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Structures of Cell Wall Mannans of Pathogenic *Candida tropicalis* IFO 0199 and IFO 1647 Yeast Strains

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We conducted a structural analysis of the cell wall mannans isolated from two *Candida tropicalis* strains, IFO 0199 and IFO 1647, exhibiting strong agglutinabilities against anti-*Candida* factor sera 5 and 6. The products released from these mannans by acid treatment were identified as the oligosaccharides, from biose to pentaose, consisting solely of β-1,2-linked mannopyranose units corresponding to common epitopes of *Candida albicans* serotypes A and B (factor 5). Mild acetolysis of acid- and alkali-treated mannans produced large amounts of hexaose and heptaose, Manpβ1-2Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man and Manpβ1-2Manpβ1-2Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, corresponding to the *C. albicans* serotype A-specific epitopes (factor 6). However, the homologous pentaose, Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, was not generated by this procedure. The oligosaccharides (biose to hexaose) obtained from the mannans by conventional acetolysis were composed exclusively of α-1,2-linked mannopyranose units. Therefore, the mannans of *C. tropicalis* IFO 0199 and IFO 1647 do not have the α-1,3-linked mannopyranose units previously observed in the mannans of *C. albicans* and *Candida stellatoidea*. The results of this study and previous findings indicate that the similarity of the antigenicities of three *Candida* species, *C. albicans* serotype A, *C. stellatoidea* type II, and *C. tropicalis*, reside in the β-1,2 and α-1,2 linkages containing oligomannosyl side chain (factor 6) in the cell wall mannan.

In addition to the species *Candida albicans* and *Candida stellatoidea*, *Candida tropicalis* is one of the important pathogenic yeasts in humans belonging to the genus *Candida*. Regarding the serological correlation between these species, it was reported by Hasenclever and Mitchell (12–14) that the immunologic behaviors of *C. albicans* serotype A strain were closely similar to those of *C. tropicalis*, while *C. albicans* serotype B strain exhibited properties closely identical to those of *C. stellatoidea*.

In previous articles, we reported the structural determination of the antigenic mannans of *C. albicans* (19–21) and demonstrated that the side chains corresponding to pentaose, hexaose, and heptaose, Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, Manpβ1-2Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, and Manpβ1-2Manpβ1-2Manpβ1-2Manpα1-2Manpα1-2Manpα1-2Man, dominate the *C. albicans* serotype A specificity (23). It is of interest that the same epitopes exist in the mannan of *C. stellatoidea* ATCC 20408 (24), which was identified by Kwon-chung et al. (27) to be type II on the basis of a chromosome analysis using pulsed-field gel electrophoresis. Furthermore, Miyakawa et al. (33) suggested that the serotype A-specific epitopes are extensively involved in the mechanisms of adherence of *C. albicans* to human buccal epithelial cells.

Additional findings that the β-1,2-linked oligomannosyl residues linked through phosphate served as major common epitopes throughout *C. albicans* serotypes A and B emphasized the importance of the β-1,2-linked mannopyranose unit as the component of epitopes of *C. albicans* (37). We also reported that both mannans of *C. stellatoidea* types I and II contain β-1,2-linked oligomannosyl side chains corresponding to common epitopes of *C. albicans* (16, 24). Li and Cutler reported that the mannotetraosyl side chain corresponding to one of the common epitopes was involved in the attachment of *C. albicans* cells to mouse spleen marginal-zone macrophage (29). Recently, Chaffin et al. (5) characterized several *C. albicans* mutant strains and reported that a mannan of these strains is deficient in these epitopes consisting of the β-1,2-linked mannopyranose unit on the basis of signals in ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra. On the other hand, Poulain et al. (35) and Trinel et al. (45) found the antigenic phospholipomannans containing these epitopes from *C. albicans* cell extract.

Although the structure of the *C. tropicalis* mannan was investigated by Hamajima et al. (11) and Kogan et al. (26) on the basis of NMR and/or methylation analysis, they could not propose the fine chemical structures. Therefore, we conducted a structural analysis of the mannans isolated from the yeast-form cells of two *C. tropicalis* strains in the hope of clarifying the location of the same β-1,2-linkage-containing epitope(s) as those detected in the mannans of *C. albicans* and *C. stellatoidea*.

MATERIALS AND METHODS

General. *C. tropicalis* IFO 0199, IFO 0589, IFO 1400, and IFO 1647 were obtained from the Institute for Fermentation Osaka, Osaka, Japan. *C. albicans* J-1012 (serotype A) and NIH B-792 (serotype B) were kindly supplied by T. Shinoda, Department of Microbiology, Meiji College of Pharmacy, Tokyo, Japan. *C. stellatoidea* ATCC 11006 (type I) and ATCC 20408 (type II) were obtained from the American Type Culture Collection, Rockville, Md. These were maintained on Sabouraud agar slants. Polyclonal rabbit anti-*Candida* factor

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sera (PFAbs; Candida Check [lot no. R156]; Iatron, Tokyo, Japan), corresponding to antigens 1, 4, 5, 6, 8, 9, 11, 13, 13b, and 34, as defined by Fukazawa et al. (10), were used. Jack bean α-mannosidase (EC 3.2.1.24) was obtained from Sigma Chemical Co. (St. Louis, Mo.). Column packing for gel filtration chromatography (Bio-Gel P-2; 400 mesh), with a fractionation range of 100 to 1,800 Da, was obtained from Bio-Rad (Richmond, Calif.).

Slide agglutination reaction. The slide agglutination reaction between the cells of yeast strains and PFAbs (Candida Check) was done by a modification (23) of the method of Miyakawa et al. (32).

Cultivation of *C. tropicalis* and preparation of mannan. Cultivation of *C. tropicalis* and preparation of mannan were performed as described for *C. albicans* J-1012 (15). The yeast forms of IFO 0199 and IFO 1647 were cultivated in Sabouraud liquid medium at 27°C for 72 h on a reciprocal shaker. Preparation of mannan was conducted by a combination of hot-water extraction and short term precipitation with Fehling solution (19). It is well known that the condition used in this procedure is as gentle as the hexadecyltrimethylammonium bromide (Cetrimid; Sigma) treatment (19, 44). The mannan fractions obtained from the cells of IFO 0199 and IFO 1647 were designated Fr 0199 and Fr 1647, respectively.

Treatment of Fr 0199 and Fr 1647 with 10 mM HCl. Treatment of Fr 0199 and Fr 1647 was done as described by Shibata et al. (40). Briefly, mannan was dissolved in 10 mM HCl, and the solution was heated in a boiling water bath for 1 h. The solution was neutralized with 100 mM NaOH concentrated in vacuo. The hydrolysate was applied to a column of Bio-Gel P-2 (2.5 by 100 cm) and eluted with water (0.25 ml/min). The acid-modified Fr 0199 and Fr 1647 were designated Fr 0199-a and Fr 1647-a, respectively.

Treatment of Fr 0199, Fr 1647, Fr 0199-a, and Fr 1647-a with 100 mM NaOH (40). Mannan was dissolved in 100 mM NaOH, and the resultant solution was kept at 25°C for 18 h. The solution was neutralized with 1 M HCl, concentrated, and applied to a column of Bio-Gel P-2 (2.5 by 100 cm). The alkali-modified Fr 0199, Fr 1647, Fr 0199-a, and Fr 1647-a were designated Fr 0199-b, Fr 1647-b, Fr 0199-ab, and Fr 1647-ab, respectively.

Conventional acetolysis of Fr 0199-ab. Conventional acetolysis of Fr 0199-ab was done by a modification (22) of the method of Kocourek and Ballou (25). A 10:10:1 (vol/vol/vol) mixture of $(CH_3CO)_2O$, CH_3COOH, and H_2SO_4 was used for the acetolysis. After de-*O*-acetylation, the resultant oligosaccharides were fractionated on a column (2.5 by 100 cm) of Bio-Gel P-2.

Mild acetolysis of Fr 0199-ab and Fr 1647-ab. Mild acetolysis of Fr 0199-ab and Fr 1647-ab was done with a 100:100:1 (vol/vol/vol) mixture of $(CH_3CO)_2O$, CH_3COOH, and H_2SO_4 as described previously (22). Separation of the region containing longer-chain oligosaccharides than hexaose was unsatisfactory in the case of mild acetolysis of Fr 0199-ab. This was due to the presence of several isomers as judged by observation of the peak shape in the elution profile. This region was further treated with jack bean α-mannosidase to degrade the isomer(s) consisting of α linkages as described below.

α-Mannosidase treatment of the fraction consisting of oligosaccharide isomers with longer chains than hexaose obtained from Fr 0199-ab by mild acetolysis. This treatment was conducted by the method of Shibata et al. (41). Briefly, each longer-chain oligosaccharide fraction was dissolved in 50 mM sodium acetate buffer (pH 4.6), to a concentration of 5 mg/ml, and 10 U of α-mannosidase per ml was added to the solution. After incubation at 37°C for 48 h, each reaction mixture was applied to a column (2.5 by 100 cm) of Bio-Gel P-2 and eluted with water.

TABLE 1. Slide agglutination assay for the whole cells of four *C. tropicalis* strains with anti-*Candida* PFAbs

Strain	Agglutination with PFAb[a]									
	1	4	5	6	8	9	11	13	13b	34
C. tropicalis										
IFO 0199	++	++	+	++	−	−	−	−	−	−
IFO 0589	++	++	+−	+	−	−	−	−	−	−
IFO 1400	++	++	+	+	−	−	−	−	−	−
IFO 1647	++	++	+	++	−	−	−	−	−	−
C. albicans[b]										
J-1012	++	++	+	+	−	−	−	−	−	−
NIH B-792	++	++	+	−	−	−	−	−	+	−
C. stellatoidea[b]										
ATCC 11006	++	++	+	−	−	−	−	−	−	−
ATCC 20408	++	++	+	+	−	−	−	−	−	−

[a] Agglutination was scored from high (++) to low (+−), and no agglutination (−).
[b] The agglutination reactions of *C. albicans* J-1012 (serotype A) and NIH B-792 (serotype B) and *C. stellatoidea* ATCC 11006 (type I) and ATCC 20408 (type II) are shown for comparative purposes.

Other methods. Total carbohydrate was determined by the phenol-sulfuric acid method (7) with D-mannose as the standard. Total protein was determined by the Folin method of Lowry et al. (31) with bovine serum albumin (Sigma) as the standard. Total phosphate was determined by the method of Ames and Dubin (3) with KH_2PO_4 as the standard. Four-hundred-megahertz ^1H NMR spectrum analyses were conducted exactly as described previously (19) with acetone as the standard (2.217 ppm). Specific rotations were determined by means of a JAS DIP-360 digital polarimeter. The sample was dissolved in water, and measurement was done after 3 h of dissolution of each sample in water.

RESULTS

Slide agglutination assay between *C. tropicalis* strain cells and PFAbs. The results of the assay with commercially available PFAbs (Candida Check) and heat-killed cell suspensions of four *C. tropicalis* strains, IFO 0199, IFO 0589, IFO 1400, and IFO 1647, are shown in Table 1. The cells of all strains agglutinated with PFAbs 1, 4, 5, and 6 in a manner identical to that of *C. albicans* serotype A and *C. stellatoidea* type II strains as reported previously (15, 24). Of the four *C. tropicalis* strains, IFO 0199 cells were agglutinated with PFAb 6 to the same extent as IFO 1647 cells, whereas the cells of IFO 0589 and IFO 1400 exhibited lower agglutinabilities than IFO 0199 and IFO 1647 cells. In the present structural study, therefore, the

TABLE 2. Chemical compositions and specific rotations of mannans Fr 0199 and Fr 1647 obtained from *C. tropicalis* strains

Fr	Total carbohydrate (%)[a]	Total protein (%)[b]	Total phosphate (%)[c]	$[\alpha]_D^{25}$ (degree)[d]	Yield (%)[e]
0199	87	1.7	0.33	+31.1	6.47
1647	85	2.3	0.53	+30.8	3.75

[a] Determined by the phenol-sulfuric acid method (7).
[b] Determined by the Folin method of Lowry et al. (31).
[c] Determined by the Ames-Dubin method as $-H_2PO_3$ (3).
[d] 1% (wt/vol) solution in water.
[e] Weight basis of the acetone-dried whole cells.

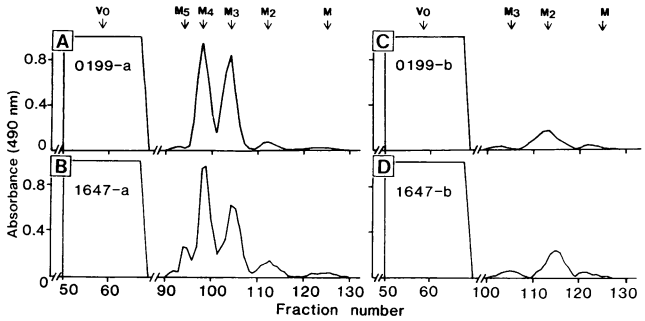

FIG. 1. Gel filtration profiles of the products obtained from Fr 0199 (A and C) and Fr 1647 (B and D) by treatment with 10 mM HCl at 100°C for 1 h (A and B) or with 100 mM NaOH at 25°C for 18 h (C and D) on a column (2.5 by 100 cm) of Bio-Gel P-2 by elution with water at 0.25 ml/min. The carbohydrate in the eluate was determined by the phenol-sulfuric acid method (7). G, M, M_2, M_3, and M_4 indicate D-glucose, D-mannose, mannobiose, mannotriose, and mannotetraose, respectively. Vo refers to the void volume.

mannans of IFO 0199 and IFO 1647 have been investigated for the existence of an epitope(s), factor 6.

Isolation of the mannans Fr 0199 and Fr 1647 from cells of IFO 0199 and IFO 1647 strains. Chemical compositions, specific rotations, and yields of Fr 0199 and Fr 1400 are given in Table 2. Both mannans have compositions nearly identical to those observed previously for *C. albicans* and *C. stellatoidea* and large amounts of carbohydrate (>85%). The low specific rotation values of these mannans indicate large numbers of β-1,2-linked mannopyranose residues in Fr 0199 and Fr 1647.

Acid treatment of Fr 0199 and Fr 1647. The mannans were treated with 10 mM HCl at 100°C for 1 h to isolate oligosaccharides linked through phosphate. Each hydrolysate was fractionated on a column of Bio-Gel P-2. As shown in Fig. 1A and B, acid treatment of Fr 0199 and Fr 1647 resulted in several oligosaccharides, from biose (M_2) to pentaose (M_5), in amounts of 0.5 and 2.6%, respectively, on the basis of parent mannans. The ^{1}H NMR spectra of these oligosaccharides were apparently identical to those of oligosaccharides isolated from mannans from *C. albicans* and *C. stellatoidea*, which were described previously (16, 19–21, 24) and identified as β-1,2-linkage-containing oligosaccharides on the basis of the assignment of H-1 signals of the same oligosaccharide series obtained from *C. albicans* mannan (38, 39) (Table 3). The acid-stable fractions Fr 0199-a and Fr 1647-a were obtained as the void-volume (V_0) regions in the gel filtration patterns.

Alkali treatment of Fr 0199, Fr 1647, Fr 0199-a, and Fr 1647-a. As shown in Fig. 1C and D, the presence of alkali-labile biosyl residues in the mannans Fr 0199 and Fr 1647 were evident because of the M_2 release corresponding to 0.2 and 0.4%, respectively, on the basis of parent mannans. This M_2 was identified as Manpα1-2Man by ^{1}H NMR analysis (Table 3). In this analysis, the presence of small amounts of epimerization product, Manpα1-2Glc, in each biose fraction was detected in accordance with the previous description (18) (data not shown). Similar treatment of the acid-stable mannan fractions Fr 0199-a and Fr 1647-a with alkali produced the acid- and alkali-stable mannan fractions Fr 0199-ab and Fr 1647-ab, respectively, lacking the *O*-linked mannobiosyl residue consisting of an α-1,2 linkage (data not shown).

^{1}H NMR analysis of mannans Fr 0199, Fr 0199-a, Fr 0199-b, Fr 0199-ab, Fr 1647, Fr 1647-a, Fr 1647-b, and Fr 1647-ab. Figures 2IA and IB show the ^{1}H NMR spectra (H-1 region) of parent mannans Fr 0199 and Fr 1647, respectively, demonstrating that these signals closely resemble those of the mannan of *C. albicans* serotype A strain (21). The weak signals, 5.542 and 5.563 ppm, and a strong signal, 4.839 ppm, indicate the presence of side chains corresponding to common epitopes throughout *C. albicans* serotypes A and B and the side chain corresponding to one of the *C. albicans* serotype A-specific epitopes, respectively. On the ^{1}H NMR spectra of Fr 0199-a and Fr 1647-a (Fig. 2IIA and IIB), loss of the weak signals mentioned above is evidence that the phosphate-bound side chains (the common epitopes) were eliminated from each parent mannan by acid treatment. On the other hand, Fr 0199-b and Fr 1647-b gave signals indistinguishable from those of Fr 0199 and Fr 1647 (Fig. 2IIIA and IIIB). Also, Fr 0199-ab and Fr 1647-ab exhibited signals closely identical to those of Fr 0199-a and Fr 1647-a (Fig. 2IVA and IVB). These findings

TABLE 3. ^{1}H chemical shifts (anomeric region) of oligosaccharides (α anomer) obtained from *C. tropicalis* mannans Fr 0199 and Fr 1647

Oligosaccharide[a]	Sugar residue[b]							Chemical shift (ppm)[c]							Distribution[d]	
	G	F	E	D	C	B	A	G	F	E	D	C	B	A	0199	1647
I																
M_2						Mβ1-2M							4.758	5.282	–	+
M_3					Mβ1-2Mβ1-2M							4.845	4.823	5.264	+	+
M_4				Mβ1-2Mβ1-2Mβ1-2M							4.910	4.917	4.819	5.272	+	+
M_5			Mβ1-2Mβ1-2Mβ1-2Mβ1-2M							4.946	5.050	4.918	4.843	5.292	–	+
II M_2						Mα1-2M							5.049	5.352	+	+
III																
M_2						Mα1-2M							5.049	5.352	+	+
M_3					Mα1-2Mα1-2M							5.051	5.268	5.336	+	+
M_4				Mα1-2Mα1-2Mα1-2M							5.051	5.269	5.255	5.338	+	+
M_5			Mα1-2Mα1-2Mα1-2Mα1-2M							5.053	5.268	5.255	5.255	5.336	+	+
M_6		Mα1-2Mα1-2Mα1-2Mα1-2Mα1-2M							5.054	5.265	5.254	5.254	5.254	5.336	+	–
		Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M							4.837	4.837	5.143	5.251	5.251	5.333	+	+
M_7	Mβ1-2Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M							4.838	4.906	4.838	5.143	5.251	5.251	5.335	+	+

[a] I, acid-labile oligosaccharide; II, alkali-labile oligosaccharide; III, acetolysis-labile oligosaccharide.
[b] M denotes a D-mannopyranose unit.
[c] Chemical shift was indicated on the basis of a value of acetone (2.217 ppm) as an internal standard (19).
[d] 0199 and 1647 indicate mannan fractions of *C. tropicalis*.

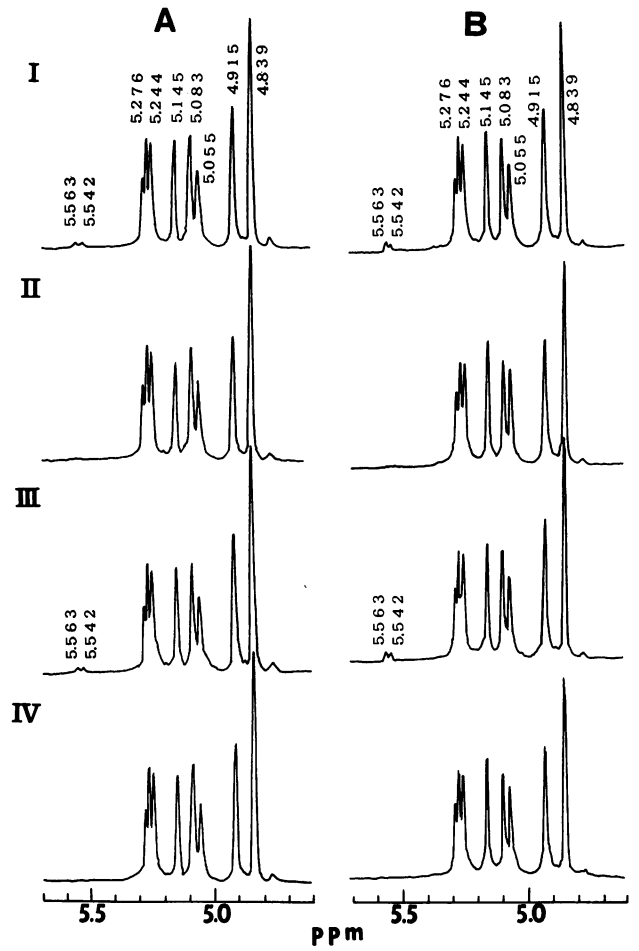

FIG. 2. ^{1}H NMR spectra in the anomeric region (H-1) resonances of parent (I), acid-modified (II), alkali-modified (III), and acid- and alkali-modified (IV) mannans isolated from two *C. tropicalis* strains. (A) Fr 0199, Fr 0199-a, Fr 0199-b, and Fr 0199-ab; (B) Fr 1647, Fr 1647-a, Fr 1647-b, and Fr 1647-ab. This analysis was conducted with a JEOL JNM-GSX 400 spectrometer in D_2O at 70°C with acetone as an internal standard (2.217 ppm).

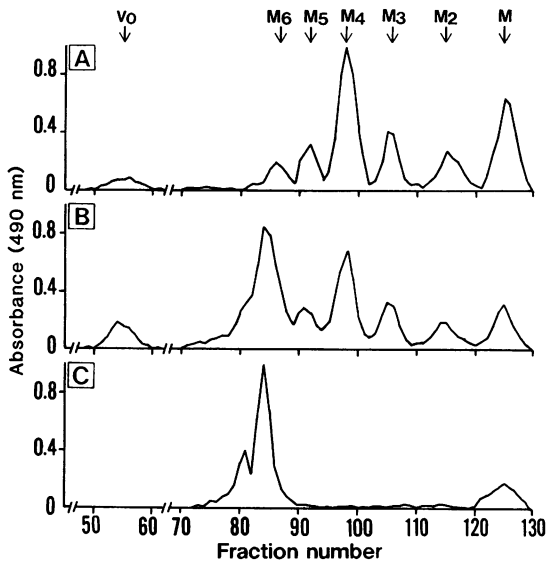

FIG. 3. Gel filtration profiles of the products obtained from Fr 0199-ab by acetolysis by using the same column and conditions as described in the legend to Fig. 1. (A) Fr 0199-ab acetolyzed under conventional conditions; (B) Fr 0199-ab acetolyzed under mild conditions; (C) enzymolysis product with a jack bean exo-α-mannosidase obtained from the fraction illustrated panel B in corresponding to oligosaccharide isomers with longer chains than hexaose. M_5 and M_6 indicate mannopentaose and mannohexaose, respectively. Other symbols are the same as those described in the legend to Fig. 1.

support the presence of small amounts of alkali-labile biose from each parent mannan as shown in Fig. 1C and D.

Degradation of acid- and alkali-stable domain of the IFO 0199 mannan Fr 0199-ab. To obtain the α-linked oligosaccharides corresponding to side chains from the acid- and alkali-stable domain of the mannan Fr 0199-ab was at first subjected to conventional acetolysis, and the acetolysate was fractionated on a column of Bio-Gel P-2 (Fig. 3A). The products isolated from this acetolysate were oligosaccharides M_2 to hexaose (M_6), mannose, and a phosphorylated oligosaccharide(s) eluted in the V_0 region. On the other hand, to isolate the β-1,2-linkage-containing oligosaccharide, Fr 0199-ab was acetolysed under mild conditions. The elution pattern of the degradation products by this procedure indicates that a large amount of phosphorylated oligosaccharide(s) was eluted in the V_0 region, and this oligosaccharide was followed by the fraction consisting of oligosaccharide isomers with longer chains than M_6, the oligosaccharides with shorter chains than M_5, and mannose (Fig. 3B). The fraction consisting of longer-chain isomers than M_6 was then digested with the α-mannosidase, and the products were fractionated by gel filtration chromatography to remove the α-linked oligosaccharides from this fraction. Consequently, the α-mannosidase-resistant oligosaccharides M_6 and heptaose (M_7) remained (Fig. 3C).

Degradation of acid- and alkali-stable domain of the IFO 1647 mannan, Fr 1647-ab. In Fig. 4, the elution profile of the products obtained from Fr 1647-ab by mild acetolysis indicates that all peaks corresponding to oligosaccharides M_2 to M_7 and phosphorylated oligosaccharides eluted in V_0 are distinct. In the case of Fr 1647-ab, therefore, it is not necessary to subject it to conventional acetolysis.

^{1}H NMR analysis of oligosaccharides obtained from Fr

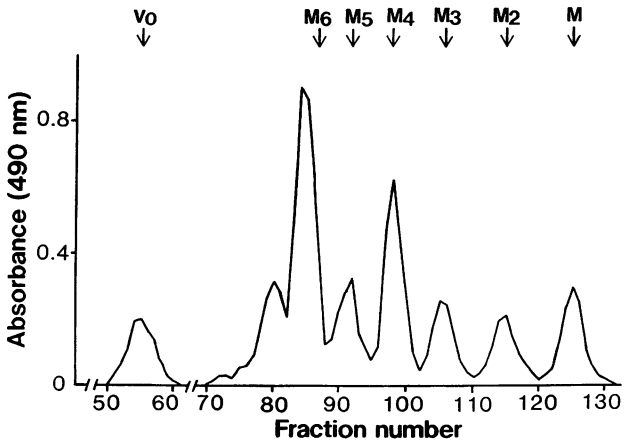

FIG. 4. Gel filtration profile of the products obtained from Fr 1647-ab by mild acetolysis by using the same column and conditions as described in the legend to Fig. 1.

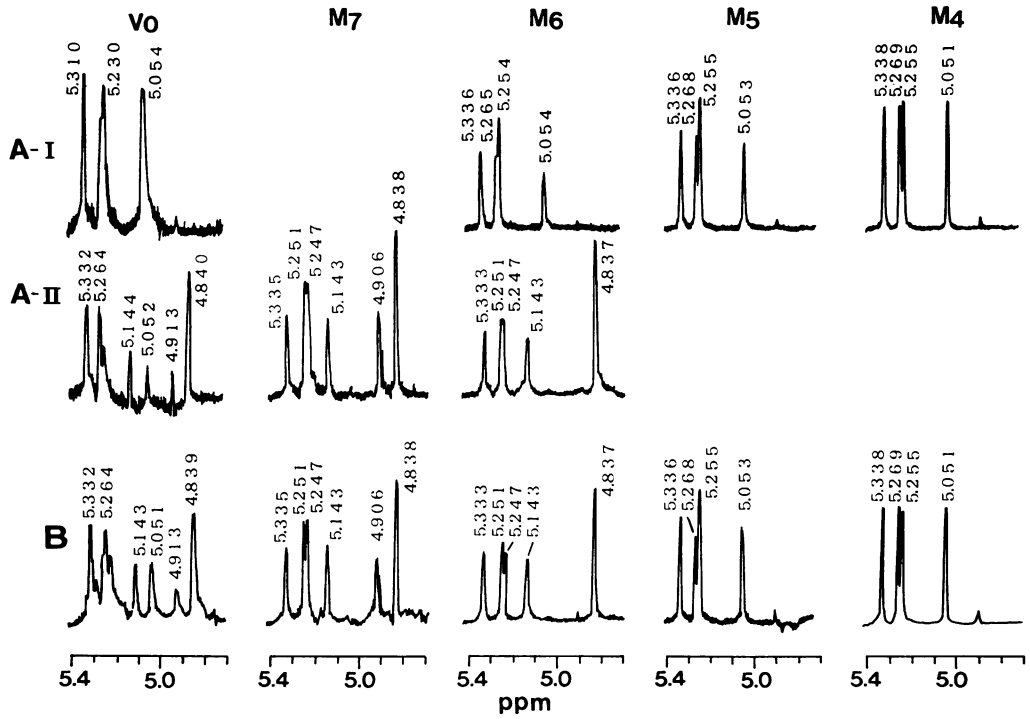

FIG. 5. ^{1}H NMR spectra in the anomeric region (H-1) resonances of oligosaccharides obtained from Fr 0199-ab by conventional acetolysis (A-I) and by mild acetolysis followed by enzymolysis with exo-α-mannosidase (A-II) and from Fr 1647-ab by mild acetolysis (B). This analysis was conducted by using the same spectrometer and conditions described in the legend to Fig. 2.

0199-ab and Fr 1647-ab by conventional and mild acetolyses. All oligosaccharides were analyzed by ^{1}H NMR (Fig. 5 and Table 3). The oligosaccharides, including a phosphorylated oligosaccharide(s) obtained from Fr 0199-ab by conventional acetolysis, were identified as homologous α-1,2-linked mannopyranose residues by correlation with data in the literature (6, 19, 42) (Fig. 5AI and Table 3; signals of M_2 to M_3 are not shown). The H-1 signals of the oligosaccharide M_5 and those with shorter chains obtained from Fr 1647-ab were identical to those of longer-chain oligosaccharides (Fig. 5B; signals of M_2 and M_3 are not shown). The H-1 signals of M_6 and M_7 obtained from Fr 0199-ab and Fr 1647-ab by mild acetolysis followed by α-mannosidase or solitary mild acetolysis revealed that these oligosaccharides were identified as Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man and Man*p*β1-2Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man, respectively, on the basis of assignment of the same oligosaccharides isolated from *C. albicans* serotype A and *C. stellatoidea* type II strains (19, 21, 24) (Fig. 5AII and B and Table 3). Additionally, the signal of the phosphorylated oligosaccharide(s) fraction (V_0) obtained from each mannan by mild acetolysis indicated that the sugar moiety of this fraction was composed of both β-1,2- and α-1,2-linked mannopyranose units (Fig. 5AII and B).

DISCUSSION

A serological identification of *C. albicans* was made by Hasenclever and Mitchell (12–14); they showed that this species could be divided into two different serotypes, A and B, and the properties of whole cells of these serotypes resembled those of the closely related species *C. tropicalis* and *C. stellatoidea*, respectively. Subsequently, Kwon-chung et al. (27, 28)

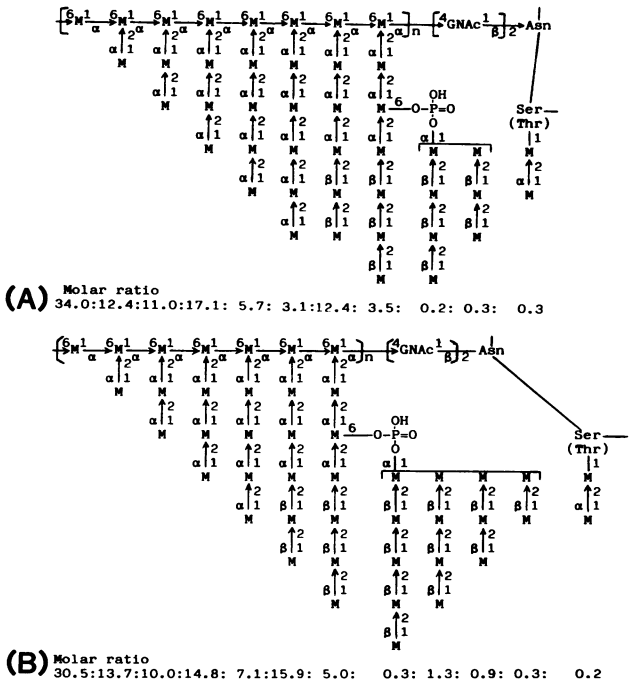

FIG. 6. Proposed structures for the cell wall mannans of *C. tropicalis* IFO 0199 (A) and IFO 1647 (B). M and GNAc denote D-mannopyranose and 2-acetamido-2-deoxy-D-glucopyranose units, respectively. The side-chain sequence is not specified.

TABLE 4. Distribution of β-1,2-linkage-containing side chains corresponding to epitopes, factors 5 and 6, in mannans of three *Candida* species, *C. albicans*, *C. stellatoidea*, and *C. tropicalis*

Epitope	Side chain[a]	Distribution				
		C. albicans[b]		*C. stellatoidea*[c]		*C. tropicalis*
		A	B	I	II	
5	Mβ1-(2Mβ1-)n2M-(H_2PO_3) (n = 1–5)	+	+	+	+	+
6	Mβ1-2Mα1-2Mα1-2Mα1-2M	+	−	−	+	−
	Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M	+	−	−	+	+
	Mβ1-2Mβ1-2Mβ1-2Mα1-2Mα1-2Mα1-2M	+	−	−	+	+

[a] M denotes a D-mannopyranose unit.
[b] This species was classified as serotypes A and B by Hasenclever and Mitchell (12).
[c] This species was classified as karyotypes I and II by Kwon-chung et al. (27).

reported that a group of *C. stellatoidea* species could be classified into two karyotypes, I and II, and that the serological properties of the former and latter strains resembled those of serotypes B and A of *C. albicans*, respectively.

A kit of PFAbs (Candida Check) was developed as an accurate serodiagnostic procedure for candidiasis on the basis of the serological specificities of medically important *Candida* species (43, 46). Each of 10 different PFAbs used in this kit is reported to recognize the antigenic structure in cell wall mannan (9).

We have reported that each of several mannans of *C. albicans* and *C. stellatoidea* strains consists of a long backbone (core) of α-1,6-linked mannopyranose units with short α-1,2-linked mannotriosyl side chains (16, 24, 44). Three long side chains in the mannans of *C. albicans* serotype A and *C. stellatoidea* type II strains corresponding to the β-1,2- and α-1,2-linkage-containing oligosaccharides Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man, Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1→2Man, and Man*p*β1-2Man*p*β1-2Man*p*β1-2Man*p*α1-2Man*p*α1-2Man*p*α1-2Man were the specific epitopes of *C. albicans* serotype A (*C. stellatoidea* type II), factor 6 (23). Additionally, it was demonstrated that the long side chains containing an α-1,3 linkage in these mannans (16, 19–21, 24) and the β-1,2-linked oligomannosyl chains attached to the phosphate groups served as the major common epitope factor 5 throughout *C. albicans* and *C. stellatoidea* (37).

The present article reports the fine chemical structures for the mannans of two *C. tropicalis* strains, IFO 0199 and IFO 1647, respectively (Fig. 6). The structures of these mannans are similar to those of *C. albicans* serotype A and *C. stellatoidea* type II strains from the viewpoint of the presence of epitopes, factors 5 and 6, except for the loss of α-1,3-linkage-containing oligomannosyl side chains, Man*p*α1-3Man*p*α1-2Man*p*α1-2Man*p*α1-2Man and Man*p*α1-2Man*p*α1-3Man*p*α1-2Man*p*α1-2Man*p*α1-2Man. This finding supports the result of the methylation analysis of same-species mannan reported by Kogan et al. (26). Moreover, Shibata et al. (42) suggested the presence of another oligomannosyl residue containing β-1,2 and α-1,3 linkages, Man*p*β1-2Man*p*α1-3Man*p*α1-, in *C. albicans* serotype A mannan on the basis of results of a two-dimensional NMR analysis. However, this oligomannosyl residue was not found in the *C. tropicalis* mannans Fr 0199 and Fr 1647.

Reiss et al. (36) showed that the monoclonal antibody CB6 against *C. tropicalis* mannan is *C. tropicalis* and *C. albicans* serotype A specific. The result obtained in this study led us to speculate that the monoclonal antibody CB6 recognizes an oligomannosyl side chain consisting of β-1,2- and α-1,2-linked mannopyranose units corresponding to a specific epitope, factor 6. The distribution of epitopes 5 and 6 among three *Candida* species, *C. albicans*, *C. stellatoidea*, and *C. tropicalis*, is summarized in Table 4. Recently, it has also been suggested that one of the antigenic determinants recognized by PFAb 1 is the O-linked sugar chain of the cell wall mannoprotein, while that recognized by PFAb 9 is the linear backbone of mannan moiety consisting of α-1,6-linked mannopyranose units (4).

An antigenic relationship between *C. tropicalis* antigen and *Salmonella* serotype Aberdeen O-antigen has been observed (1). On the other hand, Ekwall et al. (8) and Nnalue et al. (34) have described serologic cross-reactivity between *Candida* species and *Salmonella thompson* serotype C_1 with a polyclonal or monoclonal antibody specific for the O-6,7 antigen. The polysaccharide corresponding to serotype C_1 O-6,7 antigen contains β-1,2- and α-1,2-linked mannopyranose units similar to the epitope of *Candida* spp., factor 6 (30). Moreover, Aksoycan et al. (2) have described the serologic relationship between *Candida glabrata* antigen and *Salmonella* O-antigen. We have also found the oligomannosyl residues corresponding to an epitope, factor 6, in the mannan of *C. glabrata* (17). Therefore, we propose that the sugar residues containing both β-1,2- and α-1,2-linked mannopyranose units participate in the serologic cross-reactivity between *Salmonella* and *Candida* species.

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