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CHARACTERISATION OF MANNOPROTEINS FROM YEAST AND MYCELIAL FORMS OF *Mucor rouxii*

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ABSTRACT

Mannoprotein was isolated from yeast (Y) and mycelial (M) cells of *Mucor rouxii* by affinity chromatography on concanavalin A Sepharose. Mannoprotein-Y contained only mannose, whereas mannoprotein-M contained mannose and a small proportion of fucose. Each mannoprotein contained ~50% of protein. The main carbohydrate products of β-elimination reactions of the mannoproteins were α-(1→2)-linked mannobiose (24% from Y, 15% from M) and polysaccharide material (57% from Y, 79% from M). Polysaccharide-Y consisted of α-(1→2)-linked, 3,6-di-*O*-substituted, and non-reducing terminal mannopyranosyl residues, whereas polysaccharide-M contained, in addition, fucopyranosyl non-reducing end-groups and α-(1→6)-linked mannopyranosyl residues. Acetolysis of polysaccharide-Y gave mainly mannotetraose, mannotriose, and mannobiose, whereas polysaccharide-M gave mainly mannose.

INTRODUCTION

The structure of the carbohydrate component of cell-surface glycoproteins varies with the stage of cell differentiation or of morphogenesis[1–3]. The morphogenesis of micro-organisms is also strongly influenced by changes in the external environment. When the dimorphic fungus *Mucor rouxii* is incubated under air or nitrogen, it develops the mycelium (M) form, but, under CO_2, it develops the yeast (Y) form[4–5]. A striking difference of the cell-wall composition is the mannose content[6], which is about 6 times higher in the Y than in the M form. However, the structure of the carbohydrate component of the mannose-containing polymer(s) from *M. rouxii* has not been examined, except for mucoran, which is a fucoglucuronomannan[7] containing (1→3)-linked mannopyranosyl residues. The

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mannosyl transferase activity of this organism increases in both mycelium and yeast, which corresponds to the emergence of germ tubes and buds, and it has been suggested that mannoprotein is synthesised during morphogenesis[5].

We now report a partial characterisation of the carbohydrate components of mannoproteins Y and M of *M. rouxii*.

EXPERIMENTAL

Growth of M. rouxii. — *M. rouxii* ATCC-24905 was grown in a YPG medium by the method of Bartnicki-Garcia and Nickerson[6]; ~6×10^7 spores were inoculated per 100 mL of medium. The cultures were incubated at 28°, using a 10-L fermenter (New Brunswick Scientific Co.). To obtain the aerobic form, cultures were incubated under air for 12 h before arthrospora formation. To prepare the yeast-like form, stationary-growth cultures were incubated under CO_2 for 46 h. Cells were harvested by centrifugation or collection on sintered glass, washed extensively with water, lyophilised, and stored at −20°.

Analytical procedures — Total carbohydrate was determined by the phenol–sulfuric acid method[9], protein by the method of Lowry *et al.*[10], uronic acid by the method of Bitter–Muir[11], and phosphorus by the method of Chen *et al.*[12], using, as standards, mannose, bovine serum albumin, glucuronic acid, and inorganic phosphate, respectively.

Chromatography. — T.l.c. was carried out on cellulose (Merck) with ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars were detected with alkaline silver nitrate[13], amino sugars and amino acids with ninhydrin[14], and uronic acid with *p*-anisidine hydrochloride[15]. Hydrolysates (M trifluoroacetic acid, 105°, 4 h) of polysaccharides (500 μg) were reduced with $NaBH_4$ for 5 h at 23° and then neutralised, and boric acid was removed conventionally as methyl borate. The residue was treated with pyridine–acetic anhydride (1:1) at 100° for 1 h. G.l.c. was performed at 180–250° with a Shimadzu GC-6A instrument equipped with a flame-ionisation detector, a glass column (0.3 × 200 cm) of 3% of OV-225 on Gas Chrom Q, and a nitrogen flow-rate of 50 mL/min. Affinity chromatography of mannoproteins on con-A Sepharose was carried out by the method of Hayashi *et al.*[16].

Extraction of the mannose-containing polymers. — Lyophilised Y and M cells were freed from lipid by using boiling acetone, washed with acetone and ether, and dried *in vacuo*. The cells were then extracted twice with 30 vol. of 20mM citrate buffer (pH 7.0) at 125° by autoclave for 90 min, and each extract was filtered through sintered glass (G-4), dialysed against running water, and lyophilised, to give the crude extracts (7.6% from Y cells, 16.8% from M cells). Extract Y contained 12.8% of mannose, and extract M 2.5%.

Methylation analysis. — Methylation was effected by the Hakomori procedure[17], and the completeness of methylation was checked by using triphenylmethane[18]. The methylated polysaccharides were dialysed against water, remethylated, and purified[19] on a column (1 × 20 cm) of Sephadex LH-20. Methylated

oligosaccharides were extracted with chloroform and purified similarly. Each methylated product was hydrolysed, and the monosaccharide products were reduced with sodium borohydride and acetylated[20]. The resulting alditol derivatives were subjected to g.l.c.–m.s., using a Shimadzu LKB-9000 mass spectrometer equipped with a glass column packed with 3% of OV-225 on Chromosorb W, and operated at 170°; electron energy, 70 eV; trap current, 60 μA; ion-source temperature, 310°.

β-Elimination reactions. — β-Elimination of mannoproteins was carried out by the method of Nakajima and Ballou[21]. The procedure described by Tanaka and Pigman[22] was employed to investigate the involvement of serine and threonine in the carbohydrate–peptide linkage. Mannoprotein-Y (1 mg) was incubated with 0.3M $NaBH_4$ in 0.1M NaOH (1 mL) in the dark for 216 h at 4° in an atmosphere of N_2. The reaction was terminated by adjusting the pH to 5.3 with M acetic acid, and the solution was then neutralised with 0.1M NaOH and concentrated to dryness. Boric acid was removed from the residue by repeated evaporation of methanol therefrom. The residue was then dissolved in water (2 mL), and 1-octanol (1 drop), 0.08M $PdCl_2$ (2.1 mL), and 0.3M $NaBH_4$ in 0.1M NaOH (0.8 mL) were added with vigorous stirring. Reaction was allowed to proceed for 1 h at 28° in a closed tube, and then terminated and worked-up as described above. The sample was treated with constant-boiling HCl (2 mL) in a sealed, evacuated tube for 22 h at 110°, and the amino acids were quantified by using an Hitachi 835 amino acid analyser.

Acetolysis. — The method of Kocourek and Ballou[23] was used. Polysaccharides (30 mg) were treated with anhydrous pyridine (2 mL) and acetic anhydride (2 mL) at ~100° (steam bath) for 8 h, and the solvent was evaporated at 50°. The syrupy residue was treated with acetic anhydride (1 mL) and a mixture of acetic acid (1 mL) and conc. sulfuric acid (100 μL) for 12 h at 40°. The mixture was neutralised with pyridine (500 μL) and concentrated at 50°. The residue was partitioned in water–chloroform (1:1, 100 mL), the chloroform layer was separated, and the aqueous layer was extracted several times with chloroform until a negative reaction to phenol–sulfuric acid[9] was obtained. The combined chloroform solutions were washed with water, dried (Na_2SO_4), and concentrated to dryness. The amorphous residue was dried by azeotropic distillation of benzene therefrom, dissolved in dry methanol (2 mL), and treated dropwise with methanolic 0.5M sodium methoxide until precipitation was complete. After 20 min at room temperature, the mixture was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated to dryness *in vacuo*. A solution of the residue in water (1 mL) was applied to a column (2 × 150 cm) of Bio-Gel P-2.

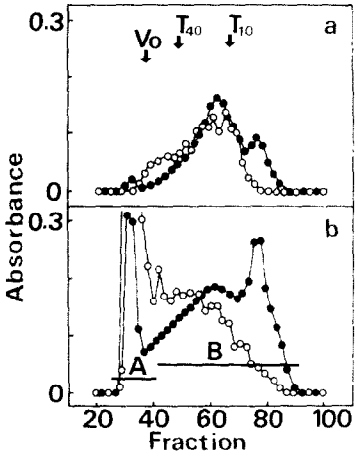
^{1}H-N.m.r. spectroscopy. — Spectra were obtained for 0.5% solutions in D_2O at 100 MHz at 80°, using an instrument equipped with a JEOL computer operated in the Fourier-transform mode. Chemical shifts were expressed relative to that of sodium 3-(trimethylsilyl)propane-1-sulfonate (TSP).

Materials. — Concanavalin A (con-A) Sepharose, Sephadex G-150, and DEAE-Sephadex A-50 were obtained from Pharmacia, Bio-Gel (-400 mesh) and

Dowex AG 50W-X8 from Bio-Rad, and Pronase from Kaken Kagaku Co. (Japan). Mycelial and extracellular polysaccharide (ACI & ACE) from *A. cylindrospora* was prepared by the published procedure[24,25]. (1→6)-Linked-α-D-mannan was prepared[26] by partial, acid hydrolysis of ACE. Fresh, whole cells of baker's yeast were supplied by the Oriental Yeast Co. Ltd. (Tokyo), and the preparation of the bulk mannan was carried out by a modification[27] of the method of Peat *et al.*[28].

RESULTS

Purification of mannoproteins Y and M. — The Y and M extracts were purified by affinity chromatography on a con-A Sepharose column, and all of the mannose-containing polymers (Y-2 and M-2) were recovered by elution with methyl α-D-mannopyranoside. Each mannose-containing polymer was further purified by gel filtration on Sephadex G-150, as shown in Fig. 1. Gel filtration of Y-2 gave a disperse peak of carbohydrate at an estimated mol. wt. of 10,000–25,000 (Fig. 1a), but M-2 was separated into a component of high molecular weight emerging in the void volume (tubes 28–38, Fraction A) and a component exhibiting a broad profile of lower molecular weights (tubes 42–70, fraction B), as shown in Fig. 1b. The carbohydrate component of 2A was mainly glucose, and that of Y-2 and M-2B was mainly mannose. Thus, M-2A was a glucan, and Y-2 and M-2B were mannans or mannoheteroglycans having mol. wt. of 10,000–30,000. These man-



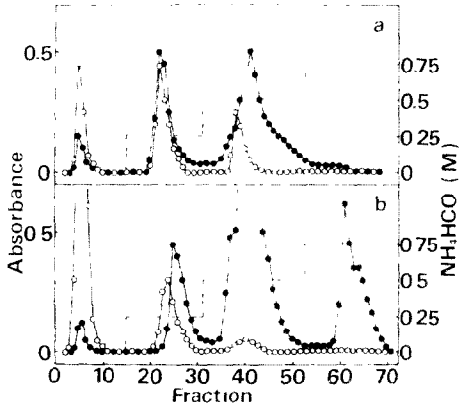


Fig. 1 Gel filtration of (a) Y-2 and (b) M-2 on Sephadex G-150. Carbohydrate, 490 nm (—○—), and protein, 280 nm (—●—), Vo, blue dextran; T_{40} dextran, mol. wt. 40,000, T_{10} dextran mol. wt. 10,000.

Fig. 2 Chromatography on DEAE Sephadex A-25 (HCO_3^- form) of (a) Y-2 and (b) M-2B. Stepwise elution was carried out with water, 0.25, 0.5, 0.75, and 1.0M NH_4HCO_3. The same elution profile was obtained by a linear-gradient elution of 0→M NH_4HCO_3. Carbohydrate, 490 nm (—○—), protein, 280 nm (—●—). For the structure analysis of both mannoproteins, each sample was rechromatographed on the same column.

TABLE I

YIELDS AND CHEMICAL COMPOSITIONS OF Y-2 AND M-2B SUB-FRACTIONS[a] OBTAINED BY ION-EXCHANGE CHROMATOGRAPHY ON DEAE-SEPHADEX A-25 (HCO_3^- FORM)

Percentage	*Y-2*			*M-2B*		
	a	*b*	*c*	*a*	*b*	*c*
Yield[b] (%)	20.7	45.9	30.7	23.8	14.4	24.8
Protein (%)	21.4	47.5	51.4	33.7	52.9	57.7
Carbohydrate (%)	60.2	48.9	43.9	72 3	48.6	35.8
Mannose[c]	51.9	48.9	32.3	63.0	45.0	6.2
Fucose[c]	0	0	trace	9.3	3.4	3.1
Uronic acid[d] (%)	2.8	2.8	11.6	4.2	2.8	26.4
Inorganic phosphorus (%)	0.2	0 2	0.1	0	0.1	0

[a]*a*, Neutral; *b*, weakly acidic; *c*, strongly acidic. [b]Based on weights of sub-fractions recovered from the chromatography of each mannoprotein on DEAE-Sephadex A-25. [c]Molar ratios determined by g.l.c. of hydrolysates as their alditol acetates. [d]No uronic acid was detected in the acid hydrolysates of the neutral (a) and mannoprotein fractions (b) by t.l.c. There may be interference by the large proportions of neutral hexose in these fractions.

nose-containing polymers were further fractionated into several components by ion-exchange chromatography on DEAE-Sephadex (HCO_3^- form) (Fig. 2). Acidic and neutral components of each mannoprotein were separated by this procedure. The acidic material retained on the column was divided into two fractions by stepwise elution with 0.25 and 0.5M NH_4HCO_3, but no significant amount of carbohydrate-containing polymer was eluted with M NH_4HCO_3. The composition of the neutral (Y-2a or M-2Ba), weakly acidic (Y-2b or M-2Bb), and strongly acidic fractions (Y-2c or M-2Bc) are summarised in Table I. The neutral fractions were fucomannans, and the weakly acidic fractions Y-2b and M-2Bb were mannoprotein and a small proportion of fucose-containing mannoprotein. The strongly acidic fraction was fucoglucuronomannan.

After treatment of mannoproteins Y-2 and M-2B with Pronase, the carbohydrate-containing materials were eluted from columns of Sephadex G-150 in the volume of lower molecular weight than Y-2 and M-2B. It was concluded that these mannans were covalently bound to protein in a glycoprotein or glycopeptide. However, no evidence was obtained as to whether the fucomannan and fucoglucuronomannan were glycoproteins. Traces of uronic acid were detected[11] in Y-2a, Y-2b, M-2Ba, and M-2Bb, but could not be detected by t.l.c. after acid hydrolysis. Extract Y contained larger amounts of mannoprotein and fucoglucuronomannan than did extract M. The latter polymer was similar to mucoran[7].

Methylation analysis of mannoproteins Y and M. — Each mannoprotein was methylated and hydrolysed, and the products were converted into the alditol acetates, each of which was identified by g.l.c.–m.s. (Fig. 3 and Table II). For mannoprotein-Y, peaks II and III were predominant, and were characterised as 2,3,4,6-tetra-*O*-methyl and 3,4,6-tri-*O*-methyl derivatives of mannitol, respectively

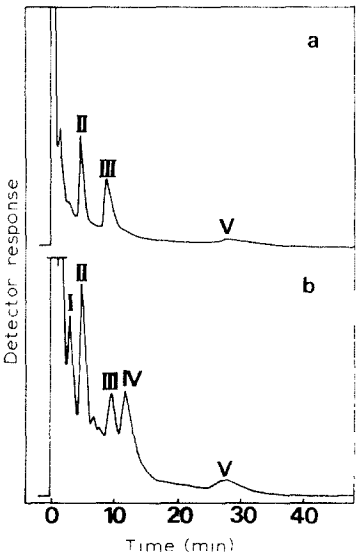


Fig. 3 G.l.c. of the partially methylated alditol acetates from mannoproteins Y (a) and M (b) The peaks are identified in Table II: peak I corresponds to A, II to B, III to C, IV to D, and V to E

(Fig. 3a). The predominant peaks for mannoprotein-M (Fig. 3b) were I–V, which were identified as 2,3,4-tri-*O*-methylfucitol, and 2,3,4,6-tetra-*O*-methyl, 3,4,6-tri-*O*-methyl, 2,3,4-tri-*O*-methyl, and 2,4-di-*O*-methyl derivatives of mannitol, respectively. Thus, mannoprotein-Y contained mannopyranosyl non-reducing end-groups, and 2-*O*- and 3,6-di-*O*-substituted mannopyranosyl residues in the chain,

TABLE II

IDENTIFICATION OF PARTIALLY METHYLATED ALDITOL ACETATES FROM MANNOPROTEINS Y AND M

Methylated alditol acetate derivatives	*Y (Molar ratios)*[a]	*M*	*Major mass-spectral fragments* (m/z)	*Linkages*
A 2,3,4-Tri-*O*-methylfucitol	—	0.30	43,101,115,117,131,175	Fuc-(1→
B 2,3,4,6-Tetra-*O*-methylmannitol	1.00	1.00	43,45,71,87,101,117, 129,145,161	Man-(1→
C 3,4,6-Tri-*O*-methylmannitol	1.40	0.76	43,45,87,99,129,161, 189	→2)-Man-(1→
D 2,3,4-Tri-*O*-methylmannitol	—	1.39	43,87,99,101,117,129, 161,233	→6)-Man-(1→ →3)
E 2,4-Di-*O*-methylmannitol	0.30	0.63	43,87,117,129,189	Man-(1→ →6)

[a]Calculated from peak areas and the molecular weights of the derivatives

whereas mannoprotein-M contained, additionally, fucopyranosyl non-reducing end-groups and 6-*O*-substituted mannopyranosyl residues. The methylation analysis revealed a difference in the molar ratios of tetra- and di-*O*-methyl sugars, suggesting the presence of a linear oligosaccharide of short chainlength *O*-glycosylically attached to the protein.

β-Elimination of mannoproteins Y and M, and characterisation of the products. — Each mannoprotein was treated with 0.1M NaOH at 27° for 18 h, and the products were fractionated on Bio-Gel P-2. As shown in Fig. 4, mannose (Y 14.4%, M 1.5%), mannobiose (Y 24.0%, M 15.0%), trisaccharide (Y 4.8%, M 5.0%), and polysaccharide (Y 57.0%, M 79.0%) were obtained. Treatment of the mannoproteins with alkali resulted in an increase in absorbance at 241 nm with the release of peptide. The number of seryl and threonyl residues decreased markedly following treatment of mannoprotein-Y with alkaline borohydride–$PdCl_2$. This treatment also resulted in an increase in alanine, and the appearance of α-aminobutyric acid after hydrolysis with 6M HCl. These observations confirmed the presence of *O*-glycosyl linkages in the mannoprotein.

(a) Disaccharide. The mannobiose, when methylated by the Hakomori procedure[17] and then hydrolysed, gave 2,3,4,6-tetra-*O*-methylmannose and 3,4,6-tri-*O*-methylmannose in the molar ratio of 1:1. The ^{1}H-n.m.r. spectrum of the mannobiose was identical with that of α-(1→2)-linked mannobiose obtained by acetolysis of baker's yeast mannan. The trisaccharide fractions contained only mannose, but the structure could not be elucidated because of the small quantity available.

(b) Polysaccharide. Each polysaccharide gave a single peak on elution from Sephadex G-75. Polysaccharide-Y contained only mannose, whereas polysaccharide-M contained mannose and fucose (molar ratio, 12:1). Because a significant

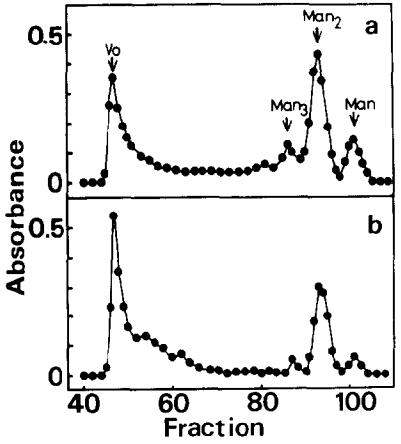


Fig. 4. Fractionation on Bio-Gel P-2 of the oligosaccharides released by β-elimination of mannoprotein Y (a) and M (b). Carbohydrate, 490 nm (—●—).

amount of protein and a trace of glucosamine were also present in each polysaccharide, it is proposed that each may be an *N*-glycosylprotein. Methylation analysis showed that polysaccharides Y and M contained the same glycosidic linkages as did mannoproteins Y and M, respectively, but the molar proportion of tetra-*O*-methyl sugar was higher for the polysaccharides than for the mannoproteins (Table III). The ^{1}H-n.m.r. spectra of polysaccharides Y and M showed several signals for anomeric protons (Fig. 5), and signals at 4.9 and 5.33 p.p.m., observed in both spectra, were assigned to

$$\begin{matrix} \rightarrow 6) \\ \quad \diagdown \\ \quad\quad \alpha\text{-Man-}(1\rightarrow \\ \quad \diagup \\ \rightarrow 3) \end{matrix} \quad \text{and} \quad \rightarrow 2)\text{-}\alpha\text{-Man-}(1\rightarrow .$$

respectively, by comparison with the spectra of fucomannan and mannoprotein from *Absidia cylindrospora*[26]. The spectrum of polysaccharide-M also showed a signal at 4.98 p.p.m. which was assigned to →6)-α-Man-(1→, in comparison with a spectrum of (1→6)-linked α-D-mannan from *A. cylindrospora*[26]. This signal was not present in the spectrum of polysaccharide-Y. The signal at 5.33 p.p.m. for polysaccharide-Y had an intensity higher than that of the corresponding signal for polysaccharide-M.

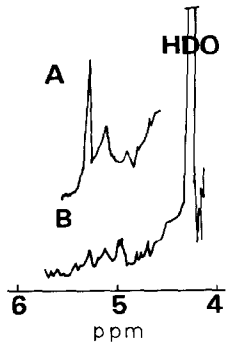
Acetolysis of polysaccharides Y and M. — On acetolysis, (1→6) linkages are cleaved much more rapidly than (1→4), (1→3), or (1→2) linkages[23,29]. Polysaccharides Y and M were severally acetolysed, and the deacetylated products were fractionated on a column of Bio-Gel P-2 (Fig. 6). The elution position of each saccharide was confirmed by t.l.c., using standard mannosaccharides. Polysaccharide Y gave mono-, di-, tri-, tetra-, penta-, hexa-, and higher oligo-saccharides. Mannose was the only product obtained on acid hydrolysis of these oligosaccharides.

TABLE III

METHYLATION ANALYSIS OF POLYSACCHARIDES Y AND M

Methylated alditol acetate derivative[a]	*Y*	*M*	*Linkages*
	(Molar ratios)[b]		
A 2,3,4-Tri-*O*-methylfucitol	—	0.74	Fuc-(1→
B 2,3,4,6-Tetra-*O*-methylmannitol	1.00	1.00	Man-(1→
C 3,4,6-Tri-*O*-methylmannitol	4.95	2.58	→2) Man-(1→
D 2,3,4-Tri-*O*-methylmannitol	—	3.32	→6)-Man-(1→
			→3)
E 2,4-Di-*O*-methylmannitol	0.72	1.67	Man-(1→
			→6)

[a]Identified by g.l.c.–m.s. [b]Calculated from the peak areas and the molecular weights of the derivatives



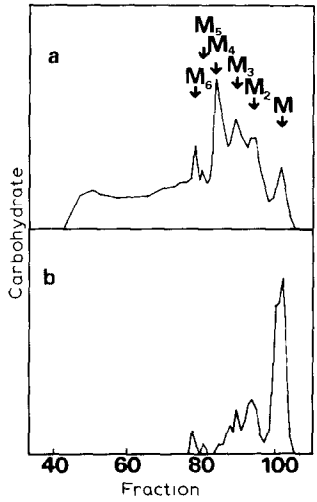


Fig. 5. ^{1}H-N.m.r. spectra of A, mannoprotein-Y; B, mannoprotein-M; HDO is the water peak.

Fig. 6. Gel filtration on Bio-Gel P-2 of the products from the partial acetolysis of mannoproteins Y (a) and M (b).

and methylation analysis showed only (1→2) linkages. Polysaccharide-Y gave mainly mannotetraose, mannotriose, and mannobiose, but polysaccharide-M gave only small proportions of these oligosaccharides, and mannose and fucose were the major degradation-products.

DISCUSSION

Dow and Rubery[30] have extracted cell wall of *M. rouxii* with EDTA, sodium hydroxide, and acetic acid, and polysaccharides of low molecular weight and glycoproteins of high molecular weight were obtained as major mannose-containing polymers. However, these polymers were not characterised. In order to ascertain the relationships between morphogenesis and the structure of the carbohydrate components of the cell-surface macromolecules, mannoprotein was isolated from yeast (Y) and mycelial (M) cells of *M. rouxii*. Extract Y contained a larger amount of mannoprotein than did extract M, which accorded with the difference between mannose content in Y and M cell-walls[6]. The mannoproteins were purified by affinity chromatography on con-A Sepharose, gel filtration, and cation-exchange chromatography. When the purified mannoprotein-Y was treated with dilute alkali, ~43% of the total carbohydrate was released, whereas 21.4% was released from mannoprotein-M. The mannoprotein of *Saccharomyces cereviseae* is composed of carbohydrate chains attached to the protein core *via* asparagine residues and by base-labile *O*-glycosylic linkages[31]. Mannoproteins of *M. rouxii* also contained such *O*-glycosylic linkages, and the structure of the major oligosac-

charide from each mannoprotein was α-(1→2)-linked mannobiose. Because β-elimination of each mannoprotein gave resistant polysaccharide that contained a trace of glucosamine, it was inferred that these polysaccharides may be *N*-glycosylically linked to the proteins. The most striking difference between β-elimination-resistant polysaccharides Y and M was their component sugars and glycosidic linkages. Polysaccharide Y contained only mannose, whereas polysaccharide-M contained mannose and fucose (molar ratio, 12:1). Polysaccharide-Y contained a large proportion of (1→2)-linked α-D-mannosyl residues, whereas polysaccharide-M contained preponderantly (1→6)-linked α-D-mannosyl residues. This difference was also shown in the structure of fucomannan[32] (neutral fraction in DEAE-Sephadex chromatography). Polysaccharide-Y was concluded to contain a (1→6)-linked α-D-mannan backbone, to all residues of which mainly (1→2)-linked α-D-mannose tetra-, tri-, and di-saccharides were attached at O-3. On the other hand, polysaccharide-M contained a (1→6)-linked α-D-mannan main-chain, to several residues of which similar mannosaccharides or (1→6)-linked α-D-mannosaccharides were attached at O-3, and had fucosyl and mannosyl non-reducing end-groups. Ballou *et al.*[33] have found a new class of mutant of *Saccharomyces cerevisiae* which appeared to lack most of the (1→6)-linked α-D-mannan outer-chain. The mannoprotein from this mutant had a much diminished content of carbohydrate and more *O*-glycosylic linkages than the wild type of mannan. This altered mannoprotein was accompanied by dramatic changes in cellular physiology, for example, distortion of the cell wall. Hamada *et al.*[34] compared the structures of the mannans from the cell-wall mutant of *Candida sp.* M-7002 and its wild type. The surface of the mutant cells was round and swollen, whereas the wild-type cells were normal, smooth, and egg-shaped. The mannan from the mutant also had a decreased content of outer chains and *N*-glycosylic linkages, as did the mannan from the mutant *S. cerevisiae*. Some of our findings are similar to these results, because the dimorphic change of *Mucor rouxii* is accompanied by a change in the structure of the carbohydrate component of the mannoprotein. Characterisation of mannose-containing polymers from a kind of *Mucorales* fungi, *Absidia cylindrospora*[24-26], was reported recently. *A. cylindrospora* develops only to the mycelium form, not the yeast form, and contains mainly fucomannan composed of (1→6)-linked α-D-mannan to which are attached single fucopyranosyl residues at O-3; the content of *O*-glycosylic linkages was negligible. This structure is similar to that of the mannan of the mycelium form of *M. rouxii*. These observations indicate that morphological change of *Mucorales* fungi is accompanied by structural change of the cell-wall mannan which may be related to the biosynthesis of high-mannose or complex type *N*-glycosylically linked glycoprotein. Most yeast cells (for example, *S. cerevisiae*[33] or *Candida albicans*[35]) contain homomannan, but most mycelial cells (for example, *Aspergillus niger*[26], *Sporthrix schenckii*[37], or *A. cylindrospora*[26]) contain such heteromannans as galactomannan, rhamnomannan, or fucomannan, suggesting that the alteration of the mannan structure may be regulated by cell morphology.

Guttierrez and Ruiz-Herrera[8] reported that the mannosyl transferase of *M.*

rouxii was not stimulated by the addition of baker's yeast mannan or mucoran, either intact or partially hydrolysed with acid, and that biosynthetic production *in vitro* by this enzyme released large amounts of *O*-glycosylically linked material. These facts strongly suggest that the mannoprotein may be synthesised by this mannosyl transferase. Furthermore, the biosynthesis of each mannoprotein may be controlled by (1→2) or (1→6) α-D-mannosyl transferase, and this may be related to the morphogenesis of *M. rouxii*.

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