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CHARACTERISATION OF FUCOMANNOPEPTIDE AND MANNOPROTEIN FROM *Absidia cylindrospora*

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ABSTRACT

Serologically active mannoprotein (MP, minor fraction) and weak or inactive fucomannopeptide (FMP, major fraction) from the mycelia of *Absidia cylindrospora* were characterised by methylation analysis and ^{1}H-n.m.r. spectroscopy. MP contained a significant proportion of (1→2)-linked α-D-mannopyranosyl residues with mannopyranosyl non-reducing terminals, whereas FMP consisted of (1→6)-linked α-D-mannopyranosyl residues. MP did not contain a significant amount of mannose branch-units; when treated with 0.1M NaOH, it was largely fragmented into mannobiose, mannotriose, mannotetraose, and higher oligosaccharides, as shown by gel-permeation chromatography. FMP released $<10\%$ of oligosaccharides on treatment by the same procedure. FMP gave a p.m.r. signal for *O*-acetyl (δ 2.2), its ^{13}C- and ^{1}H-n.m.r. data accorded with the results of the methylation analysis, and the fucopyranosyl residues were shown be α. Acetolysis of FMP gave large proportions of mannose and fucose, and small proportions of Fuc(Man)$_3$, Fuc(Man)$_2$, and Fuc-Man. Mild, acid hydrolysis of FMP released fucose and (1→6)-linked, linear α-D-mannosaccharides. Thus, FMP is composed of a (1→6)-linked α-D-mannan to which are attached single fucopyranosyl residues at O-3 of some mannosyl residues, and MP is composed of (1→2)-linked α-D-mannosaccharides which may be linked to the peptide *via O*-glycosylic linkages. Antibody-precipitating activity of MP with anti-*A. cylindrospora* serum was twice that of (1→6)-linked α-D-mannan and confirmed that the structure of the mannan moiety of FMP differs from that of serologically active MP.

INTRODUCTION

One species of *Mucorales*, *Absidia cylindrospora*, produces cell-surface, mannose-containing glycoprotein that is very heterogeneous in relation to molecular weight, affinity for concanavalin-A (con-A), acidity, and the ratio of mannose to fucose. The

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components were classified as fucomannan-type (mol. wt. 15,000–70,000) and mannan-type (mol. wt. 15,000–35,000) of which the major (90–95%) was fucomannan-type glycopeptide[1-3]. The mannan-type polymers showed higher antibody-precipitating activity than the fucomannan-type polymers against anti-*A. cylindrospora* serum[1], and both were indicated to be common antigens of the *Mucorales*[4]. A part of the immunodeterminant of this antigen appeared to be (1→6)-linked α-D-mannosaccharides larger than mannotetraose[5]. Previous results revealed that the appearance of serological reactivity of the mannose-containing polymers from *A. cylindrospora* was closely related to their heterogeneity[1]. The carbohydrate portions of these mannose-containing polymers contained mannose and fucose with molar ratios ranging from 13:1 to 2:1, and the fucomannan-type polymers having high contents of mannose showed antibody-precipitating activity[1]. However, it is not known whether MP has a homologous structure similar to that of fucomannopeptide (FMP) which has a low content of mannose.

We now report on the structures of MP and FMP.

EXPERIMENTAL

Organism. — *A. cylindrospora* IFO 4000 was obtained from the Institute for Fermentation (Osaka, Japan), and cells were cultured in modified Sabouraud's liquid medium at 27° until stationary growth[2]. The mycelium was disrupted by using a French Press in saline, and the extract was prepared as described previously[2].

Preparation of mannoprotein (MP) and fucomannopeptide (FMP). — MP and FMP were fractionated by preparative zone electrophoresis (using Pevikon) of the con-A-binding extract from the mycelia of *A. cylindrospora*, as reported previously[2]. Serologically active MP and weak or inactive FMP were further purified by column chromatography on Sephadex G-150 and DEAE-Sephadex A-50 (Cl^- form), respectively[1], and the main fractions of these polymers were used for the structural analysis. FMP was obtained as the major (90–95%), and MP as the minor (5–10%), fraction. The con-A-unbound fraction (U-FMP) was also compared (methylation analysis, ^{1}H-n.m.r. spectroscopy) with the con-A-bound polymers.

Analytical procedures. — Total carbohydrate was determined by the phenol–sulfuric acid method[6] and fucose by the cysteine–sulfuric acid method[7], with mannose and fucose as the respective standards. Protein was assayed by the method of Lowry *et al.*[8] with bovine serum albumin as the standard.

Chromatography. — T.l.c. was performed on cellulose (Merck) with ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars were detected with alkaline silver nitrate[9], amino sugar and amino acids with ninhydrin[10], and uronic acid with *p*-anisidine hydrochloride[11]. Ascending p.c. on Toyo filter paper No. 50 was performed with the above solvent. Hydrolysates of polysaccharides (500 μg) obtained using M trifluoroacetic acid (at 105° for 4 h) were reduced with $NaBH_4$ for 5 h at 23° and then neutralised, and borate ions were 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, and a glass column (0.3 × 200 cm) of 3% of OV-225 on Gas Chrom Q, with a nitrogen flow-rate of 50 mL/min.

Methylation analysis. — FMP, U-FMP, and MP were methylated by the Hakomori procedure[12], and the completeness of methylation was checked by using triphenylmethane as an indicator of the remaining carbanion[13]. Acid hydrolysates of the fully methylated polysaccharides were converted into alditol acetates[14]. G.l.c.–m.s. was performed on a Shimadzu-LKB-9000 instrument equipped with a glass column packed with 3% of Silicone OV-225 on Chromosorb W, operated at 170°; electron energy, 70 eV; trap current, 60 μA; temperature of the ion source, 310°.

Acetolysis of FMP. — The modified procedure of Kocourek and Ballou[15] was used. A suspension of FMP (20 mg) in anhydrous formamide (500 μL) was kept at ~100° until dissolution occurred. Pyridine (1 mL) and acetic anhydride (1 mL) were then added to the cooled solution which was kept for 12 h at 40°. The mixture was concentrated at 50°, and the syrupy residue was treated with acetic anhydride (1 mL) and a mixture of acetic acid (1 mL) and conc. sulfuric acid (100 μL). The solution was kept for 6 or 12 h at 40°, neutralised with pyridine (500 μL), and concentrated at 50°. The residue was dissolved 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[6] was obtained. The chloroform solutions were combined, washed with water, dried (Na_2SO_4), and concentrated to dryness. The amorphous residue was dried by azeotropic distillation of benzene, 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.

Partial, acid hydrolysis of FMP. — This was effected with 10mM HCl at 96° for 3 h. The mixture was neutralised with 10mM NaOH and then concentrated to dryness. The resulting, partially defucosylated FMP was further hydrolysed with 50mM H_2SO_4 at 96° for 2 h and neutralised with $BaCO_3$.

N.m.r. spectroscopy. — ^{1}H-Spectra were obtained for 0.5% solutions in D_2O at 100 MHz and 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). ^{13}C-Spectra were obtained at 25 MHz and room temperature, using a JEOL-FX 100 spectrometer operated in the Fourier-transform mode with complete proton-decoupling, and the field was locked on the deuterium signal. Chemical shifts were expressed in p.p.m. from the signal of methanol.

Immunological methods. — Anti-*A. cylindrospora* serum was prepared[2] by immunising rabbits with ACE. Quantitative precipitin reactions were carried out as follows. Anti-serum (0.1 mL) was mixed with saline solutions (0.4 mL) containing serial amounts of polysaccharides. The mixtures were stored at 37° for 1 h and then

at 4° for 72 h. Each precipitate was collected by centrifugation at 3,500 r.p.m. at 4° for 30 min, and a solution of each washed precipitate in aqueous 1% Na_2CO_3 was assayed[8] for protein with bovine gamma globulin as standard.

β-Elimination of MP and FMP. — Essentially the method of Nakajima and Ballou[16] was used.

Chemicals. — Extracellular polysaccharide (ACE) from *A. cylindrospora* was prepared by the published procedure[2,3,5]. The fresh, whole cells of baker's yeast were supplied by the Oriental Yeast Co. Ltd. (Tokyo, Japan) and the preparation of the bulk mannan was carried out by a modification[17] of the method of Okubo *et al.*[18]. (1→6)-Linked α-D-mannan was prepared[5] by partial hydrolysis of ACE with acid and purified by using a column of Sephadex G-75. Concanavalin-A Sepharose, DEAE-Sephadex A-50, and Sephadex G-75, G-150, and G-200 were obtained from Pharmacia Co. Ltd, and Bio-Gel P-2 (200–400 mesh) and P-2 (−400 mesh) from Bio-Rad. Pevikon (polyvinyl resin) was purchased from M & S Instrument (Osaka, Japan).

RESULTS

Methylation analysis of MP and FMP. — FMP and U-FMP have been classified, as fucomannan-type, and MP as mannan-type polymers[1]. Chemical properties of FMP, U-FMP, and MP are summarised in Table I. Fucomannan-type polymers contain traces of 2-amino-2-deoxyglucose. Each type of polysaccharide was methylated and hydrolysed, and the products were converted into the alditol acetates, each of which was identified by its retention time in g.l.c. and by its fragmentation pattern in m.s. (Fig. 1 and Table II). The results confirmed the classification of the mannose-containing polysaccharide of *A. cylindrospora*. For FMP, peaks I, IV, and V were

TABLE I

PROPERTIES OF FMP, U-FMP, AND MP

	Fucomannan-type		
	FMP	*U-FMP*	*MP*
Percentage of total polymer[a]	72.2	15.0	3.8
$[\alpha]_D$ (degrees)	−13.7	−18.6	+4.7
Monosaccharide constitution[b]			
Mannose	2.0	2.3	13.0
Fucose	1.0	1.0	1.0
2-Amino-2-deoxyglucose[c]	+	+	—
Percentage of total neutral sugar[d]	83.2	77.3	49.3
Percentage of total protein[e]	1.0	1.2	35.9

[a]Based on weights of materials recovered from purification procedures. [b]Molar ratio determined by g.l.c. of hydrolysate components as their alditol acetates. [c]T.l.c. and g.l.c.: +, traces; —, not detected. [d]Total carbohydrate content was calculated as the total quantity of mannose and fucose. [e]Determined by the Folin method of Lowry *et al.*[8].

TABLE II

IDENTIFICATION OF PARTIALLY METHYLATED ALDITOL ACETATES FROM FMP, U-FMP, AND MP

Methylated alditol acetate derivative	*FMP*	*U-FMP (Molar ratios)*[a]	*MP*	*Major mass-spectral fragments* (m/z)	*Linkages*
A 2,3,4-tri-*O*-methylfucitol	2.7	2.9	1.0	43, 101, 115, 117, 131, 175	Fuc-(1→
B 2,3,4,6-tetra-*O*-methylmannitol	1.0	1.0	20.0	43, 45, 71, 87, 101, 117, 129, 145, 161	Man-(1→
C 3,4,6-tri-*O*-methylmannitol	trace	trace	25.3	43, 45, 87, 99, 129, 161, 189	→2)-Man-(1→
D 2,3,4-tri-*O*-methylmannitol	8.3	7.0	3.0	43, 87, 99, 101, 117, 129, 161, 233	→6)-Man-(1→
E 2,4-di-*O*-methylmannitol	7.4	6.3	3.2	43, 87, 117, 129, 189	→3) \ Man-(1→ / →6)

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

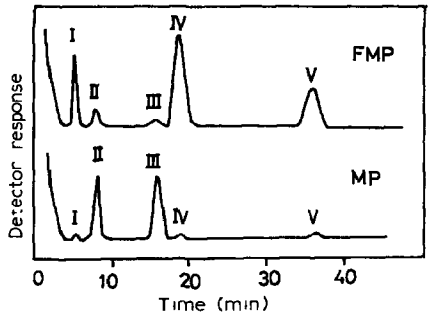


Fig. 1. G.l.c. of the partially methylated alditol acetates from FMP and MP. The peaks are identified in Table II; peak I corresponds to A, II to B, III to C, IV to D, and V to E.

predominant, and were characterised as 2,3,4-tri-*O*-methylfucitol and 2,3,4-tri-*O*-methyl- and 2,4-di-*O*-methyl-mannitol derivatives, respectively (Fig. 1a). Similar results (data not shown) were obtained for U-FMP. The predominant peaks for MP (Fig. 1b) were II and III, which were identified as 2,3,4,6-tetra-*O*- and 3,4,6-tri-*O*-methylmannitol derivatives, respectively. The fragmentation patterns agreed with these assignments (Table II). Thus, FMP contained fucopyranosyl groups and a small proportion of mannopyranosyl groups as non-reducing end-groups, and 6-, 3,6-di-, and 2-substituted mannopyranosyl residues (trace) in the chains. MP contained mannopyranosyl and a small proportion of fucopyranosyl groups as non-reducing end-groups, and 2-substituted and small proportions of 6- and 3,6-di-substituted mannopyranosyl residues in the chains. A comparison of molar ratios for the methylated sugars and the original polymers indicated that ~5% of the 2,3,4-tri-*O*-methylfucose was lost, probably because of its volatility.

^{1}H-N.m.r. spectroscopy of MP, FMP, and U-FMP. — The spectra are shown in Fig. 2. Signals in the region 4.8–5.3 p.p.m. are due to anomeric protons. The signal at 4.96 p.p.m. was due to H-1 of unsubstituted (1→6)-linked α-D-mannan which was obtained by partial, acid hydrolysis of the extracellular polysaccharide prepared from *A. cylindrospora* (Fig. 2A), and this signal appeared in the spectra of FMP (Fig. 2B and D). The signal for H-1 of 3,6-disubstituted mannopyranosyl residues has a chemical shift similar to that of unsubstituted, (1→6)-linked α-D-mannan[19-20], and it was a predominant signal in the spectra of FMP (Fig. 2B and D). These results accord with those of methylation analysis of FMP. The signals for FMP at 1.3 and 2.2 p.p.m. are characteristic of the CMe and OAc groups. The former signal is associated with fucose, and the latter disappeared after the alkaline treatment (0.1M NaOH, 22°, 16 h) of this type of polysaccharide (Fig. 2D-1). The ratio of signal intensities for CMe and OAc was 3:1. The signal at 5.08 p.p.m. (Fig. 2C) in MP may be due to H-1 of a (1→2)-linked, α-D-mannopyranosyl, non-reducing end-group because it was similar to those observed[19] in oligosaccharides. This signal was negligible for FMP. The signal at 5.14 p.p.m. for FMP may be due to H-1 of α-fucopyranosyl residues attached to O-3 of (1→6)-linked α-D-mannan. When FMP was

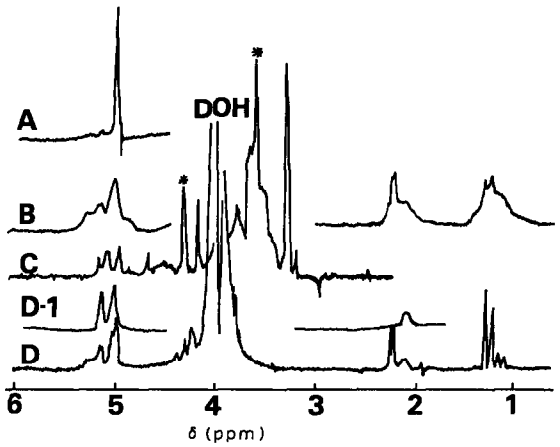


Fig. 2. ^{1}H-N.m.r. spectra of *A*, (1→6)-linked α-D-mannan; *B*, U-FMP; *C*, MP; *D-1*, FMP after treatment with dilute alkali; and *D*, FMP; *is the spinning side-band and HDO is the water peak.

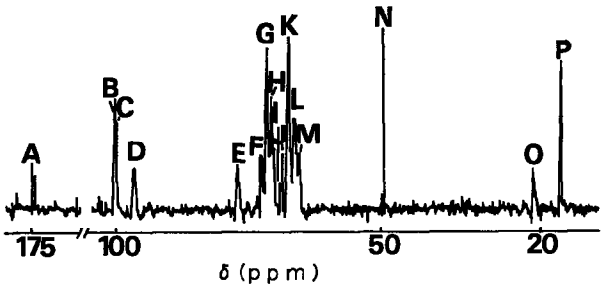


Fig. 3. ^{13}C-N.m.r. spectrum of FMP.

treated with 0.1M NaOH, the signal at 5.28 p.p.m. disappeared and that at 5.14 p.p.m. increased in intensity (Fig. 2D-1), suggesting that an OAc group may be linked to C-2 of the fucopyranosyl residue. MP also showed a signal at 4.7 p.p.m., which was not given by FMP and may be due to H-1β.

^{13}C-N.m.r. spectroscopy of FMP. — The proton-decoupled spectrum showed three resonances (Fig. 3, B–D) in the region characteristic of anomeric carbons (90–110 p.p.m.). The resonances for acetyl carbonyl and methyl carbons were present at A and O. Numerous signals (Fig. 3, E–M) in the region 65–78 p.p.m. were characteristic of C-2–C-5. The spectrum of a linear, (1→6)-linked α-D-mannotriose, obtained by partial hydrolysis of ACE with acid, was compared with that of FMP (Table III). Resonances at A, C–F, I, J, M, O, and P for FMP are not given by the trisaccharide. Signal L (66.6 p.p.m.) was assigned to C-6 of (1→6)-linked α-D-mannosyl residues, because it was downfield by ~5 p.p.m. from the signal for C-6 in methyl α-D-mannopyranoside. Signal E (77.3 p.p.m.) was assigned to C-3 of a 3,6-disubstituted α-D-mannopyranosyl residue in comparison with the chemical shift of C-3 (71.7 p.p.m.)

TABLE III

^{13}C-N.M.R. DATA

Sugar	*Chemical shift (p.p.m.)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
L-Fucopyranose	α 93.2	69.1	70.3	72.9	67.1	16.5
	β 97.3	72.7	74.0	72.4	71.7	16.5
D-Mannopyranose	α 94.7	72.1	71.2	67.8	73.3	61.9
	β 94.5	71.5	74.0	67.5	77.0	61.9
Methyl α-L-fucopyranoside[a]	100.3	68.9	70.4	72.3	66.5	16.5
Methyl α-D-mannopyranoside	101.8	70.9	71.6	67.7	73.5	61.9
The internal residue of *O*-α-D-mannopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→6)-D-mannose	100.4	70.9	71.7	67.7	71.5	66.7
Fucomannan						
6-*O*-Substituted mannopyranosyl residue	100.6 (B)	70.9 (H)	71.7 (G)	67.6 (K)	71.7 (G)	66.6 (L)
Fucopyranosyl non-reducing end-group	100.2 (C)	69.0 (J)	70.9 (H)	72.8 (F)	66.6 (L)	16.3 (D)
3,6-Di-*O*-substituted mannopyranosyl residue	100.2 (C)	67.6 (K)	77.3 (E)	65.7 (M)	71.7 (G)	66.6 (L)

[a]Values have been assigned by Tsai and Behrman[25]. The solvent was $(CD_3)_2CO$–D_2O (19:1).

in a 6-substituted α-D-mannopyranosyl residue. Signals B and C were assigned to C-1 of 6- and 3,6-di-substituted α-D-mannopyranosyl residues, because these signals are known[21] to have similar chemical shifts. Signals K and M are assigned to C-2 and C-4 of 3,6-disubstituted α-D-mannopyranosyl residues, because they are upfield from the corresponding signals for 6-substituted α-D-mannopyranosyl residues. Other signals were assigned on the basis of comparison with known compounds. Signal D (96.8 p.p.m.) may be due to C-1 of 2-*O*-acetyl-α-L-fucopyranosyl residues. These assignments (Table IV) accorded with the results of methylation analysis and the ^{1}H-n.m.r. data.

Acetolysis of FMP. — On acetolysis, (1→6) linkages are cleaved much more rapidly than (1→4), (1→3), or (1→2) linkages[15,22]. Acetolysis of FMP for 12 h gave large proportions of fucose and mannose, but negligible amounts of oligosaccharides. When acetolysis was stopped after 6 h and the products were deacetylated and fractionated on Bio-Gel P-2 (Fig. 4), peaks (IV–VI) were obtained with elution volumes intermediate to those for mannopentaose and mannotetraose, mannotetraose and mannotriose, and mannotriose and mannobiose, respectively. That

TABLE IV

SUMMARY OF THE ASSIGNMENT OF SIGNALS IN ^{13}C-N.M.R. SPECTRA OF FMP

	Chemical shifts (p.p.m.)	*Assignment*
A	174.9	Carbonyl group of acetyl units
	174.5	
B	100.6	C-1 of 6-*O*-substituted α-D-mannopyranosyl residues
C	100.2	C-1 of 3,6-di-*O*-substituted α-D-mannopyranosyl residues
		C-1 of α-L-fucopyranosyl non-reducing end-units
D	96.8	C-1 of 2-*O*-acetyl-α-L-fucopyranosyl non-reducing end-units (?)
E	77.3	C-3 of 3,6-di-*O*-substituted α-D-mannopyranosyl residues
F	72.8	C-4 of α-L-fucopyranosyl non-reducing end-units
G	71.7	C-3 and C-5 of 6-*O*-substituted α-D-mannopyranosyl residues
H	70.9	C-2 of 6-*O*-substituted α-D-mannopyranosyl residues
		C-3 of α-L-fucopyranosyl non-reducing end-units
I	69.2	Unidentified
J	69.0	C-2 of α-L-fucopyranosyl non-reducing end-units
K	67.6	C-2 of 3,6-di-*O*-substituted α-D-mannopyranosyl residues
		C-4 of 6-*O*-substituted α-D-mannopyranosyl residues
L	66.6	C-5 of α-L-fucopyranosyl non-reducing end-units
		C-6 of 6-*O*-substituted α-D-mannopyranosyl residues
M	65.7	C-4 of 3,6-di-*O*-substituted α-D-mannopyranosyl residues
N	49.0	Internal standard (MeOH)
O	21.2	Methyl group of acetyl units
P	16.3	Me-5 of α-L-fucopyranosyl residues

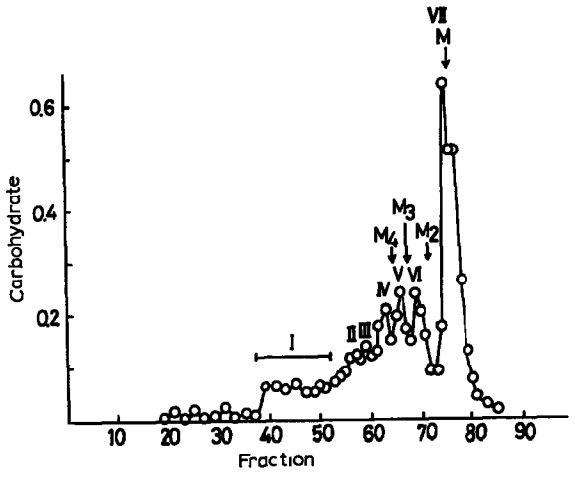


Fig. 4. Gel filtration on Bio-Gel P-2 of the products from the 6-h acetolysis of FMP.

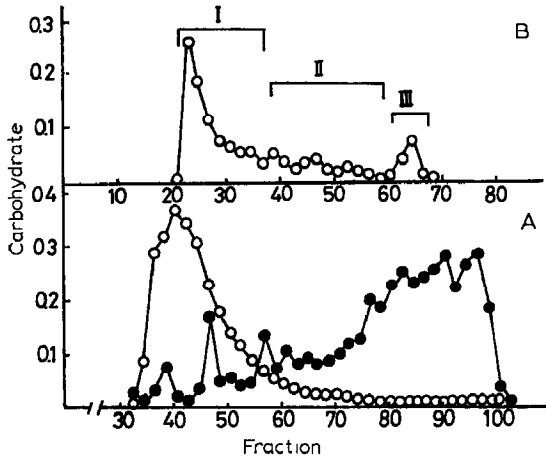


Fig. 5. Gel filtration (*A*) on a Sephadex G-100 column of FMP partially defucosylated with 10mM HCl (—○—) and the products after partial acid hydrolysis of the partially defucosylated FMP with 50mM H_2SO_4 (—●—); (*B*) of the latter products on Bio-Gel P-4. Peaks I and II contained (1→6)-linked mannosaccharides, and III contained fucose.

peaks IV–VI were due to fucose-containing oligosaccharides was suggested by the fact[23] that the presence of 6-deoxyhexose increases the elution volume by a factor of 1.5. Peaks IV–VI contained mannose and fucose, in molar ratios of 3:1, 2:1, and 1:1, respectively. Peaks II and III corresponded to pentasaccharide and hexasaccharide.

Partial, acid hydrolysis of FMP. — Treatment with 10mM HCl at 96° for 2 h released a small proportion of fucose from FMP as shown by gel filtration on Sephadex G-100, but the molecular size of the product was similar to that of FMP (data not shown). The removal of these fucosyl residues did not change the response of anti-*A. cylindrospora* serum in the quantitative precipitin reaction. The partially defucosylated FMP was further fragmentated into oligosaccharides and fucose by hydrolysis with 50mM H_2SO_4 at 96° for 2 h, as shown by gel filtration on Sephadex

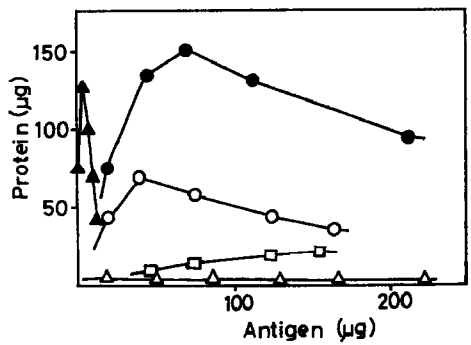


Fig. 6. Precipitin curves for *A. cylindrospora* antiserum with MP (—▲—), (1→6)-linked α-D-mannan (—○—), FMP (—□—), baker's yeast mannan (—△—), and the mixture (—●—) of MP, FMP, and serologically active FMP (see ref. 1).

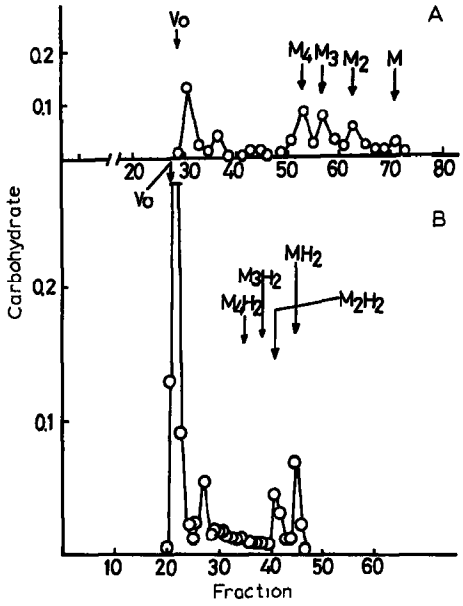


Fig. 7. Fractionation on Bio-Gel P-2 of *A*, the oligosaccharides released on treatment (*β*-elimination) of MP with 0.1M NaOH at 22° for 16 h; and *B*, after treatment of FMP with 0.1M NaOH containing borohydride and isolation of the material of low molecular weight on Sephadex G-100. Because the same column was not used for both experiments, the elution positions cannot be compared.

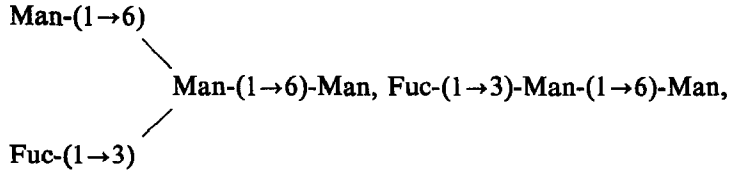
G-100 (2 × 100 cm) and Bio-Gel P-4 (2 × 150 cm) (Figs. 5A and B). These oligosaccharides contained mannose, and 2,3,4,6-tetra-*O*-methyl- and 2,3,4-tri-*O*-methylmannose were formed on methylation analysis, indicating that they were (1→6)-linked.

Immunochemical studies. — When ACE, which reacted with anti-*A. cylindrospora* serum, was hydrolysed with 50mM H_2SO_4 at 96° for 4 h, linear (1→6)-linked α-D-mannosaccharides, fucose, and mannose were obtained by gel filtration on Sephadex G-100 and Bio-Gel P-2 (data not shown). As shown in Fig. 6, the antibody-precipitating activity against anti-*A. cylindrospora* serum of MP was twice that of the (1→6)-linked α-D-mannan.

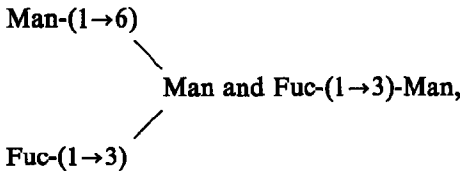
β-Elimination of MP and FMP. — Methylation analysis suggested MP to contain short, linear oligosaccharides; in order to determine if these were *O*-glycosylically linked to protein, MP was treated with 0.1M NaOH at 22° for 16 h and the products were fractionated on Bio-Gel P-2. As shown in Fig. 7A, mannobiose, mannotriose, mannotetraose, and higher oligosaccharides were obtained. FMP was also treated with 0.1M NaOH containing $NaBH_4$ at 22° for 16 h, and the products were fractionated on Sephadex G-100. The carbohydrate-containing material that was eluted in the bed volume was further fractionated on Bio-Gel P-2. As shown in Fig. 7B, a large proportion of carbohydrate-containing material was eluted in the void volume and small amounts (<10%) of mannosyl-mannitol and mannitol were obtained.

DISCUSSION

We have shown that mannose-containing polymers from the mycelia of *A. cylindrospora* are very heterogeneous and can be classified into mannoprotein (MP) and fucomannopeptide (FMP). The heterogeneity of FMP, the major component, was reflected in the ratio of fucose to mannose. FMP having a high content of mannose had antibody-precipitating activity, but this was less than that of MP. The question arises as to whether the serological reactivity depends on the degree of fucosylation of the same mannan moiety. Therefore, we have investigated the structures of FMP, which has weak or negligible serological activity and has a fucose–mannose ratio of 1:2, and MP, which is serologically active and has a fucose–mannose of 1:13. Methylation analysis and n.m.r. spectroscopy showed that FMP contained (1→6)-linked α-D-mannopyranosyl residues in the main chain and that most of the non-reducing end-groups were fucosyl groups, whereas MP contained (1→2)-linked α-D-mannosyl residues in the main chain and mannosyl groups as the non-reducing end-groups. When MP was treated with dilute alkali to effect β-elimination, the majority of the carbohydrates were released as oligosaccharides. Because di-*O*-methylmannose was not detected after methylation analysis of MP, it is suggested that MP contained (1→2)-linked α-D-mannose di- and oligo-saccharides *O*-glycosylically attached to the peptide moiety. The fraction eluted in the void volume appeared to be a β-elimination-resistant polymer, such as the *N*-glycosylmannoprotein of *Saccharomyces cerevisiae*[24], but the possibility of contamination of FMP cannot be excluded, because trace amounts of 2,3,4-tri-*O*-methylfucose and 2,3,4-tri-*O*-methyl- and 2,4-di-*O*-methyl-mannose were detected after methylation analysis of MP. Further studies of MP were not possible, because of the small quantity available. Treatment of FMP with mild alkali released traces of mannobiose and mannose, but probably did not appreciably degrade the main chain. Fucopyranosyl residues confer a negative $[\alpha]_D$ to FMP (Table I) which suggests that the anomeric linkage is α. The presence of non-reducing fucopyranosyl end-groups is shown by the formation of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol on methylation analysis. The mannan moiety of FMP consisted of (1→6)- and (1→3,1→6)-linked mannopyranosyl residues, because 2,3,4-tri-*O*-methyl- and 2,4-di-*O*-methyl-mannose were detected after methylation and hydrolysis. When FMP was subjected for 12 h to acetolysis, which readily cleaves (1→6) linkages, fucose and mannose were formed, but only a negligible amount of oligosaccharide was detected by gel filtration. Acetolysis for 6 h followed by deacetylation and chromatography on Bio-Gel P-2 revealed fucose, large proportions of mannose, and small proportions of oligosaccharides which are suggested to be



and/or



since each contained one fucose residue, the fucose–mannose ratio of FMP was 1:2, and di-*O*-methylfucose was not formed during methylation analysis of FMP. Thus, the structure of FMP is considered to involve a (1→6)-linked α-D-mannan, to some of the residues of which single fucopyranosyl residues are attached at position 3. Hydrolysis of FMP with 10mM HCl released a small proportion of fucose, but did not appreciably degrade the main mannan chain. Further hydrolysis with 50mM H_2SO_4 cleaved the remaining fucopyranosyl residues and gave a series α-D-mannosaccharides that were (1→6)-linked, because 2,3,4,6-tetra-*O*-methylmannose and 2,3,4-tri-*O*-methylmannose were the only products of methylation analysis. These results suggested that the (1→6)-linked α-D-mannan main-chain was partially hydrolysed to oligosaccharides after removal of fucopyranosyl residues from FMP. These data were consistent with the results of acetolysis of FMP. The structure inferred is similar to that of the rhamnomannan from *Sporothrix schenckii*, which involves a (1→6)-linked α-D-mannan to some residues of which are attached a single rhamnopyranosyl residue at position 3. Mendonça *et al.*[21] obtained a polysaccharide from *S. schenckii* which comprised α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl side-chains attached to a (1→6)-linked α-D-mannan main-chain. The methylation and ^{1}H-n.m.r. studies showed that the structures of con-A–unbound FMP (U-FMP) and FMP were similar. The ratios of the integrated intensities of the CMe and OAc n.m.r. signals suggested that ~33% of the fucosyl residues in FMP were acetylated, probably at O-2, whereas the proportion for U-FMP was ~100%. Previous results suggested that the immunodeterminant group of the mannose-containing polymer from *A. cylindrospora* contained (1→6)-linked α-D-mannosaccharides higher than mannopentaose[5]. MP had higher antibody-precipitating activity than FMP against antiserum to *A. cylindrospora*, but (1→6)-linked α-D-mannan prepared from ACE also reacted with this serum. The defucosylated FMP showed no precipitin activity. However, it is not known whether the fucosyl residues are cleaved from the non-reducing end-groups of the mannan main-chain in FMP or from the interior of the chains. In the latter case, FMP may not be changed to a serologically active polymer. Our results suggest that the mannose-containing polymer of *A. cylindrospora* contains two kinds of antigenic determinant and further studies on this aspect are now in progress.

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