Carbohydrate Research, 187 (1989) 239–253
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL ANALYSIS OF PHOSPHO-D-MANNAN–PROTEIN COMPLEXES ISOLATED FROM YEAST AND MOLD FORM CELLS OF *Candida albicans* NIH A-207 SEROTYPE A STRAIN

NOBUYUKI SHIBATA, SHIGEYUKI FUKASAWA, HIDEMITSU KOBAYASHI, MINEHIRO TOJO, TOSHIO YONEZU, AKIHIRO AMBO, YASUHITO OHKUBO, AND SHIGEO SUZUKI*

Second Department of Hygienic Chemistry and Department of Radiopharmacy, Tohoku College of Pharmacy, Sendai 981 (Japan)

(Received january 6th, 1988; accepted for publication, March 31st, 1988)

ABSTRACT

The immunochemical properties between phospho-D-mannan–protein complexes of yeast (Y) and mycelial (M) forms of *Candida albicans* NIH A-207 (serotype A) strain were compared. Hydrolysis of the Y-form complex gave a mixture of β-(1→2)-linked D-mannooligosaccharides consisting mainly of tri- and tetra-ose, whereas the M-form complex gave preponderantly D-mannose. The antiserum against Y-form cells exhibited a lower reactivity with the M-form than with the Y-form complex, whereas the antiserum to M-form cells could not distinguish significantly between both complexes. Moreover, these acid-modified complexes showed lower antibody-precipitating effect than each corresponding intact complex against antisera of Y- and M-form cells. Digestion of the acid-modified Y- and M-form complexes with the *Arthrobacter* GJM-1 strain α-D-mannosidase yielded 35- and 40-% degradation products, respectively. Acetolysis of each modified complex under mild conditions gave the same D-mannohexaose, 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. Because the complexes of Y- and M-form cells of *C. albicans* NIH B-792 (serotype B) strain did not give any hexaose fraction containing β-(1→2) linkages, the presence of this hexaose can be regarded as one of the dominant characteristics of the serotype-A specificity of *C. albicans* spp.

INTRODUCTION

In the preceding paper[1], we reported the structural differences between the phospho-D-mannan–protein complexes of yeast- (Y) and mold-form (M) cells of *C. albicans* NIH B-792 strain [serotype B (ref. 2), abbreviated as B-strain], and stated that a decrease of the proportion of β-(1→2)-linked D-mannooligosaccharide residues connected to other branching moieties *via* phosphoric diester linkage was evident. The immunochemical properties of the mannan–protein complexes of the Y- and M-form cells of another representative *C. albicans* strain, NIH A-207

*To whom correspondence should be addressed.

0008-6215/89/$ 03.50

[serotype A (ref. 2), abbreviated as A-strain], isolated by the action of Zymolyase followed by fractional precipitation with cetyltrimethylammonium bromide (Cetavlon), were also investigated. The mannan component of the parent complex of A-strain of Y-form cells was shown to contain β-(1→2)-linked D-mannooligosaccharide residues both labile and stable to acid treatment[3]. Therefore, it seemed of interest to investigate whether any change of density of branching moieties containing β-(1→2)-linked D-mannooligosaccharide residues in the acid-stable region of the mannan component would be observed during Y to M transformation of the parent cells. Additionally, we are proposing a chemical structure for the acid-stable domains of phospho-D-mannan moieties containing the β-D-(1→2) linkage of the parent complexes of Y- and M-form cells of A-strain.

EXPERIMENTAL

Materials. — The *C. albicans* NIH A-207 and *C. albicans* NIH B-792 strains, abbreviated as A and B strains, respectively, were kindly donated by Dr. T. Shinoda, Department of Microbiology, Meiji College of Pharmacy, Tokyo, Japan. Rabbit antisera of Y-form cells of A and B strains were the same specimens used in the previous study[3,5], and were designated as antisera YA and YB, respectively. Antiserum of M-form cells of A strain, designated as antiserum MA, was prepared by immunizing the rabbits with a saline suspension of heat-killed and sonically disrupted M-form cells of the same strain. A column packing for gel-filtration chromatography, Toyopearl HW-40 (superfine), fractionation range of 0.1 to 7 kDa, and Toyopearl HW-60 (superfine), fractionation range of 2 to 300 kDa, was purchased from Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan. Bio-Gel P-2 (−400 mesh) was obtained from Bio-Rad, and Zymolyase-100T[6,7] from Kirin Brewery, Tokyo, Japan. The *Arthrobacter* GJM-1 strain was kindly supplied by Dr. C. E. Ballou, University of California, Berkeley, *via* Dr. T. Nakajima, Tohoku University, Sendai, Japan.

Cultivation of C. albicans *strains.* — The Y- and M-form cells of A-strain were cultivated according to Shibata *et al.*[1].

Treatment of Y- and M-form cells of C. albicans *NIH A-207 strain with Zymolyase-100T.* — This treatment was conducted in accordance with the previous description by Shibata *et al.*[8]. The complexes obtained from Y- and M-form cells were designated as Fractions YA and MA, respectively.

Immunological methods. — Antibody-precipitation assay and precipitin-inhibition assays were performed in accordance with the description by Okubo *et al.*[9].

Acid treatment of phospho-D-mannan–protein complexes, Fractions YA and MA. — This treatment was conducted exactly in the same manner as that for the degradation of the D-mannans of a *S. cerevisiae* wild type strain and B-strain as described by Shibata *et al.*[1,10]. The acid-modified phospho-D-mannan–protein complexes were designated as Fractions YA-a and MA-a, respectively.

Acetolysis of acid-treated phospho-D-mannan–protein complexes, Fractions YA-a and MA-a. — The acetolysis was conducted in accordance with the previous description by Okubo and Suzuki[11].

α-D-Mannosidase treatment. — Preparation of α-D-mannosidases was conducted in accordance with the description by Jones and Ballou[12]. Fraction YA-a or MA-a (each 200 mg) was dissolved in 0.1M phosphate buffer (pH 6.8) (20 mL) and α-D-mannosidase (10 mg of protein) was added. The solution was kept at 37°, and the reducing sugar released was determined at intervals of 1 h by the method of Somogyi–Nelson[13]. When the release of reducing sugar had reached a plateau, the incubation mixture was dialyzed against water, and then lyophilized to give Fractions YA-aE and MA-aE, respectively.

Methylation analysis. — D-Mannooligosaccharides (each 1 mg) obtained by gel filtration of the acetolysis products were methylated by the Hakomori method[14], and the resultant per-*O*-methylated D-mannooligosaccharides converted into a mixture of *O*-acetyl-*O*-methyl-D-mannitols according to Lindberg[15].

Other methods. — Specific rotations were determined with an Applied Electric automatic polarimeter. ^{1}H-N.m.r. spectra of phospho-D-mannan–protein complexes were recorded with a JEOL JNM-FX 100 spectrometer for solutions in D_2O at 70°. Total carbohydrate content was determined by the phenol–H_2SO_4 method[16] with D-mannose as the standard. Total phosphate content was determined by the method of Ames and Dubin[17] using KH_2PO_4 as the standard. Total protein content was determined by the Folin method of Lowry *et al.*[18] using bovine serum albumin as the standard. G.l.c. of *O*-acetyl-*O*-methyl-D-mannitols was conducted in a glass column (5 mm × 150 cm) containing 3% OV-210 on Supelcoport (100–200 mesh) at 185°, and with N_2 as the carrier gas at a flow rate of 20 mL/min. Conversion of the peak dimensions into molar ratios of the sugar derivatives was made with a Shimadzu Chromatopac-E1A microcomputer.

RESULTS

Cultivation of M-form cells. — The M-form cells of A-strain grew in the synthetic medium during a 48-h cultivation at 37° with a small amount of Y-form cells, which could readily be removed by repeating the washing of the mycelia with saline to give homogeneous M-form cells.

Isolation of the phospho-D-mannan–protein complexes from Y- and M-form cells. — The yields of the phospho-D-mannan–protein complexes from both form of cells, designated as Fractions YA and MA, respectively, obtained by the action of the Zymolyase-100T followed by fractional precipitation with Cetavlon were 5.2 and 4.3%, respectively, on a weight basis relative to the weight of the corresponding acetone-dried whole cells. All fractions contained ~90% of carbohydrate and 2–3% of protein. The phosphate content of Fr. MA, 1.03%, was similar to that of Fr. YA, 0.93% (Table I).

^{1}H-N.m.r. spectra of phospho-D-mannan–protein complexes, Fractions YA

TABLE I

CHEMICAL COMPOSITION OF THE PHOSPHO-D-MANNAN-PROTEIN COMPLEXES OF Y- AND M-FORM CELLS OF *C. albicans* NIH A-207 STRAIN AND THEIR MODIFIED PRODUCTS

Fraction	*Total carbohydrate*[a]	*Total protein*[b]	*Total phosphate*[c]	$[\alpha]_D^{20}$ *(degrees)*[d]	*Yield*[e] *(%)*
YA	87	2.3	0.93	+40	5.2
MA	95	3.8	1.03	+50	4.3
YA-a	85	3.1	0.98	+49.5	4.7
MA-a	95	3.6	1.03	+53	4.0
YA-aE	92	3.5	1.53	+38.5	3.0
MA-aE	94	3.7	1.71	+36	2.4

[a]Determined by the phenol-sulfuric acid method[16]. [b]Determined by the Folin method of Lowry *et al.*[18]. [c]Determined by the method of Ames and Dubin[17] as H_2PO_3. [d]In water (*c* 1.0). [e]Weight basis of the acetone-dried whole cells.

and MA. — The ^{1}H-n.m.r. spectra of the H-1 region of Frs. YA and MA are illustrated in Figs. 1A and 1B, respectively. The spectrum of Fr. YA obviously resembles that of the phospho-D-mannan of the Y-form of A-strain obtained by hot-water extraction[3]. Therefore, the chemical structure of the D-mannan component in Fr. YA did not undergo a degradation to a significant extent with the con-

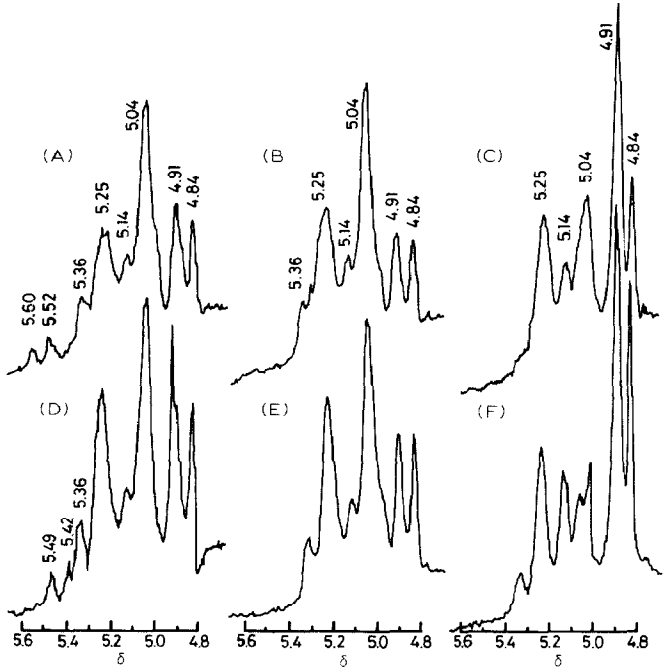


Fig. 1. ^{1}H-N.m.r. spectra (anomeric regions) of phospho-D-mannan–protein complexes isolated from yeast and mycelial forms of *C. albicans* NIH A-207 strain: (A) Fr. YA, (B) Fr. YA-a, (C) Fr. YA-aE, (D) Fr. MA, (E) Fr. MA-a, and (F) Fr. MA-aE.

taminating α-D-mannosidase in Zymolyase-100T. In addition, the presence of α-D-glycosyl phosphate groups in Fr. YA (quartet band at δ 5.60–5.52)[19] is evident. On the other hand, the ^{1}H-n.m.r. pattern of Fr. MA in this region appears somewhat different from that of Fr. YA, *i.e.*, as a very weak pair of quartet bands at δ 5.60–5.52 may be observed, as well as another fairly strong quartet band located at higher magnetic field, δ 5.49 and 5.41. This latter band may be assigned to the anomeric proton of the 1-*O*-phosphorylated and nonsubstituted α-D-mannopyranose residues and the other band located in the relatively low magnetic field corresponds to the anomeric protons of the 1-*O*-phosphorylated and 2-*O*-substituted D-mannooligosaccharides residues. These findings led us to the assumption that a considerable amount of acid-labile, phosphate-bound D-mannopyranose units exists in Fr. MA. The chemical shift at δ 4.91 observed commonly in the ^{1}H-n.m.r. patterns of Frs. YA and MA may be assigned to be the anomeric protons of the α-(1→6)-linked D-mannopyranose plus the β-(1→2)-linked D-mannopyranose units, in the form of a phosphate-bound D-mannooligosaccharide residue as shown in the preceding paper, by Shibata *et al.*[1], on the phospho-D-mannan–protein complexes of M- and Y-forms of B-strain. On the other hand, the chemical shift at δ 4.84, which appeared in both ^{1}H-n.m.r. patterns of Frs. YA and MA, corresponds presumably to be the anomeric protons of β-(1→2)-linked D-mannopyranose residues attached to α-(1→2)-linked D-mannopyranose units, including nonreducing terminal groups and intermediary β-(1→2)-linked D-mannopyranose units located in the acid-stable region of the D-mannan components of Y- and M-form complexes. These findings are substantiated by the recent observations of Kobayashi *et al.*[20] on the structure of β-(1→2)-linkage-containing phospho-D-mannan–protein complex of *Citeromyces matritensis* IFO 0651 strain: the chemical shift at δ 4.91 disappeared upon treatment of the complex with 10mM HCl at 100° with concomitant release of a D-mannotriose [β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man] and a D-mannotetraose [β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man], and the chemical shift at δ 4.85 corresponds to β-(1→2)-linked D-mannopyranose residues located in both the nonreducing terminal position and in intermediary positions of D-mannotetraose [β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man], which exists as acid-stable branches in the parent complex. The small signal at δ 5.36 was assumed to correspond to the anomeric proton of intermediary α-(1→3)-linked D-mannopyranose units[21] located in the acid-stable region of both Frs. YA and MA, the presence of which was reported by Funayama *et al.*[22] in the D-mannan of Y-form of B-strain extracted with hot aqueous alkali solution.

Treatment of Fractions YA and MA with 10mM hydrochloric acid. — In order to assess the density of acid-labile D-mannooligosaccharide residues in the phospho-D-mannan–protein complexes, Frs. YA and MA were separately treated with 10mM HCl. As shown in Fig. 2A, Fr. YA released a mixture of oligosaccharides ranging from D-mannohexaose to D-mannose in a yield of 7.8%. On the other hand, Fr. MA released a mixture of oligosaccharides ranging from D-mannotetraose to D-

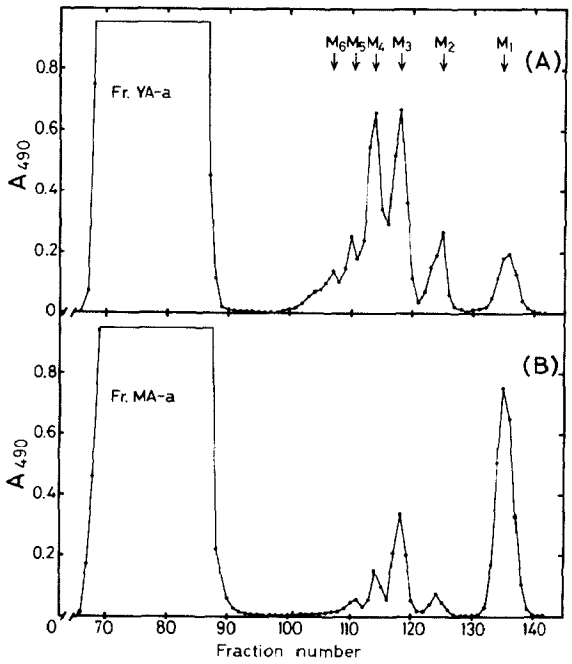


Fig. 2. Gel-filtration patterns of the reaction products of Frs. YA (A) and MA (B) with 10mM HCl on a column of Toyopearl HW-40 (2.5 × 100 cm). Elution was effected with water at 0.25 mL/min. The amounts of carbohydrate in the eluates were determined by the phenol–H_2SO_4 method[14]. Aliquots of the eluates of the void-volume and the diffusible regions (100 μL) were used for the assay: M_7, M_6, M_5, M_4, M_3, M_2, and M_1 indicate D-mannoheptaose, D-mannohexaose, D-mannopentaose, D-mannotetraose, D-mannotriose, D-mannobiose, and D-mannose, respectively.

mannose in a yield of 5.0% (Fig. 2B), and the ratio of D-mannose contained in the oligosaccharide mixture released from Fr. MA was significantly larger than that released from Fr. YA (Table II). The amount of β-(1→2)-linked D-mannooligosaccharides released from Fr. MA by treatment with hot 10mM HCl was smaller than that released from Fr. YA by the same treatment. However, the weight ratio of the acid-labile oligosaccharides released from Frs. YA and MA, (7.8 to 5.0;

TABLE II

D-MANNOOLIGOSACCHARIDES AND D-MANNOSE RELEASED FROM FRACTIONS YA AND MA BY TREATMENT WITH 10mM HCl

Fraction	*Man_6 (molar ratio)*	*Man_5*	*Man_4*	*Man_3*	*Man_2*	*Man*	*Ratio to total weight[a] (%)*
YA	0.08	0.14	0.53	0.76	0.45	1.00	7.8
MA		0.01	0.03	0.01	0.04	1.00	5.0

[a]Expressed as percentage, on a weight basis, of the parent D-mannans.

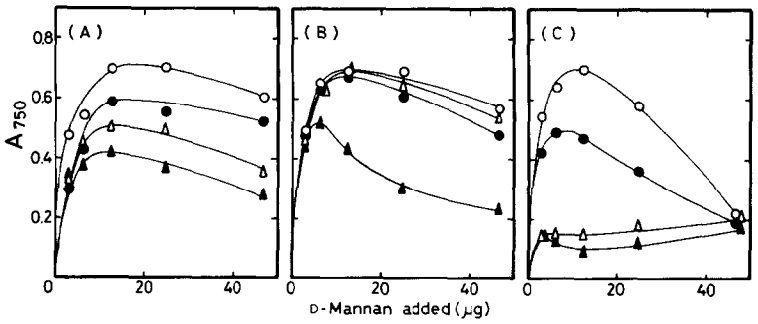


Fig. 3. Antibody-precipitating activities of phospho-D-mannan–protein complexes, Frs. YA and MA, and corresponding acid-treated mannans, Frs. YA-a and MA-a, against antisera YA (A), MA (B), and YB (C): (○) Fr. YA, (△) Fr. MA, (●) Fr. YA-a, and (▲) Fr. MA-a.

Table II), can be converted into a molar ratio of 10:9, demonstrating a significant decrease of the average chain-length of the acid-labile D-mannooligosaccharide residues in Fr. MA. Therefore, it is possible that, during Y-to-M transformation, β-(1→2)-D-mannosyltransferase, rather than D-mannopyranosyl phosphate transferase activity, is suppressed. The large peaks that appeared in the void-volume region were designated as Frs. YA-a and MA-a, respectively. They were then investigated by ^{1}H-n.m.r. spectroscopy, which showed the disappearance of the signal of the anomeric proton of the 1-*O*-phosphorylated α-D-glycose residues in a quartet band at δ 5.60–5.42 (Figs. 1C and 1D).

Antibody-precipitating activity of Fractions YA and MA and their acid-treated products. — In the quantitative precipitin test of the phospho-D-mannan–protein complexes against antiserum YA (Fig. 3A), the activity of Fr. MA was ~70% of that of Fr. YA, when comparison was made at the equivalent point of Fr. YA. Both acid-treated complexes (Frs. YA-a and MA-a) showed ~4/5 of the activity of the corresponding intact complex (Fig. 3A). Therefore, it may be assumed that the phosphoric diester- β-(1→2)-linked D-mannooligosaccharide residues participated in only a part of the antibody-precipitating activity of the corresponding intact complexes (Frs. YA and MA) and that the β-(1→2)-linked D-mannooligosaccharide residues located in the acid-stable region of Frs. YA and MA may be involved largely in this effect. Additional antibody-precipitation assays of the intact and acid-modified complexes with antiserum MA indicated that Frs. YA and MA exhibited an almost identical antibody-precipitating effect, and that the reactivity of Fr. MA-a was lower than that of the corresponding intact complex by ~30% (Fig. 3B). This suggested that the antiserum MA contained antibody(ies) against the acid-labile domain of Fr. MA. As shown in Fig. 3C, significant differences of reactivity between two pairs of phospho-D-mannan–protein complexes and their acid-degradation products, Frs. YA and YA-a, and MA and MA-a, against antiserum YB were observed. Presumably, the lower antibody-precipitating activity of Fr. MA relative to Fr. YA may be attributed to a decrease of the density of β-(1→2)-linked

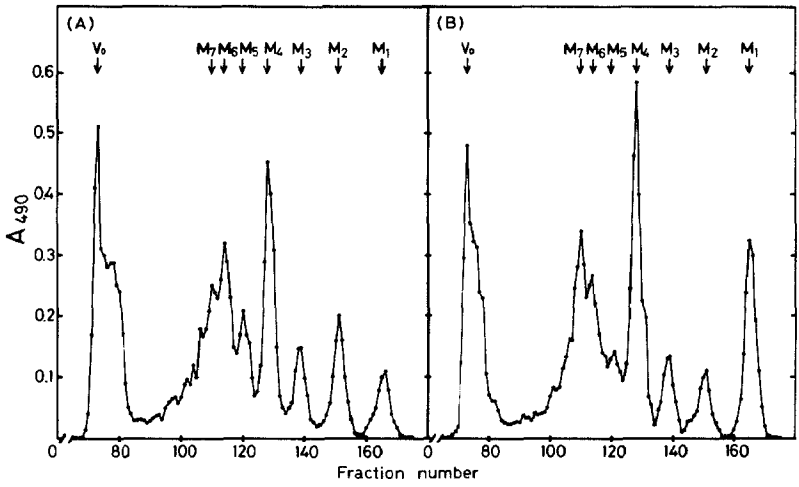


Fig. 4. Elution patterns of the oligosaccharide mixtures obtained from acid-treated phospho-D-mannan–protein complexes [Frs. YA-a (A) and MA-a (B)] by treatment with 50:50:1 (v/v) acetic anhydride–acetic acid–H_2SO_4 for 12 h at 40°, on a column of Bio-Gel P-2 (2.5 × 100 cm).

D-mannopyranose units, because the majority of antibody contained in antiserum YB is directed against the β-(1→2)-linked D-mannooligosaccharide residues[1,3].

Acetolysis of Fractions YA-a and MA-a. — As shown in Fig. 4, both acetolyzates of Frs. YA-a and MA-a gave each seven peaks from D-mannoheptaose to D-mannose, and an additional peak eluted in the void volume region. They were designated as Man_7–Man_1 and Vo, and the molar ratios of the D-mannooligosaccharides are reported in Table III. The proportions of Man_4 and Man_1 obtained from Fr. MA-a were larger than those from Fr. YA-a, whereas those of Man_6 and Man_5 from Fr. MA-a were smaller than those from Fr. YA-a. In the ^{1}H-n.m.r. spectra of the H-1 region of the D-mannooligosaccharides obtained by acetolysis, the intensity of the signal at δ 5.13 corresponding to the nonreducing terminal α-(1→3)-linked D-mannopyranosyl group residue in Man_5 obtained from Fr. MA-a (Fig. 5B) was apparently smaller than that of the corresponding D-mannooligosaccharide obtained from Fr. YA-a (Fig. 5A).

α-D-Mannosidase treatment of Fractions YA-a and MA-a. — Upon treatment

TABLE III

D-MANNOOLIGOSACCHARIDES AND D-MANNOSE PRODUCED FROM FRACTIONS YA-a AND MA-a BY ACETOLYSIS

Fraction	*Man_7 (molar ratio)*[a]	*Man_6*	*Man_5*	*Man_4*	*Man_3*	*Man_2*	*Man*
YA-a	0.51	0.80	0.78	1.80	1.00	1.88	2.12
MA-a	1.02	0.80	0.65	2.70	1.00	1.36	6.22

[a]Expressed with D-mannotriose as unit.

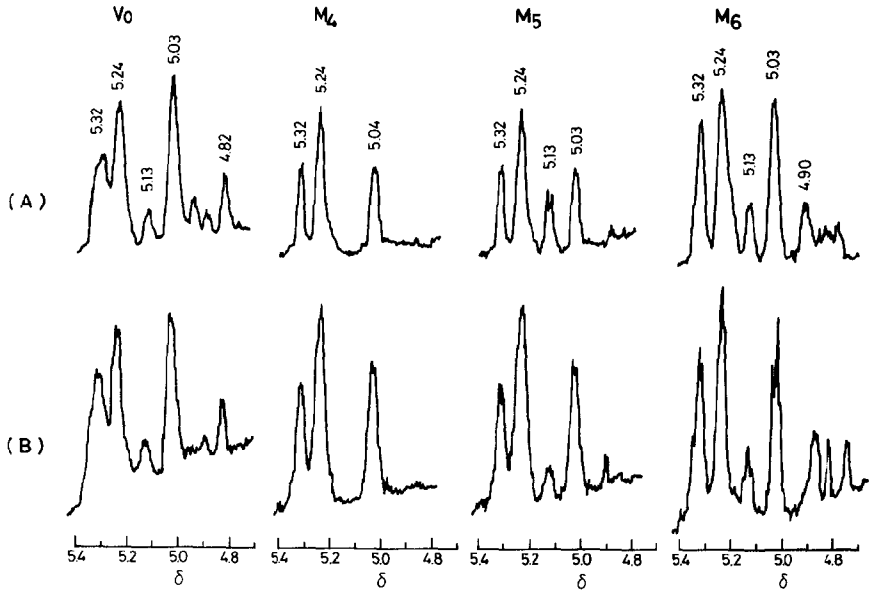


Fig. 5. ^{1}H-N.m.r. spectra of D-mannooligosaccharides obtained by acetolysis of acid-treated phospho-D-mannan–protein complexes [Frs. YA-a (A) and MA-a (B)].

with α-D-mannosidase, Frs. YA-a and MA-a released D-mannose, ~35 and 40%, to give Frs. YA-aE and MA-aE, respectively. These modified complexes corresponded to the α-D-mannosidase-resistant domains of Frs. YA-a and MA-a. This treatment caused a decrease of the specific rotations (Table I), indicating the removal of a considerable amount of α-linked D-mannopyranose residues. In the ^{1}H-n.m.r. spectra of the H-1 region of Frs. YA-aE and MA-aE (Figs. 1E and 1F), a decrease of the signals at δ 5.25 to 5.04 with a concomitant increase of the signal at δ 4.91 to 4.84 are evident. The increase of the δ 4.91 signal may be attributed to the exposure of the core moiety consisting of α-(1→6)-linked D-mannopyranose units upon elimination of part of the branches. This conclusion was substantiated by the reactivities of Frs. YA-aE and MA-aE against anti-*S. cerevisiae* X2180-1A-5 mutant strain serum[23] (data not shown), and by the results obtained in the acetolysis of Frs. YA-aE and MA-aE reported below.

Acetolysis of Fractions YA-aE and MA-aE. — In the comparison of the elution profiles of acetolyzates of Frs. YA-aE and MA-aE (Figs. 6A and 6B) with those of Frs. YA-a and MA-a (Figs. 4A and 4B), it is evident that the branches, especially those corresponding to Man_4, were eliminated by the action of *Arthrobacter* GJM-1 α-D-mannosidase. The abundance of a D-mannohexaose in both acetolyzates of Frs. YA-aE and MA-aE suggested that a part of the β-(1→2)-linked D-mannopyranose units might exist as the nonreducing terminal ends of two D-mannohexaoses designated as Man_6-YA-aE and Man_6-MA-aE. Additionally, the amounts of D-mannose in both acetolyzates were apparently larger than those in

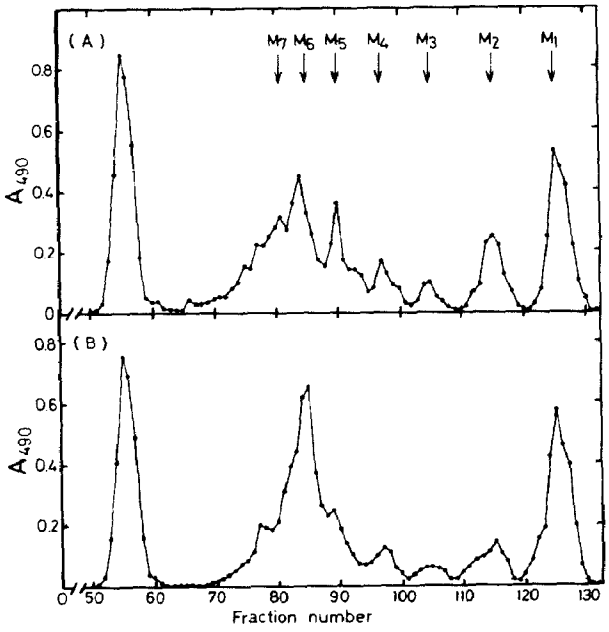


Fig. 6. Elution patterns of the oligosaccharide mixtures obtained from α-D-mannosidase-treated phospho-D-mannan–protein complexes [Frs. YA-aE (A) and MA-aE (B)] by acetolysis under the same condition as those given in the legend to Fig. 4.

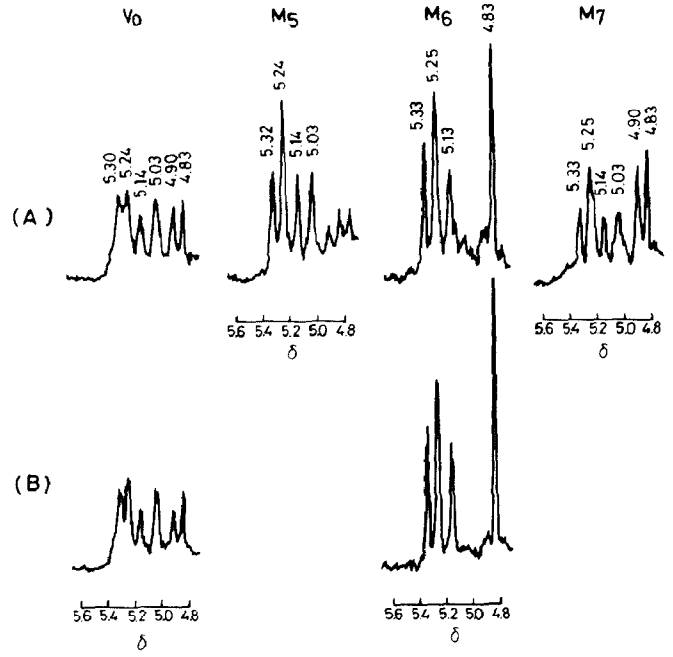


Fig. 7. ^{1}H-N.m.r. spectra of D-mannooligosaccharides obtained by acetolysis of Frs. YA-aE (A) and MA-aE (B).

TABLE IV

ANOMERIC PROTON CHEMICAL SHIFTS FOR D-MANNOOLIGOSACCHARIDES

Structure	*Chemical shifts of H-1 (δ)*						
	1^7	1^6	1^5	1^4	1^3	1^2	*1*
1			5.14	5.03	5.24	5.24	5.32
2		4.83	4.83	5.13	5.25	5.25	5.33
3	4.83	4.90	4.83	5.14	5.25	5.25	5.33

the acetolyzates of the corresponding parent complexes, Frs. YA-a and MA-a. This is in agreement with the increase of the intensity of the signal at δ 4.91 in the ^{1}H-n.m.r. patterns of Frs. YA-aE and MA-aE.

Structural investigation of D-*mannohexaoses isolated from acetolyzates of Fractions YA-aE and MA-aE.* — The ^{1}H-n.m.r. patterns of the H-1 protons of Frs. Man_6-YA-aE and Man_6-MA-aE (Figs. 7A and 7B) provided evidence that the two hexaoses are structurally identical, and that the ratio of β-(1→2)-to-α-(1→2) linkages in both hexaoses is ~1:2. Assignment of the signals according to Cohen and Ballou[24] (Table IV) suggested structure **2**. The results of methylation analysis of both hexaoses substantiated this structure, *i.e.*, the alditol acetates derived from the hexaoses were 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl- and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-mannitol, indicating that all linkages were (1→2) (Table V).

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

1

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

2

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

3

TABLE V

GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF THE MOLAR RATIOS OF *O*-ACETYL-*O*-METHYL-D-MANNITOLS DERIVED FROM D-MANNOOLIGOSACCHARIDES OBTAINED FROM FRACTIONS YA-aE AND MA-aE BY ACETOLYSIS

Partially acetylated D-*mannitol derivative*	*Relative retention time*	*Man_6*	
		YA-aE	*MA-aE*
2,3,4,6-Tetra-*O*-methyl	1.00	1.00	1.00
2,4,6-Tetra-*O*-methyl	1.66	trace	trace
3,4,6-Tetra-*O*-methyl	1.88	4.88	4.91
2,3,4-Tetra-*O*-methyl	2.21		

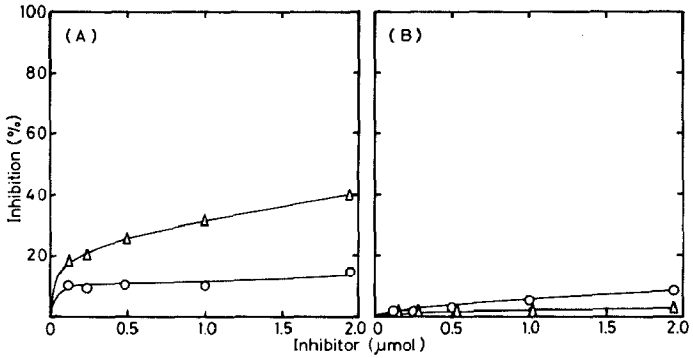


Fig. 8. Precipitin-inhibition assay of antigen-antibody systems by use of α-D-mannopyranosyl phosphate: Antiserum MA (A) or antiserum YA (B), and Fr. YA (○) or Fr. MA (△) systems.

Precipitin-inhibition assay. — The presence of a considerable proportion of D-mannose phosphoric diester units in Fr. MA by ^{1}H-n.m.r. spectroscopy and the acid hydrolysis studies allowed us to assume that the amount of antibodies against α-D-mannopyranosyl phosphate units contained in antiserum MA might be larger than that contained in antiserum YA, as reported by Raschke and Ballou[25] for the antibody response, in rabbits, of α-D-mannopyranosyl phosphate residues in the phospho-D-mannan of a *Kloeckera brevis* strain. In order to confirm this assumption, a series of precipitin-inhibition tests of antigen–antibody systems consisting of Frs. YA and MA, and antisera YA and MA was carried out with α-D-mannopyranosyl phosphate as the inhibitory hapten. In the Fr. MA–antiserum MA system, the inhibition ratio by this hapten at 1μM concentration was 30%, whereas an extremely low inhibition ratio with this hapten was observed against an antigen–antibody system consisting of Fr. YA and antiserum MA (Fig. 8).

DISCUSSION

Our study on the immunochemical properties of phospho-D-mannan–protein complexes, isolated from the Y-form cells of the three representative *C. albicans* strains, NIH A-207, NIH B-792, and J-1012, by means of a combination of hot water extraction, followed by fractional precipitation with Cetavlon, indicated the presence of β-(1→2)-linked D-mannooligosaccharide residues connected with branches of the parent complexes *via* phosphoric diester linkages[3]. However, the study of the immunochemical properties of phospho-D-mannan–protein complexes of *C. albicans* spp., including the aforementioned study, was limited to the complexes isolated from the Y-form cells.

Later, we attempted[1] the isolation of phospho-D-mannan–protein complexes of the Y- and M-form cells of the B-strain by the action of Zymolyase-100T. This study provided evidence for a structural modulation of the D-mannan component upon morphological change from the Y- to the M-form, especially a reduction of

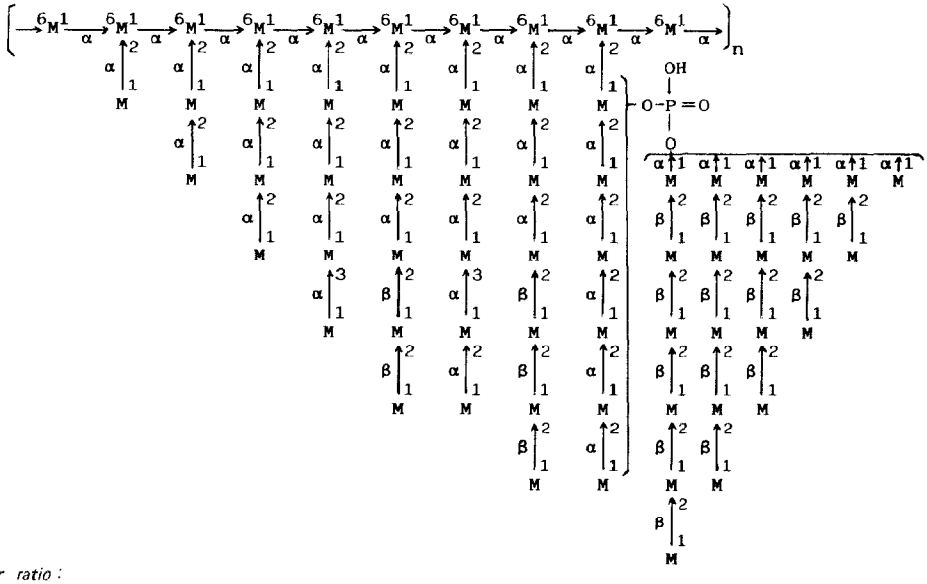
the average length of branches in the D-mannan component. In the present study, cells of another representative *C. albicans* strain, NIH A-207, in both the Y- and M-forms, were also treated with Zymolyase in order to yield each the corresponding phospho-D-mannan–protein complex. Acid degradation of these complexes, followed by acetolysis under mild conditions, also provided evidence that a remarkable suppression of D-mannan biosynthesis resulting in a decrease of the average length of branches of the D-mannan component took place upon the Y-to-M transformation, in a manner similar to that observed for the transformation of Y-form cells of B-strain[1]. The present findings are consistent with those obtained by Ansorg *et al.*[26] who assumed that the morphological change of the cells of *C. albicans* spp. from Y- to M-form was accompanied by qualitative or quantitative variation of the cell wall antigens.

The D-mannan of *C. albicans* was found to be more resistant to the digestion by the *Arthrobacter* GJM-1 α-D-mannosidase than that of *Saccharomyces cerevisiae*, as reported by Jones and Ballou[27], and Reiss *et al.*[28], who attributed this resistance to the presence of a high content of phosphate groups. In the present study, both Frs. YA and MA were found to contain β-(1→2)-linked D-mannooligosaccharide residues in acid-stable and acid-labile D-mannooligosaccharide domains, and phosphate groups connecting both domains; a D-mannohexaose (**2**) was isolated from both enzyme-digestion products by acetolysis under mild conditions. In order to provide evidence that **2** corresponds to the serotype A-specific epitopic group, it was important to confirm absence of **2** in the acetolyzate of the phospho-D-mannan–protein complexes of B-strain modified by treatment with 10mM HCl and α-D-mannosidase. Recently, evidence was obtained that this enzyme-modified complex from the B-strain gives, by mild acetolysis, a small amount of phosphate-containing D-mannooligosaccharides, eluted in the void-volume region, and a large amount of D-mannose[29]; the amount of other D-mannooligosaccharides higher than triose was quite small, and no chemical shift in the region of δ 4.84 in the ^{1}H-n.m.r. patterns of the fractions of void-volume, oligosaccharides higher than triose, and triose could be observed. On the basis of these results, the acid-stable D-mannan component of the phospho-D-mannan–protein complex of B-strain does not contain β-linked D-mannopyranose units. The previous report by Jones and Ballou[27] that treatment of the D-mannan of *C. stellatoidea* with *Arthrobacter* GJM-1 α-D-mannosidase, followed by acetolysis, gave large amounts of D-mannose with a small amount of a mixture of D-mannooligosaccharides agrees well with our results. The cause of a small decrease of antibody-precipitating activities of the acid-modified complexes may be attributed to the presence of considerable amounts of β-(1→2)-linked D-mannooligosaccharide residues in the acid-stable D-mannan component, as well as in the acid-labile oligosaccharide component of the parent complexes.

Although the previous[1,3] and present studies demonstrated the importance of β-(1→2)-linked, acid-labile D-mannooligosaccharide residues as antigenic determinants in the phospho-D-mannan–protein complexes of A- and B-strains, we assumed that these D-mannooligosaccharide residues were the common determin-

ants for the complex of both A and B strains of either form, Y or M, and therefore they were unable to dominate neither serotype specificity, A nor B. As the chemical shift at δ 4.84, which corresponds to the anomeric proton of the D-mannopyranose unit attached β-(1→2) to an α-(1→2)-linked D-mannopyranose unit, was not observed in the ^{1}H-n.m.r. pattern of the B-strain complex, it is indicative that the D-mannohexaose residue **2** corresponds to a characteristic structure of the phospho-D-mannan components of both Frs. YA and MA. This conclusion seems to be preferable to that of Suzuki and Fukazawa[4] who proposed a D-mannohexaose residue containing one nonreducing terminal group linked α-(1→3) and four α-(1→2)-linked residues as the serotype A-specific determinant group[30]. However, the presence of a nonreducing, α-(1→3)-linked terminal group in the phospho-D-mannan–protein complexes of B-strain, based on the results of ^{1}H-n.m.r. analysis of the D-mannohexaose and D-mannopentaose, is evident.

The results of the precipitin-inhibition study with α-D-mannopyranosyl phosphate as the inhibitory hapten indicated that the phosphate groups bound to the acid-labile β-(1→2)-linked D-mannooligosaccharide residues might be included in the integral part of the antigenic determinant groups [even in the phosphorylated, β-(1→2)-linked, higher D-mannooligosaccharide] for all phospho-D-mannan–protein complexes regardless of the forms of cells (Y or M) of *C. albicans* and its related species.



Molar ratio :

Yeast	22	:	20	:	10	:	19	:	8	:	8	:	5	:	0.2:0.4:1.4:2.0:1.2:2.6
Mold	43	:	9	:	7	:	19	:	4	:	6	:	7	:	0 : 0 :0.1: 0 :0.2:4.6

Scheme 1. Representative structure of the phospho-D-mannan–protein complexes of Y- and M-form cells of *C. albicans* NIH A-207 strain, Frs. YA and MA. M denotes a D-mannopyranose residue.

In summary of the previous[3] and present studies, a representative chemical structure of the phospho-D-mannan–protein complex of A-strain is depicted in Scheme 1. Upon morphological change from Y- to M-form, the suppression of some enzymes involved in the biosynthesis of the D-mannan component, such as the β-(1→2)- and α-(1→3)-D-mannosyltransferases[31], takes place resulting in D-mannan component(s) with a less complete structure.

REFERENCES

1 N. SHIBATA, H. KOBAYASHI, M. TOJO, AND S. SUZUKI, *Arch. Biochem. Biophys.*, 251 (1986) 697–708.
2 H. F. HASENCLEVER AND W. O. MITCHELL, *J. Bacteriol.*, 82 (1961) 570–573.
3 N. SHIBATA, T. ICHIKAWA, M. TOJO, M. TAKAHASHI, N. ITO, Y. OKUBO, AND S. SUZUKI, *Arch. Biochem. Biophys.*, 243 (1985) 338–348.
4 M. SUZUKI AND Y. FUKAZAWA, *Microbiol. Immunol.*, 26 (1982) 387–402.
5 Y. OKUBO, Y. HONMA, AND S. SUZUKI, *J. Bacteriol.*, 137 (1979) 677–680.
6 K. KITAMURA, T. KANEKO, AND Y. YAMAMOTO, *J. Gen. Appl. Microbiol.*, 20 (1974) 323–344.
7 K. KITAMURA, *Agric. Biol. Chem.*, 46 (1982) 963–969.
8 N. SHIBATA, K. MIZUGAMI, K. TAKANO, AND S. SUZUKI, *J. Bacteriol.*, 156 (1983) 552–558.
9 Y. OKUBO, T. ICHIKAWA, AND S. SUZUKI, *J. Bacteriol.*, 136 (1978) 63–68.
10 Y. OKUBO, N. SHIBATA, T. ICHIKAWA, S. CHAKI, AND S. SUZUKI, *Arch. Biochem. Biophys.*, 212 (1981) 204–215.
11 Y. OKUBO AND S. SUZUKI, *Carbohydr. Res.*, 62 (1978) 135–141.
12 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 243 (1968) 2442–2446.
13 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19–23.
14 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1946) 205–208.
15 B. LINDBERG, *Methods Enzymol.*, 28B (1972) 178–195.
16 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
17 B. N. AMES AND D. T. DUBIN, *J. Biol. Chem.*, 235 (1960) 769–775.
18 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
19 T. R. THIEME AND C. E. BALLOU, *Biochemistry*, 10 (1971) 4121–4129.
20 H. KOBAYASHI, N. SHIBATA, T. YONEZU, AND S. SUZUKI, *Arch. Biochem. Biophys.*, 256 (1987) 381–396.
21 W. ZHANG AND C. E. BALLOU, *J. Biol. Chem.*, 256 (1981) 10073–10079.
22 M. FUNAYAMA, A. NISHIKAWA, T. SHINODA, M. SUZUKI, AND Y. FUKAZAWA, *Microbiol. Immunol.*, 28 (1984) 1359–1371.
23 W. C. RASCHKE, K. A. KERN, C. ANTALIS, AND C. E. BALLOU, *J. Biol. Chem.*, 248 (1973) 4660–4666.
24 R. E. COHEN AND C. E. BALLOU, *Biochemistry*, 19 (1980) 4345–4358.
25 W. C. RASCHKE AND C. E. BALLOU, *Biochemistry*, 10 (1971) 4130–4135.
26 R. ANSORG, D. GUNESCH, B. BANDELOW, AND R. THOMSSEN, *Mykosen*, 20 (1976) 167–177.
27 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 244 (1969) 1052–1059.
28 E. REISS, D. G. PATTERSON, L. W. YERT, J. S. HOLLER, AND B. K. IBRAHIM, *Biomed. Mass Spectrom.*, 8 (1981) 252–255.
29 H. KOBAYASHI, unpublished data.
30 Y. MIYAKAWA, K. KAGAYA, Y. FUKAZAWA, AND G. SOE, *J. Clin. Microbiol.*, 23 (1986) 881–886.
31 C. ANTALIS, S. FOGEL, AND C. E. BALLOU, *J. Biol. Chem.*, 248 (1973) 4655–4659.