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Structural determination of D-mannans of pathogenic yeasts *Candida stellatoidea* Type I strains: TIMM 0310 and ATCC 11006 compared to IFO 1397*

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ABSTRACT

The structures of the cell-wall D-mannans of pathogenic yeasts of *Candida stellatoidea* Type I strains, IFO 1397, TIMM 0310, and ATCC 11006, were investigated by mild acid and, alkaline hydrolysis, by digestion with the *Arthrobacter* GJM-1 strain exo-α-D-mannosidase, and by acetolysis. The modified D-mannans and their degradation products were studied by ^{1}H- and ^{13}C-n.m.r. analyses. D-Manno-oligosaccharides released by acid treatment from the parent D-mannans were identified as the homologous β-(1→2)-linked D-manno-oligosaccharides from biose to hexaose, whereas those obtained by alkaline degradation were the homologous α-(1→2)-linked D-mannobiose and D-mannotriose. The acid- and alkali-modified D-mannans lacking ^{1}H-n.m.r. signals above 4.900 p.p.m. [corresponding to β-(1→2)-linked D-mannopyranose units] were acetolyzed with 10:10:1 (v/v) Ac_2O–AcOH–H_2SO_4, and the resultant D-manno-oligosaccharides were also analyzed. It was found that the longest branches of these D-mannans, corresponding to hexaosyl residues, had the following structures: α-D-Man*p*-(1→3)-α-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 α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man. These results indicate that the D-mannans of *C. stellatoidea* Type I strains possess structures in common with the D-mannans of *Candida albicans* serotype B strain (see ref. 4) containing phosphate-bound β-(1→2)-linked oligo-D-mannosyl residues.

INTRODUCTION

In previous papers, we reported the structures of the antigenic phospho-D-mannans of three representative *Candida albicans* strains, NIH A-207 serotype A (refs. 1,2), NIH B-792 serotype B (refs. 3,4), and J-1012 serotype A (formerly serotype C) (ref. 5) as elucidated by a sequential degradation-procedure involving treatment with hot 10mM HCl and/or 100mM NaOH at 25°, acetolysis under conventional and mild conditions, enzymolysis with *Arthrobacter* GJM-1 α-D-mannosidase, and ^{1}H- and ^{13}C-n.m.r. analyses. These studies demonstrated a structural identity among these D-mannans in that each one possesses a long α-(1→6)-linked-D-mannopyranose backbone having a large number of α-(1→2)-linked D-mannotriosyl branches, each of which

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is attached to the backbone by an α-(1→2) linkage[2]. The branch in the D-mannan of serotype A strain corresponds to a D-mannopentaose, 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, displayed as one of the serotype A-specific epitopes. Subsequently, Tojo *et al.*[9] of our laboratory reported the structure of the D-mannan of *C. stellatoidea* IFO 1397 strain, providing evidence for the absence of phosphate group and β-(1→2)-linked D-mannopyranose units. This strain appears important, as Suzuki and Fukazawa[7] used heat-killed whole cells of this strain as the absorbing cells of the antiserum of *C. albicans* M-1012 (J-1012) strain during the preparation of the factor serum corresponding to antigen 6, the serotype A-specific epitope of *C. albicans* spp. In this subsequent study, we have attempted the structural determination of mannans of other *C. stellatoidea* species including two karyotypes, designated as Types I and II in accordance to the description by Kwon-Chung *et al.*[8,9]. This paper reports the structural determination of the D-mannans of three *C. stellatoidea* Type I strains, IFO 1397, TIMM 0310, and ATCC 11006, using the D-mannan of the first strain as the reference material.

EXPERIMENTAL

Materials. — The *C. stellatoidea* IFO 1397 strain was obtained from the Institute for Fermentation (Osaka, Japan). The TIMM 0310 strain was obtained from the University of Teikyo (Tokyo, Japan). The ATCC 11006 strain was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Column packing for gel-filtration chromatography, Bio-Gel P-2 (400 mesh), fractionation range 100–1800 Da, was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). A kit of polyclonal rabbit anti-*Candida* factor antisera ("Candida Check", Iatron, Tokyo, Japan), corresponding to antigens 1, 4, 5, 6, 8, 9, 11, 13b, and 34, as defined by Fukazawa *et al.*[10], was used.

Cultivation of C. stellatoidea *strains and preparation of the* D-*mannans.* — Cultivation of these strains and preparation of the D-mannans were performed as previously described by Kobayashi *et al.*[5] for isolation of the *C. albicans* J-1012 strain. The D-mannan fractions obtained from cells of *C. stellatoidea* IFO 1397, TIMM 0310, and ATCC 11006 strains are designated as Frs. If, Ti, and A1, respectively; yields: If, 7.6%; Ti, 6.7%; and A1, 6.5%, on a weight basis for the acetone-dried whole cells of the corresponding strains.

Treatment of Frs. If, Ti, and A1 with 10mM HCl. — This procedure was conducted by exactly as described by Shibata *et al.*[11] The acid-modified Frs. If, Ti, and A1 are designated as Frs. If-a, Ti-a, and A1-a, respectively.

Treatment of intact D-*mannans, Frs. If, Ti, and A1, and acid-modified* D-*mannans, If-a, Ti-a, and A1-a with 100mM NaOH.* — This procedure was exactly as previously described[11]. The alkali-modified Frs. If, Ti, A1, If-a, Ti-a, and A1-a are designated as Frs. If-b, Ti-b, A1-b, If-ab, Ti-ab, A1-ab, respectively.

Treatment of Frs. If, Ti, and A1, with Arthrobacter *GJM-1 strain exo-*α-D-*mannosidase.* — This was conducted exactly as previously described[1]. This treatment of

Fr. If, Ti, and A1 released D-mannose corresponding to 76.8, 75.2, and 56.4% of parent D-mannans on a weight basis (data not shown). The enzyme-modified Frs. If, Ti, and A1 are designated as Frs. If-e, Ti-e, and A1-e, respectively.

Acetolysis of Frs. If-ab, Ti-ab, and A1-ab. — This was conducted exactly as described by Kobayashi *et al.*[12,13] in a modification of the method of Kocourek and Ballou[14].

Calculation of average chain-length of the branches moieties of Frs. If, Ti, and A1. — The average chain-length of the branches in Frs. If, Ti, and A1 were calculated as described by Kobayashi *et al.*[15] for the partial acid-degradaded D-mannans of *Pichia pastoris* IFO 0948 strain.

N.m.r. studies of D-mannans and D-manno-oligosaccharides. — ^{1}H-N.m.r. spectra were measured as described by Gorin and Spencer[16] by use of a Jeol JNM-GSX 400 spectrometer. D-Mannans and D-manno-oligosaccharides were separately dissolved in D_2O in concentrations of 10 mg/0.7 mL and 5 mg/0.7 mL at 70°, respectively, and acetone was used as the internal standard (2.217 p.p.m.). The ^{13}C-n.m.r. spectra were measured with the same spectrometer as described by Kobayashi *et al.*[4] The concentrations of D-mannans and D-manno-oligosaccharides in D_2O were 25 and 15 mg/0.7 mL at 55°, respectively, and CD_3OD was used as the internal standard (49.00 p.p.m.).

Slide-agglutination reaction. — This was conducted as described by Miyakawa *et al.*[17], using heat-killed cell suspensions of *C. albicans* and *C. stellatoidea* strains.

Other methods. — Total carbohydrate was determined by the phenol–H_2SO_4 method of Dubois *et al.*[18] with D-mannose (Wako Pure Chemicals, Co. Ltd., Osaka and Tokyo, Japan) as the standard. Total protein was determined by the Folin method of Lowry *et al.*[19], using bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) as the standard. Total phosphate was determined by the method of Ames and Dubin[20], using KH_2PO_4 (Nakarai Chemicals, Ltd., Kyoto, Japan) as the standard. Specific rotations were determined by means of a JAS DIP-360 digital polarimeter. The sample was dissolved in water, and measurements made 3 h after dissolution.

RESULTS

Treatment of the D-mannans with 10mM HCl. — Fractions If, Ti, and A1 were first examined for the presence of acid-labile oligomannosyl residues, which were assumed to connect with phosphate groups via their reducing-terminal groups. After treatment with 10mM HCl for 1 h at 100°, each mixture was fractionated on a column of Bio-Gel P-2. As shown in Figs. 1A–1C, Frs. If, Ti, and A1 released a mixture of D-manno-oligosaccharides ranging from hexaose to biose, and D-mannose in amounts of 0.03, 0.72, and 1.22%, respectively, on a weight basis of the parent D-mannans. The ^{1}H-n.m.r. spectra of these D-manno-oligosaccharides were apparently 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 a homologous oligosaccharide series composed solely of β-(1→2)-linked D-mannopyranose units (data not shown).

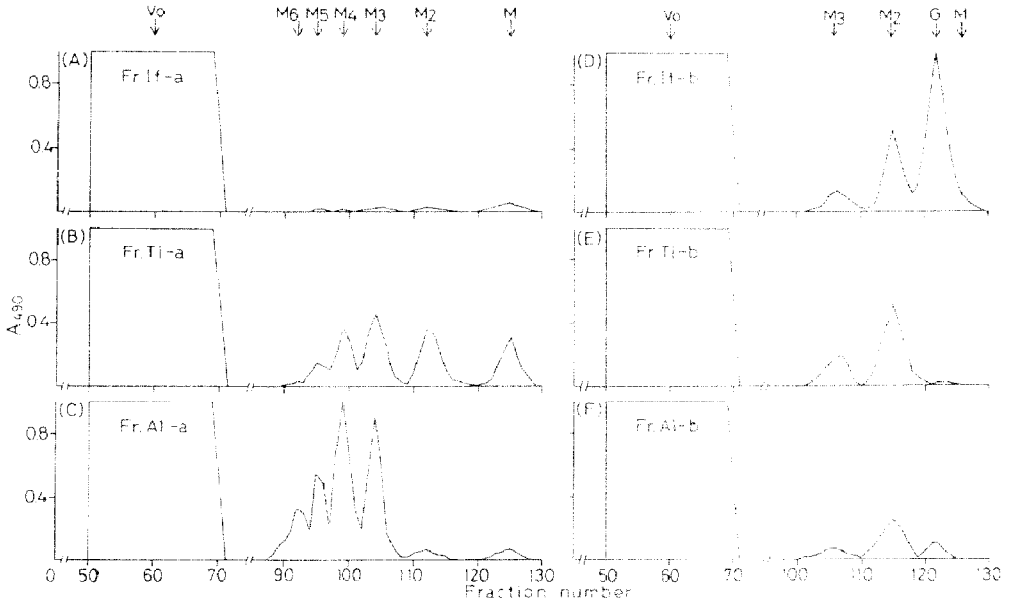

Fig. 1. Gel-filtration patterns of the reaction products of Frs. If (A, D), Ti (B, E), and Al (C, F) by acid treatment with 10mM HCl (A–C) or alkali treatment with 100mM NaOH (D–F) on a column of Bio-Gel P-2 (2.5 x 100 cm). Elution was effected with water at 0.25 mL/min. The amounts of carbohydrate in a eluates were determined by the phenol-H_2SO_4 method[18]. M_6, M_5, M_4, M_3, M_2, M, and G indicate D-mannohexaose, D-mannopentaose, D-mannotetraose, D-mannotriose, D-mannobiose, D-mannose, and D-glucose, respectively. Vo refers to void volume.

Treatment of each D-*mannan with 100mM NaOH.* — To investigate the presence of alkali-eliminable oligo-D-mannosyl residues in Frs. If, Ti, and Al, these D-mannans were treated with 100mM NaOH for 18 h at 25°, and each mixture was fractionated on a column of Bio-Gel P-2. As shown in Figs. 1D–1F, Frs. If, Ti, and Al released a mixture of oligosaccharides, triose to biose, and monosaccharide in the respective amounts of 1.40, 0.61, and 0.39%, on a weight basis of the parent D-mannans. It is pointed out in Fig. 1D that a monosaccharide having the same retention time as that of the standard (D-glucose) was present in the oligosaccharide mixture. This presumably arises from D-mannose by the Lobry de Bruyn–Alberda van Ekenstein isomerization[24] by the action of 100mM NaOH (pH ~ 12). The ^{1}H-n.m.r. analyses of biose and triose released from each D-mannan by the action of 100mM NaOH indicate that their the reducing terminal D-mannopyranose residues units were likewise epimerized to D-glucopyranose residues (data not shown). The ^{1}H-n.m.r. signals of biose and triose released from each D-mannan provided evidence that the alkali-eliminable oligo-D-mannosyl residues belonged to the homologous α-(1→2)-linked series.

N.m.r. and chemical analyses of each intact, acid-modified, alkali-modified, acid and alkali-modified, and enzyme-modified D-*mannans.* — Fig. 2I-A to 2I-C show the 400-MHz ^{1}H-n.m.r. spectra (H-1 region) of Frs. If (A), Ti (B), and Al (C), respectively. As may be observed in Figs. 2I-B and 2I-C, the signals of the α-anomeric proton of

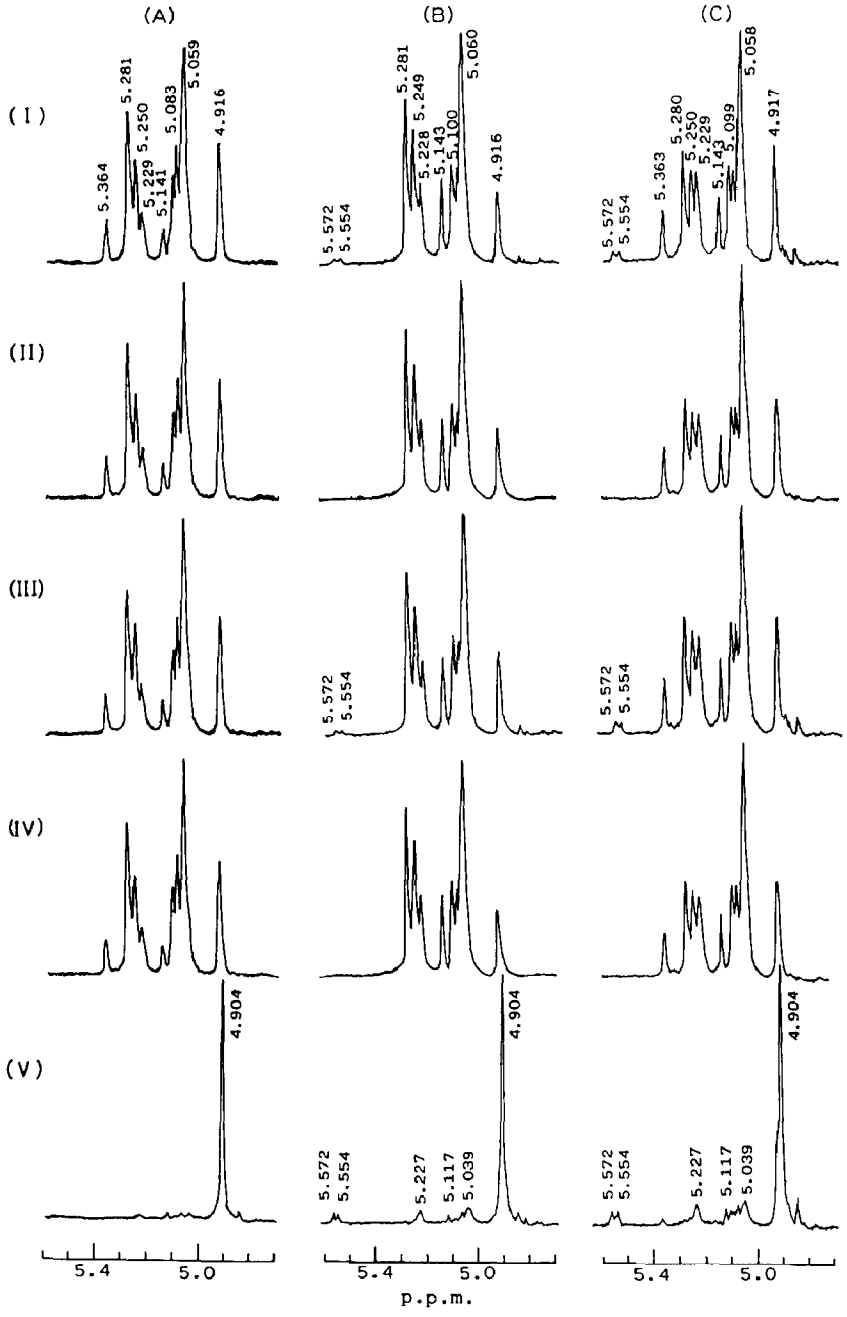

Fig. 2. ^{1}H-N.m.r. spectra (anomeric regions) of intact (I), acid-modified (II), alkali-modified (III), acid and alkali-modified (IV), and enzyme-modified (V) D-mannans isolated from three *C. stellatoidea* strains. (A): Frs. If, If-a, If-b, If-ab, and If-e; (B): Frs. Ti, Ti-a, Ti-b, Ti-ab, and Ti-e; (C): A1, A1-a, A1-b, A1-ab, and A1-e. These were recorded using a Jeol-GSX 400 spectrophotometer in D_2O solution at 70° with acetone as the standard (2.217 p.p.m.).

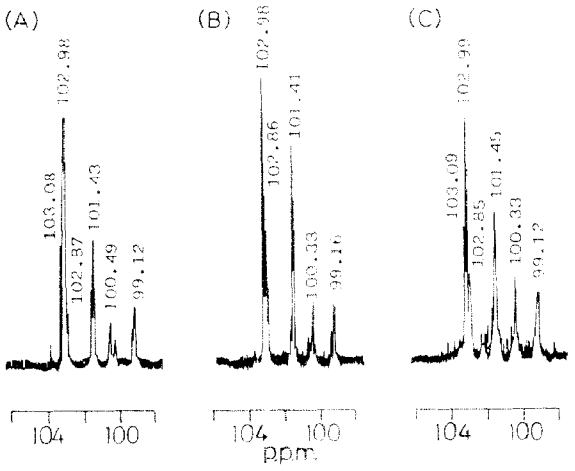

Fig. 3. ^{13}C-N.m.r. spectra (C-1 region) of intact D-mannans, Frs. If (A), Ti (B), and A1 (C). These were recorded using a Jeol-GSX 400 spectrophotometer in D_2O solution at 55° with CD_3OD as the standard (49.00 p.p.m.).

1-*O*-phosphorylated glycose residues in the 5.554–5.572 p.p.m. region in these signals of Frs. Ti and A1 are much weaker than those of phospho-D-mannans of *C. albicans* spp. Moreover, (Fig. 2I-B) a signal at 5.364 p.p.m. was not found in the ^{1}H-n.m.r. spectrum of Fr. Ti. Similarly the ^{13}C-n.m.r. spectrum (C-1 region) of Fr. Ti is devoid of a signal at 103.08 or 103.09 p.p.m. (Fig. 3B).

Complementary evidence for structural change of these parent D-mannans upon digestion by the *Arthrobacter* GJM-1 exo-α-D-mannosidase was obtained by ^{1}H-n.m.r. analysis (Fig. 2IV). The existence of a backbone consisting only of α-(1→6)-linked D-mannopyranose units is evident, because Frs. If-e, Ti-e, and A1-e each exhibited a strong signal at 4.905 p.p.m. corresponding to an unsubstituted α-(1→6)-linked D-mannopyranose unit, whereas those corresponding to α-(1→2)- and α-(1→3)-linked D-mannopyranose residues disappeared upon this enzymolysis. These findings indicate that Frs. If, Ti, and A1 have comb-like branched structures similar to those of the D-mannans of *C. albicans* J-1012 and NIH B-792 strains as recently reported by Kobayashi *et al.*[4,5] Table I shows the chemical compositions of the intact D-mannans and their acid-, alkali-, acid and alkali-, and α-D-mannosidase-modification products. Although no significant difference has been observed between the chemical compositions of Frs. If, Ti, and A1, and their modification products obtained by treatment with acid and/or alkali (Frs. If-a, Ti-a, A1-a, If-b, Ti-b, A1-b, If-ab, Ti-ab, and A1-ab), and increase of protein and phosphate contents was evident in the enzyme-modified products (Frs. If-e, Ti-e, and A1-e). It appears that the increase of the content of those components is because of the decrease of total carbohydrate content. This finding indicates that enzymolytic activity of the *Arthrobacter* GJM-1 α-D-mannosidase does not undergo interference in the presence of phosphate-bound β-(1→2)-linked oligo-D-mannosyl residues.

TABLE I

Chemical compositions and specific rotations of D-mannan fractions obtained from *C. stellatoidea* strains.

Fraction	*Total carbohydrate (%)*[a]	*Total protein (%)*[b]	*Total phosphate (%)*[c]	$[\alpha]_D^{20}$ (°)[d]
If	87.1	2.6	trace	+65.3
If-a	86.9	2.4	trace	+65.8
If-b	87.0	2.1	0.01	+65.1
If-ab	87.1	2.2	0.01	+65.3
If-e	81.3	3.8	0.06	+53.8
Ti	87.8	2.1	0.06	+65.4
Ti-a	86.0	2.0	0.05	+65.7
Ti-b	87.1	1.9	0.05	+65.6
Ti-ab	85.9	2.0	0.05	+65.8
Ti-e	80.7	3.5	0.18	+52.0
Al	84.2	2.0	0.23	+64.0
Al-a	84.2	2.2	0.25	+69.3
Al-b	86.7	2.0	0.23	+65.2
Al-ab	84.3	2.2	0.25	+68.8
Al-e	80.0	3.3	0.52	+50.2

[a] Determined by the phenol–H_2SO_4 method[18]. [b] Determined by the Folin method of Lowry *et al.*[19]
[c] Determined by the method of Ames and Dubin[20] as H_2PO_3. [d] In water (*c* 1.0).

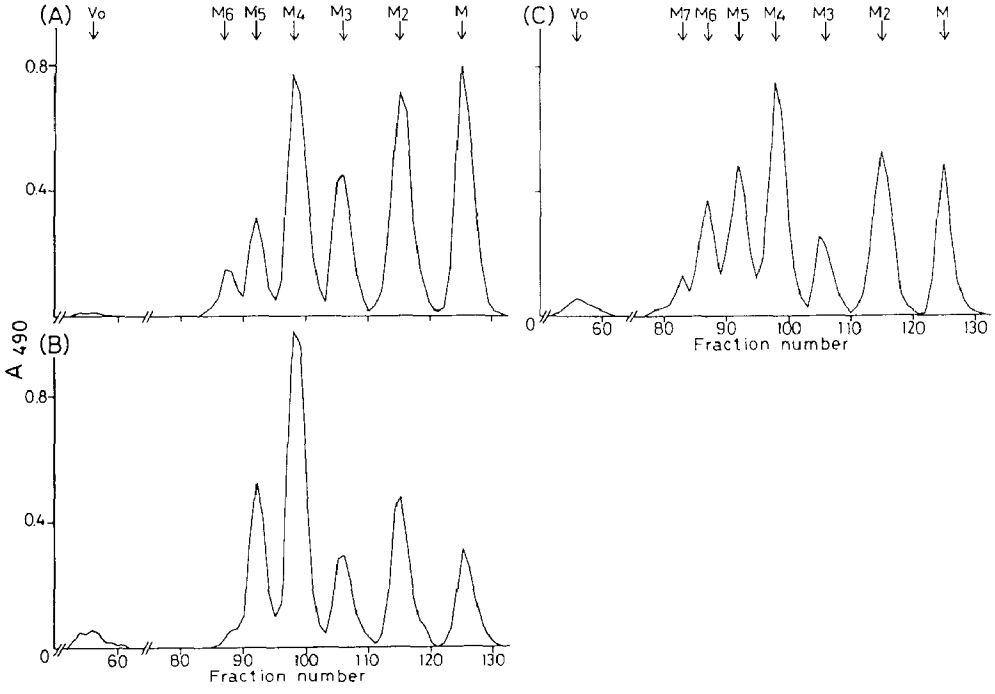

Fig. 4. Gel-filtration patterns of the acetolyzates of Frs. If-ab (A), Ti-ab (B), and Al-ab (C) by 10:10:1 (v/v) Ac_2O–AcOH–H_2SO_4 on a column of Bio-Gel P-2. M_7 indicates D-mannoheptaose. Other symbols are as in Fig. 1.

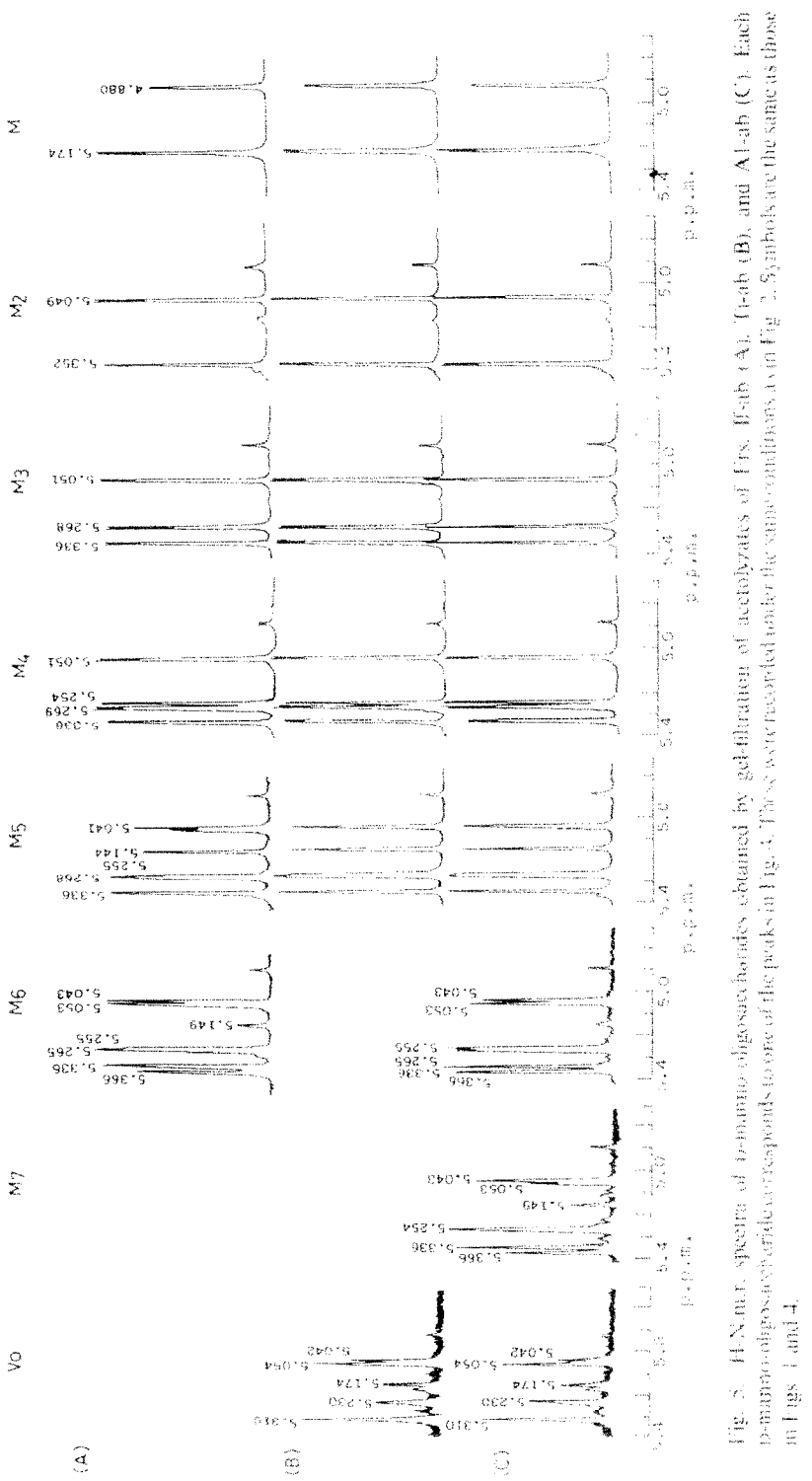

FIG. 5. ^{1}H-NMR spectra of oligosaccharide fragments obtained by gel filtration of acetolysates of Fr. II-ab (A), II-ab (B), and A1-ab (C). Each oligosaccharide corresponds to one of the peaks in Fig. 4. The spectra recorded under the same conditions as in Fig. 3. Symbols are the same as those in Figs. 1 and 4.

Acetolysis of D-*mannans modified by acid and alkali.* — In order to analyze the structures of the acid- and alkali-stable domains of the phosphomannans, the acid- and alkali-modified D-mannans (Frs. If-ab, Ti-ab, and A1-ab) were subjected to acetolysis[12,14] with 10:10:1 Ac_2O–AcOH–H_2SO_4. Figs. 4A to 4C show the elution profiles of the acetolyzates of Frs. If-ab, Ti-ab, and A1-ab. As may be seen, the oligosaccharides isolated from these acetolyzates were neutral D-manno-oligosaccharides, ranging from heptaose to biose, D-mannose, and phosphate-containing component (Frs. Ti-ab and A1-ab). These oligosaccharides were also investigated by ^{1}H-n.m.r. analysis (Fig. 5). Structural identification through assignment of the anomeric-proton signals of each D-manno-oligosaccharide was conducted by correlation with the findings by Gorin *et al.*[22], Cohen and Ballou[23], and Zhang and Ballou[24], and the structures of the D-manno-oligosaccharides identified are depicted in formulas **1**–**9**.

α-D-Man*p*-(1→2)-D-Man
1

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man
2

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D--Man*p*-(1→2)-D-Man
3

α-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
4

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man
5

α-D-Man*p*-(1→3)-α-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
6

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man
7

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-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
8

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-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
9

It is noteworthy that oligosaccharides of the same molecular weight obtained from the three different D-mannans had the same chemical structure, although the oligosaccharides higher than pentaose were each found to contain one isomeric oligosaccharide. The D-manno-oligosaccharides resulting from the acetolysis of Fr. Ti-ab were relatively low members corresponding to structures **1**, **2**, **3**, and **5**. These four oligosaccharides lacked signals at 5.363 and/or 5.364 p.p.m., as C-1 of an α-(1→3)-linked D-mannopyranose residue had another D-mannopyranose unit attached via an α-(1→2) linkage. However these signals were present in the higher D-manno-oligosaccharides 7 and 9 as isolated from the acetolyzates of Frs. If-ab and A1-ab; both of these have a signal at 5.366 p.p.m. (Figs. 2A and 2C). It was therefore evident that Fr. Ti had the simplest chemical structure among the D-mannans of three *C. stellatoidea* strains, in terms of average chain-length and chemical structure of the branches.

In contrast, acetolysis of Fr. A1-ab gave homogeneous biose, triose, tetraose, pentaose, and hexaose corresponding to structures **1**, **2**, **3**, **5**, and **7**, and these oligosaccharides were investigated by ^{13}C-n.m.r. spectrometry. The ^{13}C-n.m.r. spectrum of the D-mannohexaose, **7**, showed a 103.09-p.p.m. signal, which was observed in ^{13}C-n.m.r. spectra of Frs. If and A1, but was absent that of Fr. Ti.. This finding agrees well with that found in the acetolysis study of Fr. Ti-ab, namely that this D-mannohexaose

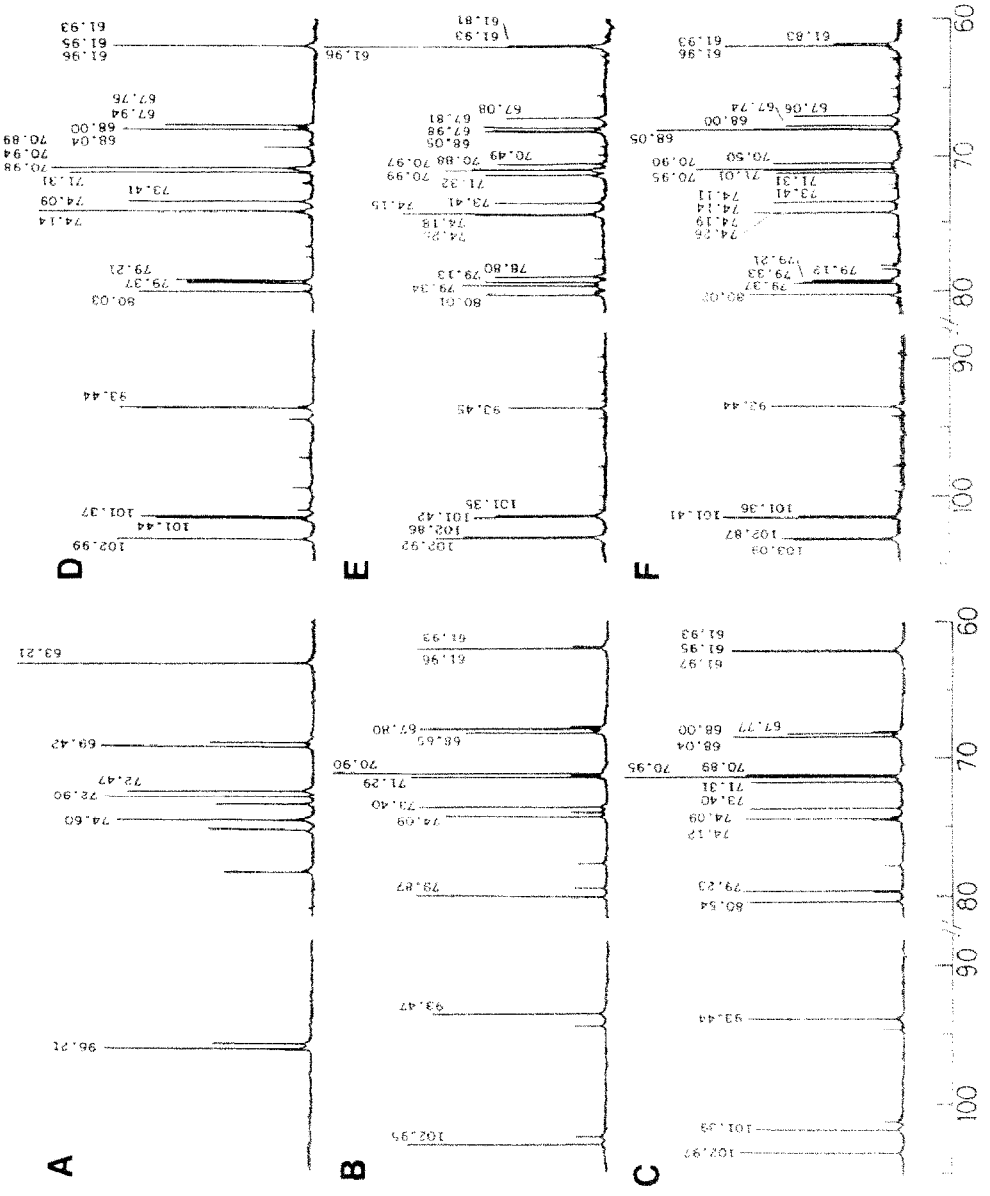

Fig. 6. ^{13}C-N.m.r. spectra of D-mannose (A), and D-mannose-oligosaccharides, structures 1 (B), 2 (C), 3 (D), 5 (E), and 7 (F), obtained from Fr. A1-ab by acetolysis. Each D-mannose-oligosaccharide corresponds to one of the peaks in Fig. 4C. These were recorded under the same conditions as in Fig. 3.

TABLE II

^{13}C Chemical shifts of D-manno-oligosaccharides (α anomers) obtained from Fr. A1-ab by acetolysis

	D-Mannose	**1**		**2**			**3**			
	1	*1*^{2}	*1*	*1*^{3}	*1*^{2}	*1*	*1*^{4}	*1*^{3}	*1*^{2}	*1*
C-1	96.21	102.95	93.47	102.97	101.39	93.44	102.99	101.37	101.44	93.44
C-2	72.90	70.90	79.87	70.89	79.23	80.54	71.31	79.37	79.21	80.03
C-3	72.47	71.29	70.90	71.31	70.95	70.95	70.98	70.89	70.89	70.94
C-4	69.42	67.80	68.05	67.77	68.00	68.04	67.76	67.94	68.00	68.04
C-5	74.60	74.09	73.40	74.09	74.12	73.40	74.09	74.14	74.14	73.41
C-6	63.21	61.93	61.96	61.93	61.95	61.97	61.93	61.95	61.95	61.96

	5					**7**					
	1^5	*1*^{4}	*1*^{3}	*1*^{2}	*1*	*1*^{6}	*1*^{5}	*1*^{4}	*1*^{3}	*1*^{2}	*1*
C-1	102.92	102.86	101.35	101.42	93.45	103.09	101.41	102.87	101.36	101.41	93.44
C-2	70.97	70.49	79.34	79.13	80.01	71.01	79.37	70.50	79.33	79.12	80.02
C-3	71.32	78.80	70.99	70.97	70.88	71.31	70.95	79.21	70.90	70.90	70.90
C-4	67.81	67.08	67.98	68.05	68.05	67.74	68.00	67.06	68.00	68.05	68.05
C-5	74.25	74.18	74.15	74.15	73.41	74.11	74.26	74.19	74.14	74.14	73.41
C-6	61.93	61.81	61.96	61.96	61.96	61.93	61.93	61.83	61.96	61.96	61.96

was absent from this acetolyzate. Assignment of the signals in ^{13}C-n.m.r. spectra of the lower α-(1→2)-linked D-manno-oligosaccharide, biose, triose, and tetraose (structures **1**, **2**, and **3**), were as described by Gorin[25], Ogawa and Yamamoto[26], and Allerhand and Berman[27]. However, the ^{13}C-n.m.r. data for the D-mannopentaose and D-mannohexaose (structures **5** and **7**) are new. Figs 6E and 6F, and also Table II, show signals at 78.80 and 79.21 p.p.m. (corresponding to the C-3 carbon atoms of the 1^4 residues of structures **5** and **7**) at lower magnetic fields (6.33 and 6.74 p.p.m.) than that of the α-anomeric carbon atom of free D-mannose (72.47 p.p.m.) in Fig. 6A and Table II. Additionally, the C-2 signals of the 1^4 residues of structures **5** and **7** are shifted by 2.41 and 2.40 p.p.m. to the higher magnetic fields, giving rise 70.49 and 70.50-p.p.m. signals, and the C-4 signals of the 1^4 residues of these D-manno-oligosaccharides showed upfield shifts, by 2.34 and 2.36 p.p.m., as compared with the spectrum of free D-mannose.

Slide-agglutination assay for C. stellatoidea *Type I strains with polyclonal factor sera.* — We also conducted a series of slide-agglutination reactions between commercially available polyclonal factor sera, "Candida Check", and heat-killed cell suspensions of yeasts, including three *C. albicans* strains, NIH A-207 (serotype A), NIH B-792 (serotype B), and J-1012 (serotype A), and three *C. stellatoidea* Type I strains, IFO 1397, TIMM 0310, and ATCC 11006.

Table III summarizes the results of this assay. The *C. stellatoidea* Type I strain cells used in the present study were not agglutinated by either factor serum 6 or 13b. In the previous paper[2], we suggested the possibility that a D-mannopentaosyl residue, 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, corresponds to one of epitopes dominating serotype-A specificity of *C. albicans* spp. The results of the present structural investigation of mannans of the *C. stellatoidea* Type I strain cells agree well with those of the present agglutination assay, because no β-(1→2)-linkage-containing oligosaccharide was isolated from the acetolyzates of Frs. If-ab, Ti-ab, and A1-ab.

TABLE III

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									
IFO 1397	+	+	+	−	−	−	−	−	−
TIMM 0310	+	+	+	−	−	−	−	−	−
ATCC 11006	+	+	+	−	−	−	−	−	−

[a] + agglutination; − no agglutination.

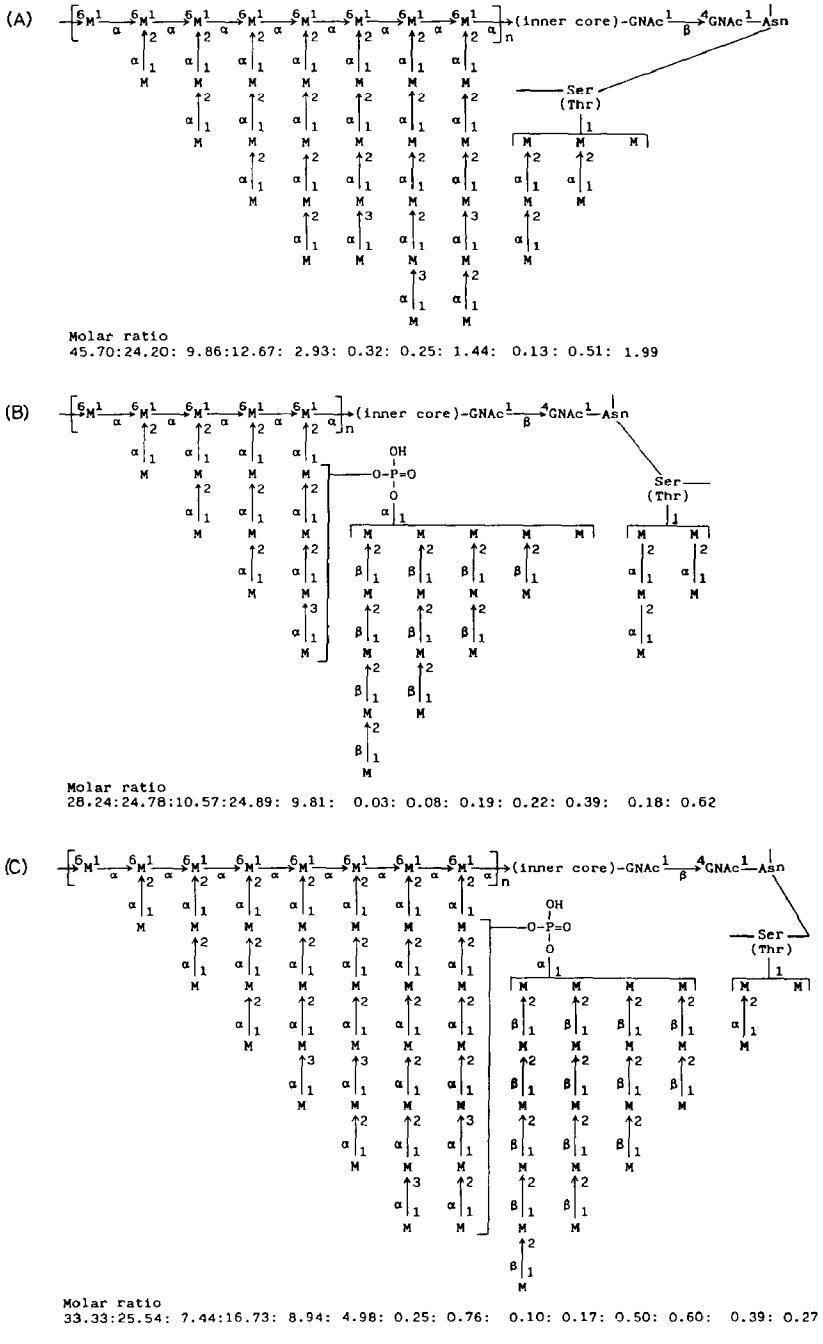

Fig. 7. Possible structures for the cell-wall D-mannans of *C. stellatoidea* IFO 1397 (A), TIMM 0310 (B), and ATCC 11006 (C) strains. M (Man) and GNAc (GlcNAc) denote a D-mannopyranose and 2-acetamido-2-deoxy-D-glucopyranose units, respectively. The side-chain sequence is not specified.

In 1984, Funayama *et al.*[28] stated that the specific antigen corresponding to factor serum 13b was a D-mannohexaose constituted as shown as structure 7. However, it should be recalled that no *C. stellatoidea* cells thus far examined in this assay were agglutinated by factor serum 13b. This fact suggests that the specific epitope corresponding to this factor is not the D-mannohexaose 7, but another sugar residue not revealed by the present analytical procedure, or some exposed peptidic moiety on the cell surface.

DISCUSSION

It was recently reported by Kwon-Chung *et al.*[6,7] that a group of pathogenic yeasts of genus *Candida*, *C. stellatoidea* spp. could be classified into two karyotypes designated as Types I and II, and that the serological properties of the former and the latter strains resembled those of serotypes B and A of *C. albicans* spp. respectively. These workers further stated that the *C. stellatoidea* Type I strains showed pulse-field electrophoresis patterns distinctly different from those of Type II, weaker virulence for mice, lower resistance to u.v. irradiation, slower production of proteinase, and negative reactivity with the anti-*Candida tropicalis* D-mannan monoclonal antibody[29].

Quite recently, it was demonstrated by Tojo *et al.*[5] that the D-mannan of *C. stellatoidea* IFO 1397 (Type I) strain did not possess any acid-labile, phosphate-bound oligo-D-mannosyl residue, and that this D-mannan did not react with an anti-*C. albicans* NIH B-792 D-mannan monoclonal antibody[30], which has been shown to react strongly with β-(1→2)-linked oligo-D-mannosyl residues in the D-mannan of *C. albicans* NIH B-792 strain. Based on the results of the present study, however, the D-mannans of *C. stellatoidea* TIMM 0310 and ATCC 11006 strains are shown to contain both phosphate and phosphate-bound β-(1→2)-linked oligo-D-mannosyl residues, demonstrating the existence of two groups of structural difference in the D-mannans in *C. stellatoidea* Type I strains.

Summarizing the present study, the chemical structures for the three D-mannans of *C. stellatoidea* (Type I) spp. are depicted in Fig. 7. Taking into account the finding of Type II strains exhibiting serotype A-like serological specificity for *C. albicans* spp., it may be stated that the *C. stellatoidea* spp. can be regarded as possessing at least three structurally different D-mannans. It is likely that the Type II specificity corresponds to the branches possessing β-(1→2)-linked oligo-D-mannosyl residues in the non-reducing terminal sites in the acid- and alkali-stable domain of the parent D-mannan (as reported by Shibata *et al.*[1] and Kobayashi *et al.*[2,5] on the D-mannans of *C. albicans* serotype A strains), and virulency to mice. Analysis of the location of genes dominating Type II specificity would be of interest, because it is highly probable that these genes are located in close proximity in the same chromosome.

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