Carbohydrate Research, 231 (1992) 105–116
Elsevier Science Publishers B.V., Amsterdam

Structure of the D-mannan of the pathogenic yeast, *Candida stellatoidea* ATCC 20408 (Type II) strain, in comparison with that of *C. stellatoidea* ATCC 36232 (Type I) strain

Hidemitsu Kobayashi, Masako Takaku, Yuko Nishidate, Shin-ichi Takahashi, Masashi Takikawa, Nobuyuki Shibata and Shigeo Suzuki

Second Department of Hygienic Chemistry, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Sendai Aoba-ku, Miyagi 981 (Japan)

(Received October 2nd, 1991; accepted January 3rd, 1992)

ABSTRACT

Acid treatment of the cell-wall D-mannans of *Candida stellatoidea* strains ATCC 36232 (Type I, A3 strain) and ATCC 20408 (Type II, A2 strain) gave (1 → 2)-linked β-D-manno-oligosaccharides (dp 2–5), whereas treatment with alkali gave the (1 → 2)-linked α-D-mannobiose. Conventional acetolysis of the acid- and alkali-treated D-mannan of the A3 strain gave oligosaccharides consisting of (1 → 2)- and (1 → 3)-linked α-D-mannopyranose residues, similar to those of *Candida albicans* serotype B strain. Mild acetolysis of the acid- and alkali-treated D-mannan of the A2 strain gave higher oligosaccharides that were digested by the *Arthrobacter* GJM-1 strain exo-α-D-mannosidase. The results of ^{1}H- and ^{13}C-NMR analyses indicated this D-mannan to contain branches with the following structures: β-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-D-Man, β-D-Man*p*-(1 → 2)-β-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-D-Man, and β-D-Man*p*-(1 → 2)-β-D-Man*p*-(1 → 2)-β-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-D-Man, in common with the D-mannans of *C. albicans* serotype A strains.

INTRODUCTION

We have reported that the antigenic D-mannans of three representative *Candida albicans* strains, namely, NIH A-207 (serotype A)[1,2], NIH B-792 (serotype B)[3–5], and J-1012 (serotype A, formerly serotype C)[6] each comprises a long backbone of (1 → 6)-linked α-D-mannopyranose residues with many (1 → 2)-linked α-D-mannotriosyl branches. The branches in the D-mannan of the serotype A strain corresponding to β-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-D-Man and β-D-Man*p*-(1 → 2)-β-D-Man*p*-(1 → 2)-α-D-Man*p*-(1

Correspondence to: Dr. S. Suzuki, Second Department of Hygienic Chemistry, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Sendai Aoba-ku, Miyagi 981, Japan.

→ 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-D-Man were the serotype A-specific epitopes[7,8].

Kwon-Chung et al.[9,10] reported that *Candida stellatoidea* could be classified into Type I and II karyotypes, the serological properties of which resembled those of serotypes B and A of *C. albicans*, respectively, and Type II was more pathogenic in mice than Type I.

Tojo et al.[11] and Kobayashi et al.[12] have demonstrated that the D-mannans of the *C. stellatoidea* Type I strains, IFO 1397, TIMM 0310, and ATCC 11006, had a common structure that lacked (1 → 2)-linked β-D-mannopyranosyl units in the non-reducing terminal sites of relatively longer branches.

We now report on the structure of the cell-wall D-mannan isolated from *C. stellatoidea* ATCC 20408 (Type II) strain, in comparison with that of *C. stellatoidea* ATCC 36232 (Type I) strain.

EXPERIMENTAL

Materials.—The *C. stellatoidea* ATCC 36232 and ATCC 20408 strains, designated as A3 and A2 strains, respectively, were obtained from the American Type Culture Collection (Rockville, MD, USA). Bio-Gel P-2 (−400 mesh) was purchasd from Bio-Rad Laboratories (Richmond, CA, USA). A kit of polyclonal rabbit anti-*Candida* factor sera (Candida Check) corresponding to antigens 1, 4–6, 8, 9, 11, 13b, and 34, as defined by Fukazawa et al.[13], was purchased from Iatron (Tokyo, Japan).

Cultivation of C. stellatoidea *strains and preparation of the* D-*mannans.*—These were performed as described by Kobayashi et al.[6] for the *C. albicans* J-1012 strain. The D-mannan fractions obtained from the cells of the A3 and A2 strains were designated as Frs. A3 and A2, respectively, and were obtained in yields of 6.5 and 5.6% (dry-weight basis), respectively.

Treatment of Frs. A3 and A2 with acid.—The procedure of Shibata et al.[14], using 10 mM HCl at 100° for 1 h, was followed: Frs. A3 and A2 gave Frs. A3-a and A2-a, respectively.

Treatment of Frs. A3, A2, A2-a, and A2-a with alkali.—The procedure described[14] was followed, using 100 mM NaOH at 25° for 18 h: Frs. A3, A2, A3-a, and A2-a gave Frs. A3-b, A2-b, A3-ab, and A2-ab, respectively.

Conventional acetolysis of Frs. A3-ab and A2-ab.—The method of Kobayashi et al.[15], as modified by Kocourek and Ballou[16], was used.

Mild acetolysis of Fr. A2-ab and digestion of the resulting O-*deacetylated higher oligosaccharides with exo-α-*D-*mannosidase.*—The procedure followed was that described[6].

NMR spectroscopy.—^{1}H-NMR spectra (internal acetone, 2.217 ppm) were measured[6] with a Jeol JNM-GSX 400 spectrometer on solutions (5–10 mg/0.7 mL) in D_2O at 70°. ^{1}H,^{13}C COSY spectra (internal acetone, 2.217 ppm; and MeOH, 49.00

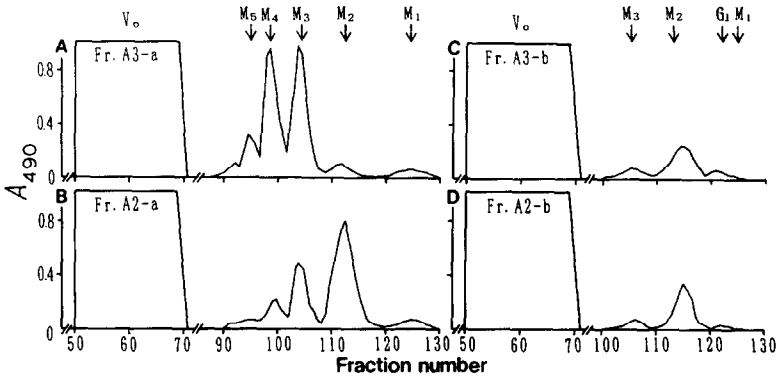


Fig. 1. Gel filtration of the products obtained on treatment of Frs. A3 (A and C) and A2 (B and D) with 10 mM HCl at 100° for 1 h (A and B) or 100 mM NaOH at 25° for 18 h (C and D) on a column (2.5 × 100 cm) of Bio-Gel P-2 by elution with water at 0.25 mL/min. The carbohydrate in the eluate was determined by the phenol–H_2SO_4 method[18]: G, M, and M_2–M_5 indicate D-glucose, D-mannose, and D-manno-oligosaccharides of dp 2–5, respectively; V_0 refers to the void volume.

ppm) were recorded as described by Kobayashi et al.[7] on solutions (14–21 mg/0.7 mL) in D_2O at 55°.

Slide agglutination reaction.—The procedure of Miyakawa et al.[17] was used with heat-killed cell suspensions of *C. albicans* and *C. stellatoidea* strains.

Other methods.—Carbohydrate was determined by the phenol–H_2SO_4 method[18], using D-mannose as the standard; protein by the Folin method of Lowry et al.[19], using bovine serum albumin (Sigma Chemical Co.) as the standard; and phosphate by the method of Ames and Dubin[20], using KH_2PO_4 as the standard. Optical rotations were determined with a JAS DIP-360 digital polarimeter on aqueous solutions that had been stored for 3 h.

RESULTS

Treatment of the D-mannans with 10 mM HCl.—Treatment of Frs. A3 and A2 with 10 mM HCl at 100° for 1 h and fractionation of the products on Bio-Gel P-2 gave the results shown in Fig. 1 (A and B). Frs. A3 and A2 each released a mixture of D-manno-oligosaccharides (dp 2–5) and D-mannose corresponding to 1.50 and 0.98%, respectively, of the parent D-mannans. The ^{1}H-NMR spectra of these oligosaccharides were identical to those of the D-manno-oligosaccharides isolated from the D-mannan of *C. albicans* NIH B-792 strain by Kobayashi et al.[4] and identified as (1 → 2)-linked β-D-manno-oligosaccharides (data not shown). The acid-resistant fractions obtained from A3 and A2 are designated A3-a and A2-a, respectively.

Treatment of D-mannans with alkali.—Treatment of Frs. A3 and A2 with 100 mM NaOH for 18 h at 25° and fractionation of the products on Bio-Gel P-2 gave the results shown in Fig. 1 (C and D). Frs. A3 and A2 each released a disaccharide

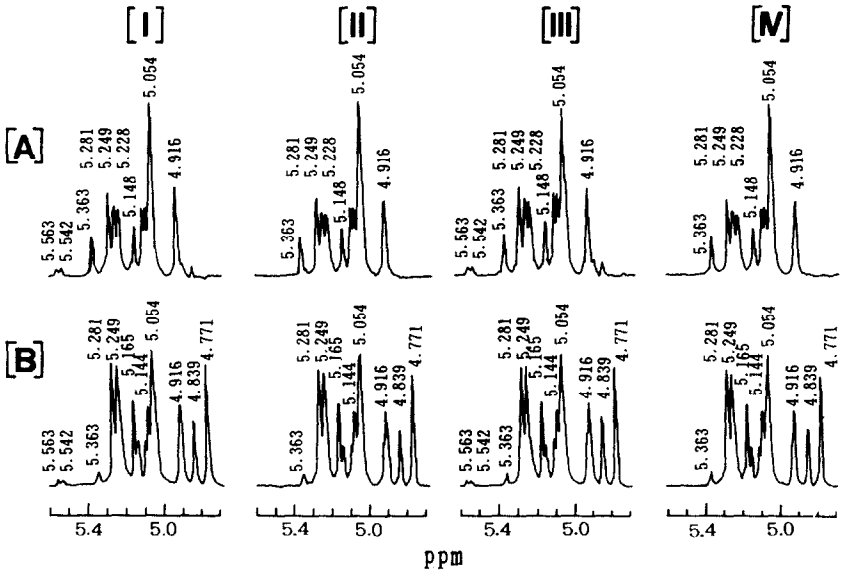


Fig. 2. ^{1}H-NMR spectra [D_2O, 70°, internal acetone (2.217 ppm)] in the region for H-1 resonances of intact (I), acid-modified (II), alkali-modified (III), and acid- and alkali-modified (IV) D-mannans isolated from two *C. stellatoidea* strains: (A) Frs. A3, A3-a, A3-b, and A3-ab; (B) Frs. A2, A2-a, A2-b, and A2-ab.

corresponding to 0.38 and 0.36%, respectively, of the parent D-mannans. ^{1}H-NMR spectroscopy indicated the structure to be α-D-Man*p*-(1 → 2)-D-Man (data not shown). Small proportions of an epimerisation product[21], α-D-Man*p*-(1 → 2)-D-Glc, were also present.

NMR spectroscopy.—Fig. 2 (A and B) shows the 400-MHz ^{1}H-NMR spectra (H-1 region) of Frs. A3 and A2, respectively. The resonances of H-1α of the glycose 1-phosphate residues in the range 5.542–5.563 ppm are weak in comparison with those of the D-mannans of *C. albicans*[2,4,6]. Fig. 3 (A and B) shows the ^{1}H, ^{13}C COSY spectra of Frs. A3 and A2, respectively. Comparison of H-1/C-1 resonances in these spectra indicated that the terminal (1 → 2)-linked β-D-manno-oligosaccharide residues present for Fr. A2 (4.771/99.52, 4.839/101.82, 4.916/99.86, and 5.165/100.81 ppm) were absent for Fr. A3. The differences between the chemical compositions of Frs. A3 and A2, and the modified products (Frs. A3-a, A2-a, A3-b, A2-b, A3-ab, and A2-ab), as shown in Table I, are not significant.

Acetolysis of Frs. A3-ab and A2-ab.—In order to analyse the structures of the acid- and alkali-stable domains, the D-mannans (Frs. A3-ab and A2-ab) were subjected to conventional acetolysis. Fig. 4 (A and B) shows the elution profiles of the respective *O*-deacetylated acetolysates. The products isolated from these acetolysates were D-manno-oligosaccharides (dp 2–6), D-mannose, and phosphorylated oligosaccharide(s) ($V_0$3-I and $V_0$2-I), respectively, eluted in the void volumes.

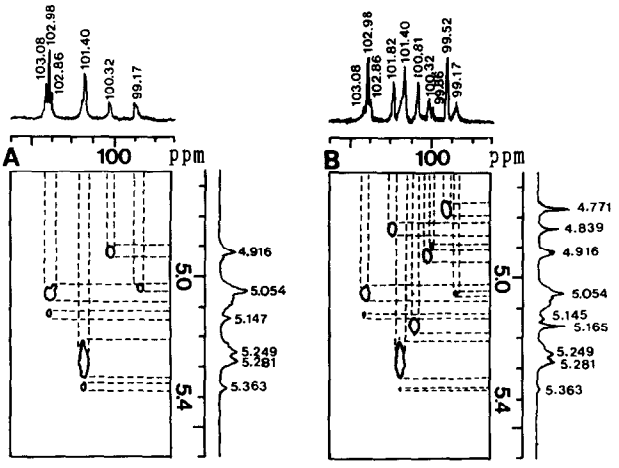


Fig. 3. $^{1}H,^{13}C$ COSY spectra [D_2O, 55°, internal acetone (2.217 ppm) and MeOH (49.00 ppm)] in the regions for H-1 and C-1 resonances of D-mannans; Frs. A3 (A) and A2 (B).

The chemical analyses of V_o3-I and V_o2-I are given in Table II, and the ^{1}H-NMR spectra in Fig. 5. The D-manno-oligosaccharides were also investigated by ^{1}H-NMR spectroscopy (data not shown). The H-1 signals of each D-manno-oligosaccharide were identified by correlation with data in the literature[22–24] and the structures **1–7** were assigned. Conventional acetolysis of Fr. A3-ab gave **5** and **7**, and the same acetolysis of Fr. A2-ab gave **4–7**.

Fig. 6 (A) shows the elution profile of the *O*-deacetylated products obtained from Fr. A2-ab by mild acetolysis. A large peak for phosphorylated oligosaccharide(s) (V_o2-II) was eluted in the void volume followed by oligosaccharides higher

TABLE I

Chemical compositions of the D-mannan fractions obtained from *C. stellatoidea* ATCC 36232 (Type I) and ATCC 20408 (Type II) strains

Fraction	Total carbohydrate (%) [a]	Total protein (%) [b]	Total phosphate (%) [c]	$[\alpha]_D^{20}$ (degrees) [d]
A3	86.5	2.1	0.30	+62.8
A3-a	84.8	2.4	0.31	+68.0
A3-b	85.0	2.2	0.31	+63.2
A3-ab	84.8	2.2	0.30	+67.6
A2	92.1	2.0	0.16	+46.3
A2-a	90.2	2.2	0.17	+49.5
A2-b	89.8	1.9	0.18	+45.5
A2-ab	89.9	2.0	0.18	+49.2

[a] Determined by the phenol–H_2SO_4 method[18]. [b] Determined by the method of Lowry et al.[19]. [c] Determined by the Ames–Dubin method[20] as $-H_2PO_3$. [d] (*c* 1.0, H_2O).

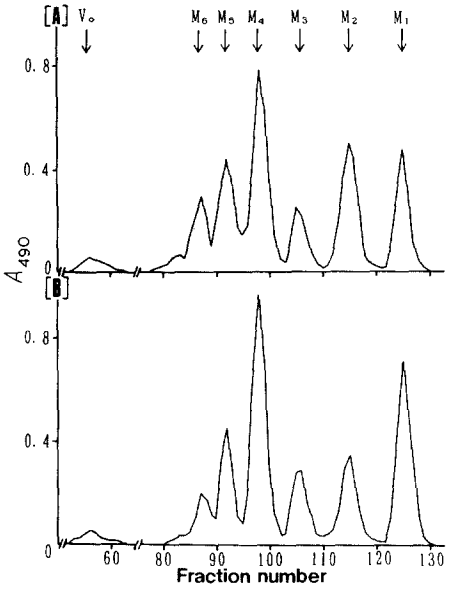


Fig. 4. Gel filtration of the *O*-deacetylated products of the conventional acetolysis of Frs. A3-ab (A) and A2-ab (B) with 10:10:1 Ac_2O–AcOH–H_2SO_4 for 13 h at 40°, using the same column and conditions as in Fig. 1: M_6 indicates D-mannohexaose; other symbols are as in Fig. 1.

than pentaose, which were resolved poorly. D-Mannose and D-manno-oligosaccharides (dp 2–4) were resolved satisfactorily. The ^{1}H-NMR spectra of these D-manno-oligosaccharides were identical to those of the structures **1–3** isolated after conventional acetolysis of Fr. A2-ab (data not shown). The fraction containing the higher oligosaccharides was digested with the exo-α-D-mannosidase and the products were fractionated (Fig. 6B). The separation of the components of a mixture of pentaose (M_5-IIe), hexaose (M_6-IIe), and heptaose (M_7-IIe) can be achieved by a combination of this enzymic procedure and gel-filtration chromatog-

TABLE II

Compositions of phosphorylated oligosaccharide(s) fractions obtained from Frs. A3-ab and A2-ab by acetolysis

Fraction	Total carbohydrate (%) [a]	Total phosphate (%) [b]	Man/P molar ratio [c]	$[\alpha]_D^{20}$ (degrees) [d]	Yield (%) [e]
$V_0$3-I	80.7	8.01	4.59	+61.8	3.5
$V_0$2-I	79.4	10.40	3.39	+59.8	2.9
$V_0$2-II	85.6	5.71	7.32	+33.6	24.2
$V_0$2-IIe	77.1	5.29	6.47	+32.2	22.5

[a] Determined by the phenol–H_2SO_4 method[18]. [b] Determined by the Ames–Dubin method[20] as $-H_2PO_3$. [c] Ratio of D-mannose residues to phosphate groups. [d] (*c* 1.0, H_2O). [e] Weight basis of the acid- and alkali-treated D-mannan.

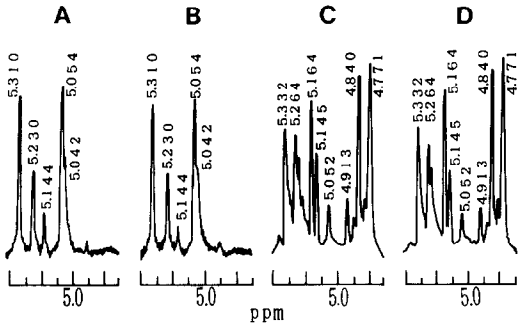


Fig. 5. ^{1}H-NMR spectra (see Fig. 2 for the conditions) in the region for H-1 resonances of phosphorylated oligosaccharide(s) fractions V_o3-I (A), V_o2-I (B), V2-II (C), and V_o2-IIe (D). These products were eluted in the void volume of the gel filtration shown in Figs. 4 (A and B) and 6 (A and C).

raphy. The ^{1}H-NMR spectra of these oligosaccharides were identical to those of the D-manno-oligosaccharides isolated from the D-mannan of *C. albicans* J-1012 strain[6] and identified as **8–10** (data not shown). M_5-IIe (**8**) and M_6-IIe (**9**) correspond to the serotype A-specific epitopes of genus *Candida*[8] and were investigated by ^{1}H,^{13}C COSY spectrometry (Fig. 7 and Table III).

α-D-Man*p*-(1 → 2)-{α-D-Man*p*-(1 → 2)}$_n$D-Man

1 $n = 0$
2 $n = 1$
3 $n = 2$
4 $n = 3$

α-D-Man*p*-(1 → 3)-{α-D-Man*p*-(1 → 2)}$_n$D-Man

5 $n = 3$
6 $n = 4$

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-{α-D-Man*p*-(1 → 2)}$_3$D-Man

7

β-D-Man*p*-(1 → 2)-{α-D-Man*p*-(1 → 2)}$_3$D-Man

8

β-D-Man*p*-(1 → 2)-β-D-Man*p*-(1 → 2)-{α-D-Man*p*-(1 → 2)}$_3$D-Man

9

β-D-Man*p*-(1 → 2)-{β-D-Man*p*-(1 → 2)}$_2$-{α-D-Man*p*-(1 → 2)}$_3$D-Man

10

When V_o2-II was treated with the exo-α-D-mannosidase (Fig. 6C), only 7% of the D-mannose was released, so that a large proportion was resistant to the enzyme. The results of chemical and ^{1}H-NMR analyses for V_o2-II and V_o2-IIe are given in Table II and Fig. 5 (C and D).

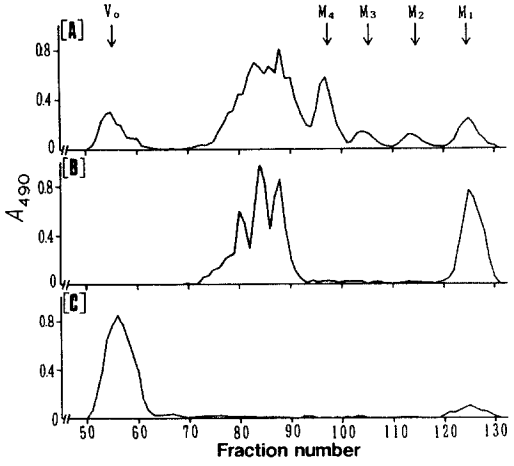


Fig. 6. Gel filtration of the *O*-deacetylated products of the mild acetolysis of Fr. A2-ab with 100:100:1 Ac_2O–AcOH–H_2SO_4 for 36 h at 40°, using the same column and conditions as in Fig. 1 (A); digestion products of the oligosaccharides in (A) higher than pentaose with exo-α-D-mannosidase (B); and digestion products [phosphorylated oligosaccharide(s), V_o2-II] in the void volume in (A) with exo-α-D-mannosidase (C).

Structures of phosphate-containing oligosaccharide(s), V_o3-I, V_o2-I, V_o2-II, and V_o2-IIe.—Fig. 5 (A–D) shows the ^{1}H-NMR spectra of the three phosphorylated oligosaccharide(s) fractions. Because V_o3-I and V_o2-I were obtained by conventional acetolysis, only weak resonances relating to β-(1 → 2) linkages could be detected. On the other hand, V_o2-II and V_o2-IIe gave nearly identical ^{1}H-NMR

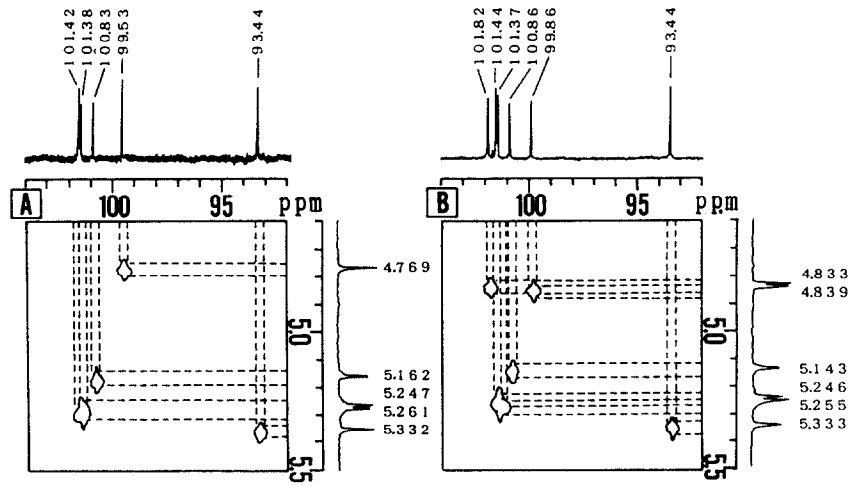


Fig. 7. ^{1}H, ^{13}C COSY spectra (see Fig. 3 for the conditions) in the regions for H-1 and C-1 resonances of M_5-IIe (A) and M_6-IIe (B).

TABLE III

Chemical shifts of H-1 and C-1 signals in the NMR spectra of M_5-IIe and M_6-IIe

Atom	**8** (M_5-IIe)				
	1''''	*1'''*	*1''*	*1'*	*1*
H-1	4.769	5.162	5.261	5.247	5.332
C-1	99.53	100.83	101.38	101.42	93.44
	9 (M_6-IIe)				
	1''''	*1''''*	*1'''*	*1''*	*1'*	*1*
H-1	4.839	4.833	5.143	5.255	5.246	5.333
C-1	99.86	101.82	100.86	101.37	101.44	93.44

spectra, including strong resonances in the range 4.770–4.916 ppm. Therefore, digestion with the exo-α-D-mannosidase did not alter the structural features surrounding the phosphate group, indicating that the phosphate groups might be located mainly on branches longer than M_5-IIe. Since a resonance at 5.145 ppm corresponding to a non-reducing, terminal (1 → 3)-linked α-D-mannopyranosyl unit can be detected in the ^{1}H-NMR spectra of $V_0$2-II and $V_0$2-IIe, it is presumed that phosphorylated branches longer than pentaose in the parent D-mannan are resistant to the enzyme.

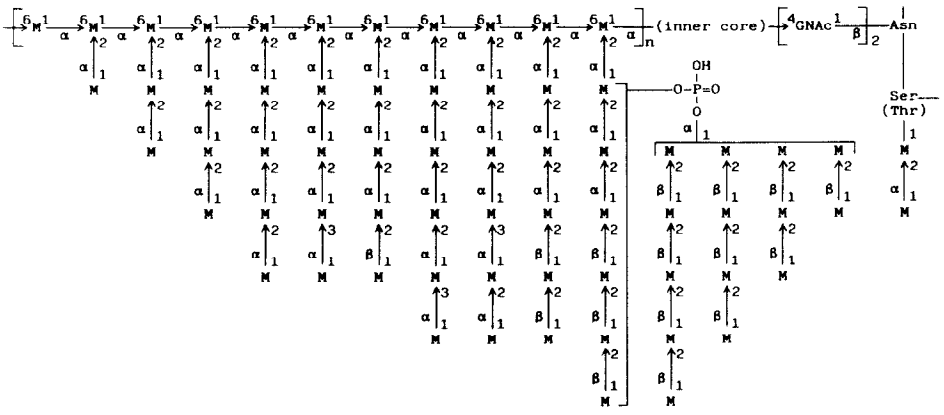
Slide agglutination assay for C. stellatoidea *strains with polyclonal factor sera (PFAb).*—The results of the assay with commercially available factor sera (Candida Check) and heat-killed cell suspensions of yeasts, including three *C. albicans* strains, NIH A-207 (serotype A), J-1012 (serotype A), and NIH B-792 (serotype B), and two *C. stellatoidea* strains, A3 (Type I) and A2 (Type II), are shown in Table IV. The A2-strain cells were equally agglutinated with a factor serum 6, corresponding to the epitopes of *Candida* spp., as the cells of *C. albicans* J-1012 strain. On the other hand, the A3-strain cells were not agglutinated. Therefore, this

TABLE IV

Slide agglutination assay for *C. albicans* and *C. stellatoidea* strains with polyclonal factor sera (PFAb) [a]

Strain	PFAb								
	1	4	5	6	8	9	11	13b	34
C. albicans									
NIH A-207 (serotype A)	++	++	+	+−	−	−	−	−	−
J-1012 (serotype A)	++	++	++	+	−	−	−	−	−
NIH B-792 (serotype B)	++	++	+	−	−	−	−	+−	−
C. stellatoidea									
ATCC 36232 (Type I)	++	++	+	−	−	−	−	−	−
ATCC 20408 (Type II)	++	++	++	+	−	−	−	−	−

[a] ++, +, and +−, agglutination; −, no agglutination.



Molar ratio

(A) 34.30:25.24: 8.09:17.87: — : 8.37: — : — : 3.71: — : — : 0.15: 0.74: 0.98: — : 0.55

(B) 27.80: 7.48: 6.75:15.98: 3.52: 8.21:11.56: 2.43: 3.65: 9.99: 5.23: — : 0.15: 0.39: 0.85: 0.63

Fig. 8. Possible structures for the cell-wall D-mannans of *C. stellatoidea* ATCC 36232 Type I (A) and ATCC 20408 Type II (B) strains: M (Man) and GNAc (GlcNAc) denote D-mannopyranose and 2-acetamido-2-deoxy-D-glucopyranose residues, respectively. The side-chain sequence is not specified.

finding provides evidence that the specific epitopes corresponding to factor serum 6 are M_5-IIe (**8**) and M_6-IIe (**9**), as assumed by Kobayashi et al.[8].

DISCUSSION

Kwon-Chung et al.[9,10] reported that *C. stellatoidea* spp. could be classified into two karyotypes (Types I and II) which showed a distinct difference in their chromosome patterns. The Type I strains were less virulent in mice, and showed lower resistance to UV irradiation, slower production of proteinase, and negative reactivity with the anti-*Candida tropicalis* D-mannan monoclonal antibody[25].

We have demonstrated[12] that the D-mannans of the *C. stellatoidea* Type I strains, IFO 1397, TIMM 0310, and ATCC 11006, have the same structure as the D-mannan of *C. albicans* NIH B-792 strain (serotype B), which lacks (1 → 2)-linked β-D-mannopyranosyl units in the non-reducing terminal sites of the branches corresponding to the structures **8**–**10**. The results in this study indicate that the D-mannan of the A2 strain contained branches corresponding to the serotype A-specific epitopes of genus *Candida*, the structures of which have been proposed as formulas **8** and **9** (Fig. 8). The D-mannan of the A3 strain did not contain these branches, in common with those of the other Type I strains, IFO 1397, TIMM 0310, and ATCC 11006. This is the most characteristic difference between the structures of D-mannans of *C. stellatoidea* strains of both Types I and II, which possess structures in common with the D-mannans of *C. albicans* serotype B and A strains.

Meyer et al.[26] reported that *C. stellatoidea* differs from *C. albicans* in the assimilation of sucrose. Furthermore, Kwon-chung et al.[27] and Iwaguchi et al.[28] demonstrated that *C. stellatoidea* spp. are stable mutants of *C. albicans* spp., on the basis of chromosome patterns. Therefore, it is concluded that the two pairs of serological identities observed between *C. albicans* serotype A and *C. stellatoidea* Type II strains and between *C. albicans* serotype B and *C. stellatoidea* Type I strains are due to the common structural features depending on the presence of branches corresponding to structures **8–10** in the acid-stable domains of the parent D-mannans.

ACKNOWLEDGMENT

We thank Dr. K. Hisamichi for recording the NMR spectra.

REFERENCES

1 N. Shibata, S. Fukasawa, H. Kobayashi, M. Tojo, T. Yonezu, A. Anbo, Y. Ohkubo, and S. Suzuki, *Carbohydr. Res.*, 187 (1989) 239–253.
2 H. Kobayashi, N. Shibata, T. Osaka, Y. Miyagawa, Y. Ohkubo, and S. Suzuki, *Phytochemistry*, 31 (1992) in press.
3 N. Shibata, H. Kobayashi, M. Tojo, and S. Suzuki, *Arch. Biochem. Biophys.*, 251 (1986) 697–708.
4 H. Kobayashi, N. Shibata, M. Nakada, S. Chaki, K. Mizugami, Y. Ohkubo, and S. Suzuki, *Arch. Biochem. Biophys.*, 278 (1990) 195–204.
5 N. Shibata, H. Kobayashi, S. Takahashi, Y. Okawa, K. Hisamichi, S. Suzuki, and S. Suzuki, *Arch. Biochem. Biophys.*, 290 (1991) 535–542.
6 H. Kobayashi, N. Shibata, H. Mitobe, Y. Ohkubo, and S. Suzuki, *Arch. Biochem. Biophys.*, 272 (1989) 364–375.
7 H. Kobayashi, P. Giummelly, S. Takahashi, M. Ishida, J. Sato, M. Takaku, Y. Nishidate, N. Shibata, Y. Okawa, and S. Suzuki, *Biochem. Biophys. Res. Commun.*, 175 (1991) 1003–1009.
8 H. Kobayashi, N. Shibata, and S. Suzuki, *Infect. Immun.*, 60 (1992) in press.
9 K.J. Kwon-chung, B.L. Wickes, and W.G. Merz, *Infect. Immun.*, 56 (1988) 1814–1819.
10 K.J. Kwon-chung, W.S. Riggsby, R.A. Uphoff, J.B. Hicks, W.L. Whelan, E. Reiss, B.B. Magee, and B.L. Wickes, *Infect. Immun.*, 57 (1989) 527–532.
11 M. Tojo, N. Shibata, Y. Ban, and S. Suzuki, *Carbohydr. Res.*, 199 (1990) 215–226.
12 H. Kobayashi, T. Kojimahara, K. Takahashi, M. Takikawa, S. Takahashi, N. Shibata, Y. Okawa, and S. Suzuki, *Carbohydr. Res.*, 214 (1991) 131–145.
13 Y. Fukazawa, T. Shinoda, and T. Tsuchiya, *J. Bacteriol.*, 95 (1968) 754–763.
14 N. Shibata, T. Ichikawa, M. Tojo, M. Takahashi, N. Ito, Y. Ohkubo, and S. Suzuki, *Arch. Biochem. Biophys.*, 243 (1985) 338–348.
15 H. Kobayashi, N. Shibata, and S. Suzuki, *Arch. Biochem. Biophys.*, 245 (1986) 494–503.
16 J. Kocourek and C.E. Ballou, *J. Bacteriol.*, 100 (1969) 1175–1181.
17 Y. Miyakawa, K. Kagawa, and Y. Fukazawa, *J. Clin. Microbiol.*, 23 (1986) 881–886.
18 M. Dubois, K. A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
19 O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.
20 B.N. Ames and D.T. Dubin, *J. Biol. Chem.*, 235 (1960) 769–775.
21 H. Kobayashi, N. Shibata, S. Konno, K. Hisamichi, and S. Suzuki, *Carbohydr. Res.*, 229 (1992) 369–375.
22 P.A.J. Gorin, J.F.T. Spencer, and R.J. Magus, *Can. J. Chem.*, 47 (1969) 3569–3576.
23 R.E. Cohen and C.E. Ballou, *Biochemistry*, 19 (1980) 4345–4358.
24 W. Zhang and C.E. Ballou, *J. Biol. Chem.*, 256 (1981) 10073–10079.

25 E. Reiss, L. De Repentigny, R.J. Kuykendall, A.W. Carter, R. Galindo, P. Auger, S.L. Bragg, and L. Kaufman, *J. Clin. Microbiol.*, 24 (1986) 796–802.
26 S.A. Meyer, D.G. Ahearn, and D. Yarrow, *Yeast: A Taxonomic Study*, 3rd edn., Elsevier, Amsterdam, 1984, p. 585.
27 K.J. Kwon-chung, J.B. Hicks, and P.N. Lipke, *Infect. Immun.*, 58 (1990) 2804–2808.
28 S. Iwaguchi, M. Homma, and K. Tanaka, *J. Gen. Microbiol.*, 136 (1990) 2433–2442.