigations by several groups. Apparently, the adhesin site



P phosphate bond

* acid-labile linkage

a oligomannosyl chains (α-1,2- and α-1,3-linked mannose units)

b oligomannosyl chains (β-1,2-linked mannose units)

protein

FIG. 7. Simplified model of the cell wall phosphomannoprotein complex of *C. albicans* serotype B based on a proposed structure by Kobayashi et al. (25). The number of mannose units in each oligomannosyl side chain ranges from 1 to 7. The backbone phosphomannan portion may be tandemly repeated. Asp, asparagine.

may vary depending on the particular tissue examined. The protein portion of the complex may function in attachment of *C. albicans* to buccal epithelial cells (29), extracellular matrix proteins (23), or to other tissue sites because of hydrophobic properties (18). As alluded to above, oligomannosyl residues in the acid-labile portion of the complex also act as adhesins, and our findings reported here are the first to indicate an adhesin role for carbohydrate moieties associated with the acid-stable portion.

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