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Structural variability in the glucuronoxylomannan of *Cryptococcus neoformans* serotype A isolates determined by ^{13}C NMR spectroscopy *

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ABSTRACT

Cryptococcus neoformans, the etiologic agent of cryptococcal meningoencephalitis, produces glucuronoxylomannan (GXM) as the major capsule component. Purified GXMs obtained from eight serotype A isolates of *C. neoformans* were treated by ultrasonic irradiation and then *O*-deacetylated prior to their comprehensive chemical analysis by GLC, GLC–MS, and ^{13}C NMR spectroscopy. The average xylose : mannose : glucuronic acid molar ratio of the eight isolates is $1.96 \pm 0.25 : 3.00 : 0.58 \pm 0.10$. Methylation analyses and ^{13}C NMR spectroscopy show a general structure for GXM that is comprised of a linear (1 → 3)-α-D-mannopyranan substituted with β-D-Glc*p*A and with β-D-Xyl*p* at O-2. Variable quantities of unsubstituted (1 → 3)-α-D-Man*p* were observed between the eight isolates studied. In several isolates some of the (1 → 3)-α-D-Man*p* residues are disubstituted with β-D-Glc*p*A at O-2 and with β-D-Xyl*p* at O-4; this type of substitution was not previously thought to occur in serotype A isolates. Heterogeneity, between isolates, in the disposition of the substituents along the mannopyranan backbone was revealed by ^{13}C NMR spectroscopy. The eight isolates, and three isolates previously studied, were each assigned to one of four distinct groups based on the ^{13}C NMR chemical shifts of the anomeric carbons. Six of the eleven isolates gave identical spectra (Group I). The six major anomeric resonances from Group I were assigned to specific glycosidic linkages present in GXM. The remaining five isolates gave more complex spectra that are indicative of additional linkages and comprise the remaining three groups. Three of these five isolates contain substantial amounts of linkages previously thought to be distinctive of serotypes B and C, i.e., Man*p* residues that are 4-*O*-glycosylated with β-D-Xyl*p*. Methylation analyses only predicted an average repeating unit, whereas ^{13}C NMR spectroscopy demonstrated that GXM from each isolate may be categorized into four groups by the occurrence of distinct sequences of carbohydrate residues.

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INTRODUCTION

Cryptococcus neoformans has emerged as a major opportunistic pathogen in patients diagnosed with AIDS[1]. It is the cause of the fourth most common life-threatening infection in AIDS after *Pneumocystis carinii, Cytomegalovirus*, and *Mycobacterium avium-M. intercellulare*[2]. Up to 10% of the patients diagnosed with AIDS are also suffering from cryptococcosis[3].

C. neoformans was originally divided into three and later into four (A, B, C, and D) serotypes by agglutination reactions with whole yeast cells[4,5]. The occurrence of a fifth type, serotype A-D, has been described more recently[6]. Additionally, *C. neoformans* is subdivided into two varieties, *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gatti* (serotypes B and C)[7].

The cell envelope of *C. neoformans* is composed of a rigid cell wall, constituted mainly of glucans[8]; a capsular polysaccharide, glucuronoxylomannan (GXM), composed of mannose (Man), xylose (Xyl), glucuronic acid (GlcA), and *O*-acetyl[9–13], and at least two minor carbohydrate antigens, galactoxylomannan (GalXM) and mannoprotein (MP)[14,15]. GXM, GalXM, and MP are isolated from growth medium by selective precipitation with ethanol and differential complexation with hexadecyltrimethylammonium bromide[12,15,16].

The antigenic basis for serotype specificity is a set of structurally related capsular polysaccharides (GXM)[12–14]. The inference that the capsular polysaccharide governs serotype specificity was deduced from the observation that acapsular mutants are untypeable[17,18]. Monocloncal antibodies, produced in response to pure GXM or its conjugate, react specifically with GXM in dot-enzyme immunoassay and enzyme immunoassay[19–21]. These data substantiate the role GXM plays in conferring serotype to a particular *C. neoformans* isolate. However, cross-reactivity of these monoclonal antibodies with heterologous purified GXM[19,20] or whole cells[21] indicates that some antigenic epitopes on GXM may be shared among strains of each serotype.

Current models of GXM depict a general structure consisting of a linear (1 → 3)-α-D-mannopyranan bearing β-D-xylopyranosyl (Xyl*p*), β-D-glucopyranosyluronic acid (Glc*p*A), and 6-*O*-acetyl substituents[9–11]. A simple structural relationship between the model polysaccharides of the four serotypes exists. They are all comprised of a core repeating unit,

to which (1 → 2)-linked β-D-Xyl*p* and (1 → 4)-linked β-D-Xyl*p* units are added in increments of one to four residues. Serotypes A and D GXM are mainly substituted at O-2, whereas serotypes B and C GXM are substituted with Xyl*p* at O-2 and at O-4. By GLC and GLC–MS, precise molar ratios of Xyl:Man:GlcA of

serotypes D, A, B, and C were originally determined to be 1:3:1, 2:3:1, 3:3:1, and 4:3:1, respectively[9]. Additional analytical data[11,16], obtained recently from the study of a larger number of isolates of each serotype, show that the precise molar ratio and substitution patterns as proposed in the original models of GXM structure are an oversimplification except in the case of serotype B[22]. In addition, substituent dispositions previously thought to be characteristic of one serotype have been identified in heterologous isolates[11,23,24].

Herein we present a comprehensive evaluation of the structure of GXM from eight isolates of *C. neoformans* serotype A. Preliminary ^{13}C NMR studies have indicated that extensive structural heterogeneity exists between serotype A isolates[25]. The present study shows that serotype A isolates can be divided into four distinct groups based on ^{13}C NMR analyses.

EXPERIMENTAL

Native and modified O-*acetyl*-D-*glucuronoxylomannan (GXM).*—One of the authors (K.J.K.-C.) provided the crude polysaccharide from the following *C. neoformans* NIH isolates: 201, 196, 68, and 371–3 that were obtained from cells cultured in Sabouraud dextrose broth[26]. Another author (E.R.) provided the crude polysaccharide obtained from the following *C. neoformans* isolates: 9104, NIH 355, NIH 271, and 9759 from cells that were cultured in a chemically defined medium[12]. The GXM from each isolate was isolated and purified as previously described[15]. The mol wt of each purified GXM was reduced by ultrasonic irradiation (GXM-S), and then a portion of each GXM-S was chemically *O*-deacetylated (GXM-D) as previously described[16]. Xylomannan (XM) was obtained from GXM by selective cleavage of Glc*p*A with lithium metal as previously described[27,28].

Analytical methods.—(The experimental details of the following methods are either described or appropriately cited in refs. 16 and 22.) Uronic acid was determined by the method of Blumenkrantz and Asboe-Hansen[29]. *O*-Acetyl content was quantitated by the procedure of Hestrin[30] using D-mannitol hexaacetate as the standard. GXM-S was analyzed by ion-exchange column chromatography using DEAE Sepharose CL-6B (Pharmacia) and a linear elution gradient of 0.01 M Na_2HPO_4 to 0.01 M Na_2HPO_4–01.0 M NaCl, pH 7.1. The constituent monosaccharides of GXM-S were obtained by acid-catalyzed hydrolysis of the polysaccharide in 2 M trifluoroacetic acid for 1 h at 120°. Sugars were identified and quantified as their per-*O*-acetylated aldononitrile (PAAN) derivatives by GLC on a RSL-300 (Alltech) capillary column. Per-*O*-methylation of GXM-S was done by the method of Hakomori[31] as modified by Darvill et al.[32]. GLC–MS analyses of per-*O*-methyl PAAN derivatives were done with a capillary gas chromatography equipped with an ion-trap detector (Perkin–Elmer GC–ITD) as previously described using a SPB-5 0.25 μm capillary column (30 m × 0.25 mm, Supelco). NMR spectra were recorded at 70° with a Varian VXR-400 spectrometer, equipped with a 10-mm multinuclear probe, at 100.58 MHz (^{13}C). GXM-D (~ 100 mg) was

dissolved in 3.1 mL of D_2O. The pH was adjusted to pH 7.6 with NH_4OH if required. Chemical shifts were measured relative to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate taken as 0.00 ppm.

RESULTS AND DISCUSSION

Gel filtration and ion-exchange chromatography.—The GXM-S purified from each isolate eluted as a single peak by gel-filtration column chromatography on Sepharose CL-6B[16,22]. The apparent mol wt varied from 6.0×10^4 (isolate 371–3) to 1.55×10^5 (isolate 68). GXM-S from each isolate eluted from DEAE Sepharose CL-6B as a single peak at approximately 0.2 M NaCl as was observed in previous studies[16,22,23].

Composition analyses.—The Xyl:Man:GlcA molar ratio of each GXM-S was similar between isolates (Table I). Isolate 371–3 was higher in Xyl (molar ratio = 2.50) than one standard deviation from the mean of all isolates (coefficient of variation, $CV = 13\%$). The amount of GlcA acid determined in each GXM-S also was similar between isolates ($CV = 17\%$). The percent *O*-acetyl differed significantly between isolates (Table I). Three isolates (201, 196, and 68) had an *O*-acetyl content of only 6–9%, whereas the remaining isolates contained approximately 12.5% *O*-acetyl.

Methylation analyses.—The *O*-deacetylated polymers of all isolates were methylated and analyzed by GLC–MS as previously described. The derivatives obtained from all isolates were: 2,3,4-tri-*O*-methylXyl, 2,4,6-tri-*O*-methylMan, 4,6-di-*O*-methylMan, and 6-*O*-methylMan (Table II). The molar ratio of disubstituted mannan, as indicated by the 6-*O*-methylMan derivative, varied significantly ($CV = 72\%$) between isolates; 0.07 (isolate 68) to 0.64 (isolate 271). Two isolates (68 and 355) had a significantly higher molar ratio of unsubstituted Man than the other isolates as indicated by the molar ratio of the 2,4,6-tri-*O*-methylMan derivative.

TABLE I

Molar ratios of GXM from *C. neoformans*, serotype A[a]

Isolate	D-Man	D-Xyl	D-GlcA[b]	*O*-Acetyl (%)[b]
201	3.00	1.97	0.58	8.8
196	3.00	1.96	0.47	6.3
68	3.00	1.70	0.50	6.4
9759	3.00	1.91	0.43	12.8
9104	3.00	1.71	0.65	12.2
271	3.00	2.02	0.71	12.5
355	3.00	1.92	0.64	12.8
371–3	3.00	2.50	0.64	12.3
Averages ± SD[c]		1.96 ± 0.25	0.58 ± 0.10	10.5 ± 2.9

[a] Composition data were obtained on sonicated GXM (GXM-S). [b] Uronic acid and *O*-acetyl were determined colormetrically. [c] Mean of all isolates for each substituent ± one standard deviation.

TABLE II

GLC–MS methylation analysis of GXM from *c. neoformans*, serotype [a]

Isolate	Methylated PAAN derivatives (mol ratios)			
	Tri-*O*-Me		Di-*O*-Me	*O*-Me
	2,3,4-D-Xyl [b]	2,4,6-D-Man	4,6-D-Man	6-D-Man
201	1.66	0.39	2.50	0.11
196	1.58	0.33	2.50	0.17
68	1.51	0.62	2.31	0.07
9759	1.57	0.39	2.38	0.22
9104	1.42	0.41	2.42	0.17
371–3	1.91	0.26	2.43	0.32
271	2.01	0.31	2.05	0.64
355	1.54	0.67	2.02	0.31
Average ± SD [c]	1.65 ± 0.20	0.42 ± 0.15	2.33 ± 0.19	0.25 ± 0.18

[a] Methylation analysis was done on *O*-deacetylated GXM (GXM-D). [b] Identified by GLC–MS and molar ratios calculated based on the degree of substitution of mannose. [c] As Table I.

Methylation analyses were also performed on XM derived from four serotype A isolates: 9759, 9104, 371–3, and 271. Except for isolate 371–3, the reduction in the molar ratio of 6-*O*-methylMan was paralleled by the appearance of an almost equal amount of a new derivative, 2,6-di-*O*-methylMan (Table III). The reduction in 4,6-di-*O*-methylMan was almost equal to the increase in unsubstituted mannan as reflected by the increase in the molar ratio of 2,4,6-tri-*O*-methylMan. These results indicate that Glc*p*A is linked to O-2 of the mannan backbone. Glc*p*A and Xyl*p* are linked O-2 to individual Man*p* residues of the mannan backbone in most isolates, but in some isolates the mannan backbone also has Man*p* residues that are disubstituted with D-Glc*p*A at O-2 and with D-Xyl*p* at O-4.

Nuclear magnetic resonance spectroscopy.—The ^{13}C NMR spectra of selective GXM-D samples are shown in Fig. 1. Tabulation of the chemical shifts of the anomeric carbons for all of the serotype A isolates examined in this study and in those from a previous investigation[16] are given in Table IV along with their putative linkage assignments. The assignments of Table IV were specified by

TABLE III

GLC–MS methylation analysis of mannose derivatives of XM from *C. neoformans*, serotype A [a]

Isolate	Methylated PAAN derivatives (mol ratios)			
	Tri-*O*-Me	Di-*O*-Me		*O*-Me
	2,4,6-D-Man	2,6-D-Man	4,6-D-Man	6-D-Man
9759	1.10	0.20	1.56	0.05
9104	1.31	0.08	1.54	0.06
371–3	1.06	0.15	1.79	0.00
271	0.85	0.38	1.66	0.12

[a] Methylation analysis was done on xylomannan (XM) derived from GXM-S.

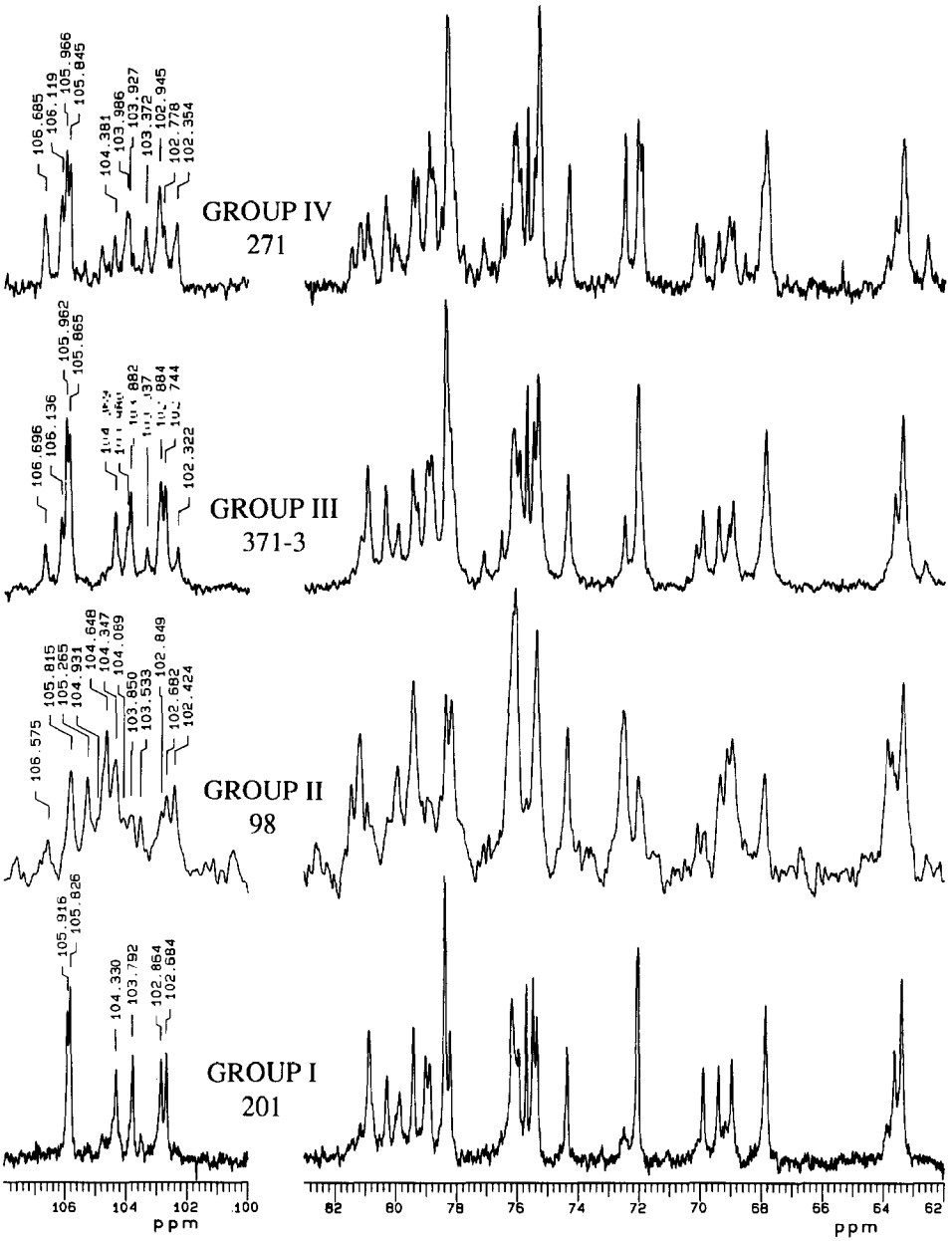


Fig. 1. Proton-decoupled ^{13}C NMR spectra (70°, 100 MHz) of *O*-deacetylated GXM-S. ^{13}C NMR chemical shifts are in ppm. Serotype A isolates from the bottom are as follows: Group I, 201; Group II, 98; Group III, 371–3; Group IV, 271.

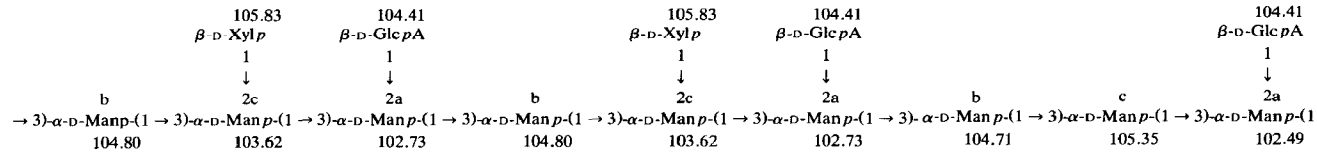
comparison to ^{13}C NMR chemical shift data obtained from related polysaccharides for which chemical shift assignments have already been related to saccharide sequence: Structures **1** (ref. 33), **2** (ref. 28), and **3** (ref. 34). Examination of the

TABLE IV

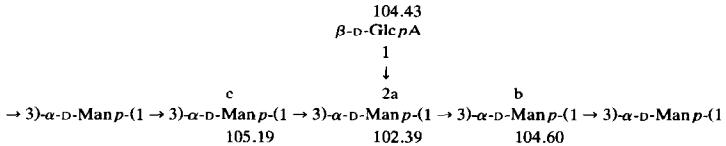
^{13}C NMR chemical shift [a] data for the anomeric carbons of GXM-D of *C. neoformans* serotype A

Isolate	MaGX4 [b]	MaGX4MbX2	MbX2	McX2	Mb	*Mc*	MaG	MaGX4	MaG	*Ma*GX4	MbX2	*Mc*X2
Group I isolates												
201			105.83	105.91			104.33		102.68		102.86	103.79
196			106.03	105.93			104.40		102.73		102.92	103.90
9759			105.88	105.98	104.81 [c]		104.38		102.75		102.90	103.91
6			105.85	105.94			104.34		102.69		102.88	103.83
9104			105.87	105.97	104.81		104.37		102.74		102.89	103.89
												103.61
68			105.89	105.98	104.81		104.39		102.73		102.89	103.89
												103.61
Group II isolates												
98	106.58		105.82		104.65	105.27	104.35		102.68	102.42	102.85	103.85
110	106.59	105.93	105.85	105.94	104.67	105.26	104.36		102.69	102.43	102.87	103.84
Group III isolates												
371–3	106.70	106.14	105.87	105.96			104.37	103.98	102.74	102.32	102.88	103.88
												103.34
Group IV isolates												
271	106.69	106.12	105.85	105.97	104.81		104.38	103.99	102.78	102.35	102.95	103.93
												103.39
355	106.70	105.97	105.82	105.87	104.80		104.37	103.91	102.73		102.97	103.62
											102.89	
Previously assigned chemical shift data												
9375 D [d]				105.83	104.80		104.41		102.73			103.62
430 D [d]				105.81	104.80	105.35	104.40		102.72			103.61
					104.71				102.49			
409 B [e]	106.70	106.07		105.95				103.99		102.36	102.95	103.35
GM [f]					104.60	105.19	104.43		102.39			

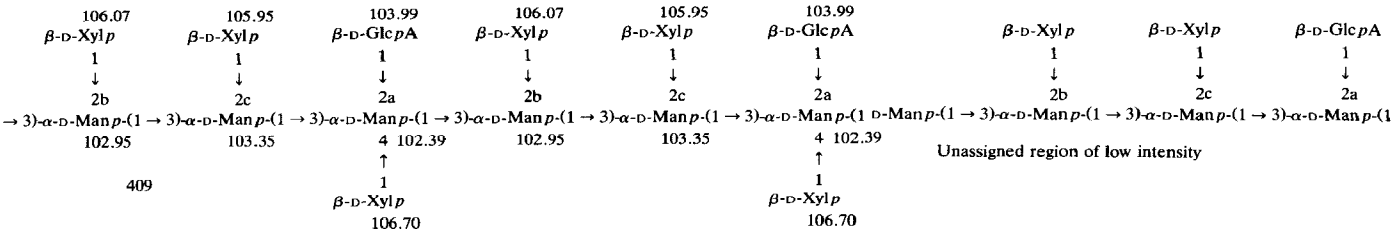
[a] In ppm relative to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate. [b] MaGX4 = chemical shift of the underlined residue, (1 → 4)-linked-β-D-Xyl*p*, in → 3)-Man*p*a substituted at O-2 and O-4 with Glc*p*A and Xyl*p*, respectively. See Structures 1–7. Other entries in this table follow the same nomenclature. [c] Underlined values are of low intensity. [d] Serotype D from refs. 23 and 33. [e] Serotype B from refs. 22 and 34. [f] Glucuronomannan from ref. 28.



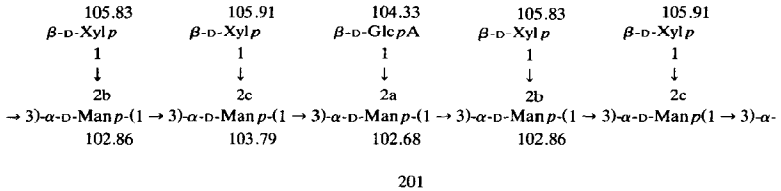
Structure 1 Serotype D



Structure 2 GM



Structure 3 Serotype B



Structure 4 Group I

anomeric regions showed that the serotype A isolates could be categorized into 4 groups — Group I: isolates 68, 196, 201, 9759, 9104, and 6 (ref. 16); Group II (ref. 16): isolates 98 and 110; Group III: isolates 371–3; and Group IV: isolates 271 and 355.

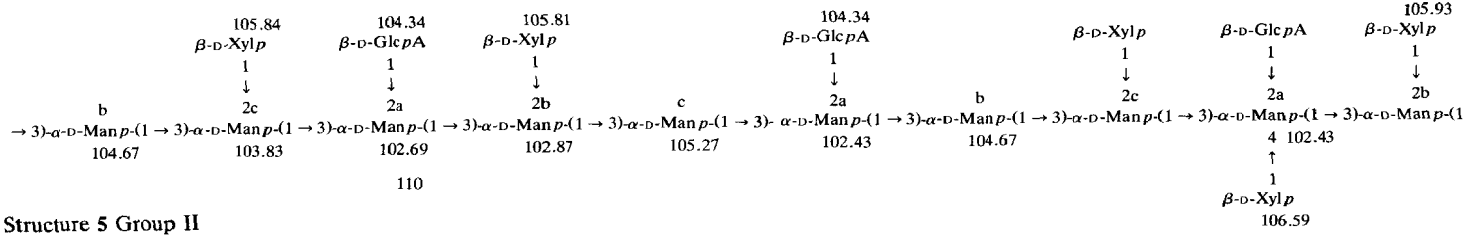
The GXM-D from Group I isolates (e.g., isolate 201) gave the simplest spectrum (Fig. 1). The two resonances observed at average values of 105.86 and 105.95 were assigned to Xyl*p* linked O-2 to Man*p* in two different environments: ManbXyl2 (MbX2) and MancXyl2 (McX2), respectively (Table IV, and Structure **4**). Each Group I isolate also gave a major resonance at an average value of 104.37 ppm. This resonance corresponds to GlcA linked O-2 to Man*p*: ManaGlcA2 (MaG). The three remaining major resonances for Group I were observed at average values of 103.87, 102.89, and 102.72 ppm. These resonances are assigned to the three Man*p* residues substituted with β-D-Xyl*p* or β-D-Glc*p*A at O-2; ManaGlcA2 (MaG), ManbXyl2 (MbX2), and MancXyl2 (McX2) (Structure **4**). Low-intensity resonances, observed for isolate 201[35] in TOCSY (total correlation spectroscopy)[36] and HMQC (heteronuclear multiple quantum coherence spectroscopy)[37] experiments, probably reflect the presence of additional linkage environments (Structure **4**, right hand portion). These minor signals cannot be assigned at this time.

Group II is composed of two isolates, 98 and 110, that are deficient in Xyl*p* in comparison to isolates from other Groups. Their molar composition, reported previously[16], resembles that observed for serotype D (Structure **1**), but serological analysis showed that these two isolates are serotype A[16]. The signals observed for these isolates are listed in Table IV. The major linkage environments of Group II can tentatively be assigned to a mixture of serotype A as found in Group I (Structure **4**), glucuronomannan (GM, Structure **2**)[28] and serotype B (Structure **3**)[22,34]. The signals observed at 104.67 and 105.26 ppm are characteristic of the GM structure. Additional signals arise at 106.59, 105.93, and 102.43 ppm from residues present in a serotype B (Structure **3**) environment. The composite model of the Group II GXM-D along with the putative chemical shift assignments are given in Structure **5**.

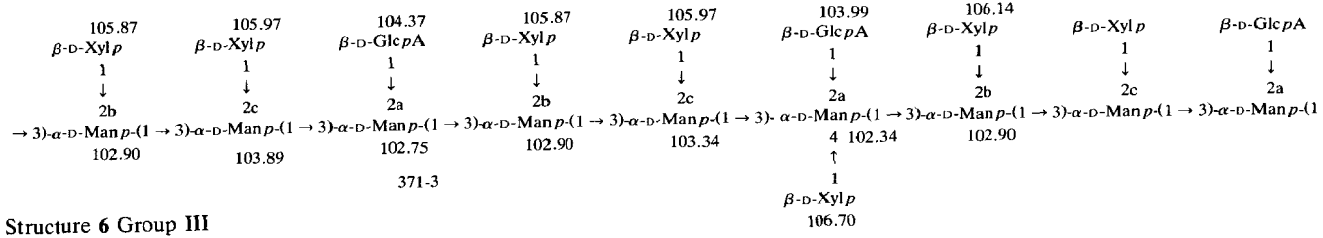
Group III has one member, isolate 371–3. This GXM-D has a mix of resonances found in serotypes A and B. The prominent anomeric signals were those assigned to serotype A (Structure **4**). However, there are four low-intensity signals, 106.70, 105.96, 103.98, and 103.34 ppm (Table IV), that are characteristics of serotype B (structure **3**). A model of the Group III GXM-D is given in Structure **6**. Regions containing unsubstituted Man*p* are not shown.

Group IV isolates, 271 and 355, have a similar distribution of resonances as observed for Group III, except that Group IV has a much higher molar ratio of β-D-Xyl linked at O-4. This difference is reflected in the relatively higher intensity of the signals assignable to serotype B, 106.70, 105.97, 103.91, and 102.36 ppm (Fig. 1). A model for Group IV GXM-D is given in Structure **7**.

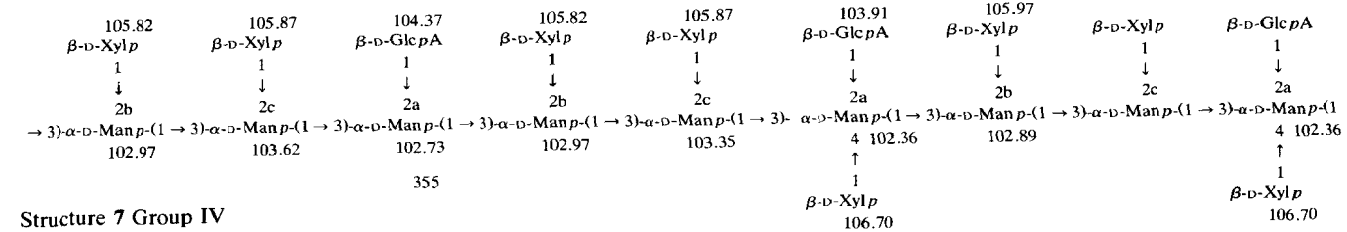
The comparative relationship of Group II, Group III, and Group IV is the



Structure 5 Group II



Structure 6 Group III



Structure 7 Group IV

increase in their substitution with β-D-Xyl*p* at O-4. There is a loss of the signals characteristic of glucuronomannan (GM) at 105.27 and 104.67 ppm, and the concurrent appearance of resonances at 106.70, 105.97, and 103.91 ppm assigned to GXM-D of serotype B (Structure **3**). As in GXM-D of serotype B, the resonance at 106.70 ppm present in Groups II, III, and IV, was assigned to β-D-Xyl*p* linked at O-4. The resonance at ~ 106.13 ppm observed in these three groups was due to the downfield shift of the β-D-Xyl*p* linked O-2 to Manb adjacent to a Mana disubstituted with β-D-Glc*p*A at O-2 and with β-D-Xyl*p* at O-4. In addition, the signal for Glc*p*A in this environment was shifted upfield to 103.99 ppm. This resonance was of greater intensity in isolate 271, which by methylation analyses contained the highest molar ratio of disubstituted Man*p* (Table II). The chemical shift assignments are generally consistent with the linkage distribution determined by methylation analysis (Table II). The presence of two types of Glc*p*A residues (104.38 and 103.99 ppm) and two types of Man*p* substituted with Glc*p*A (102.36 and 102.73 ppm) correlated with the methylation data since removal of Glc*p*A reduced the molar ratio of 6-*O*-methylMan*p* and 4,6-di-*O*-methylMan*p*. These results confirm that within Groups II, III, and IV, (1 → 2)-linked β-D-Glc*p*A was the sole substituent on some Man*p* residues, while others were disubstituted with β-D-Glc*p*A at O-2 and with β-D-Xyl*p* at O-4.

Signals corresponding to unsubstituted Man*p* residues of the mannan backbone were not observed in 1D ^{13}C NMR spectra for Groups I, III, and IV, although they were detected by methylation analyses. HMQC experiments identified resonances of relatively low intensity for unsubstituted Man*p* that overlapped with other major signals for GXM-D of isolate 201 (ref. 35) (Group I). The disubstituted Man*p*, observed by GLC–MS, in Group I GXM-D was present below a critical molar ratio threshold and was not observed in 1D ^{13}C NMR spectra nor by the more sensitive HMQC experiment. Obviously, the sensitivity of methylation analysis gives linkages information not detected by NMR spectroscopy. However, NMR spectroscopy identified the majority of the linkage when the number of different environments was not extremely diverse. More importantly, NMR spectroscopy provides sequence information, and, in conjunction with molecular modeling, the conformation of the polysaccharide can be formulated. The complexity of the serotype A structure has allowed unequivocal chemical shift assignments for only those particular sequences present in greatest density. This complication was not observed in the study of serotype D, isolate 9375 (ref. 33) or serotype B, isolate 409 (refs. 22, 34). These results punctuate the microheterogeneity in the GXM-D structure of serotype A isolates and show that it is naive to think that each serotype can be characterized by a single uniform model structure.

An interesting question arises from this data pertaining to serotype A isolates obtained from AIDS patients. In the pre-AIDS era both varieties of *C. neoformans* (*C. neoformans* var. *neoformans* and *C. neoformans* var. *gatti*) were isolated from clinical specimens[38]. *C. neoformans* isolates from AIDS patients, with a few exceptions[39], were always of the *neoformans* variety[38,40,41]. There appears to be a

selective infection of AIDS patients with the *neoformans* variety[38,41], and 99% of these are of the A serotype[42,43]. Will the GXM-D from AIDS isolates correspond to a single group or will examples from each group be observed? Work to answer this question is in progress.

An isolate of *C. neoformans*, recently serotypes as A-D (ref. 6), contained a molar ratio of 2,4,6-tri-*O*-methylMan*p* intermediate between that of serotype A and serotype D. The structure proposed, based on methylation analyses, was a composite of the model structures for serotype A and serotype D. It is easy to speculate that a range of serotypes corresponding to the *neoformans* variety exist in nature — serotype A to serotype A-D to serotype D — because of the variation in the quantity of unsubstituted Man*p* observed in these studies. Three studies[44–46] showed, at least under laboratory conditions, that successful mating occurs not only among mating paris within a serotype, but also between serotypes, e.g., between serotypes B and C, A and D, C and A, and C and D. These crosses also may occur at random in nature. This may result in an isolate which possess the genes necessary to produce a GXM-containing epitopes from each parent strain distributed uniquely along the polysaccharide. The mating between serotype A and D may explain the heterogeneity observed in the electrokaryotyping of serotype D isolates[47].

A recent study showed that methylation analysis alone cannot predict serotype[16]. Two of the three serotype A isolates in that previous study (Group II, Table IV) had molar ratios of Xyl*p* and 2,4,6-tri-*O*-methyMan equal to the ratios reported for serotype D, yet these isolates were retyped as serotype A. However, ^{13}C NMR spectra from the four serotype A groups lacked the resonance at 103.61 ppm, which is a characteristic of serotype D isolates[22,33].

CONCLUSIONS

The comprehensive analyses of serotype A (data presented herein) serotype B (refs. 22, 34), serotype C (ref. 24), and serotype D (ref. 23), show that it is not accurate to portray a particular serotype by a single model structure. Eleven serotype A isolates are assigned to four distinct groups based on the presence of specific glycosyl linkages observed by methylation analyses, and based on the disposition of these linkages in GXM as established by ^{13}C NMR spectroscopy. Several minor linkages observed by methylation analyses were not detectable by NMR spectroscopy. However, NMR spectroscopy identified key sugar dispositions which may be important in the serological typing of *C. neoformans*. In the future, serological typing reagents should be prepared by using *C. neoformans* isolates for which the GXM-D has been thoroughly characterized by ^{13}C NMR spectroscopy and by methylation analyses.

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