INFECTION AND IMMUNITY, May 1995, p. 1810–1819 Vol. 63, No. 5
0019-9567/95/$04.00+0

Determination of Antigen Binding Specificities of *Cryptococcus neoformans* Factor Sera by Enzyme-Linked Immunosorbent Assay

TESFAYE BELAY AND ROBERT CHERNIAK*

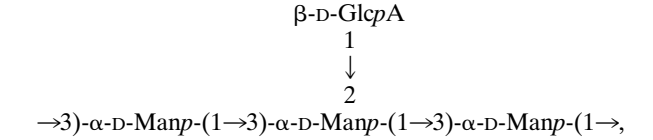
Department of Chemistry, Laboratory for Biological and Chemical Sciences, Georgia State University, Atlanta, Georgia

Received 25 October 1994/Returned for modification 12 December 1994/Accepted 20 February 1995

The competitive binding specificities of glucuronoxylomannan (GXM) and its derivatives to factor sera of *Cryptococcus neoformans* were studied by enzyme-linked immunosorbent assay. An effort was made to determine the epitope specificity of each factor serum. Despite the presence of antigenic factor 1 on all serotypes of *C. neoformans*, variations in inhibition ability were observed with different native GXMs. The panspecific component of factor serum 1 (antibody 1) appeared to be due to the presence of more than one antibody component. The activity was dependent on the 6-*O*-acetyl substituent. GXMs of serotypes A and D inhibited factor serum 2 equally well, indicating a low titer for the antibody 7 component. Serotype B GXM was a poor inhibitor, and serotype C GXM did not inhibit factor serum 2. The activity of factor serum 2 was 6-*O*-acetyl dependent. GXMs from typical serotype A and serotype D isolates were excellent inhibitors of factor serum 3. GXMs from serotype B were poorly inhibitory and serotype C did not inhibit factor serum 3. The activity of factor serum 3 was 6-*O*-acetyl dependent. The activity of factor serum 4 was due predominantly to antibody component 6. The activity of factor 4 was directed mainly against serotype C, and it was independent of 6-*O*-acetyl substitution. Factor serum 5 was specific for serotype B GXMs. The inhibitory effect was independent of 6-*O*-acetyl substitution, but the effect was diminished by reduction of the glucuronic acid. The GXMs with a typical serotype C structure inhibited antibody 6. O deacetylation of the GXMs did not affect their inhibitory activity. However, reduction of glucuronic acid reduced factor serum 6 binding. Factor serum 8 was specific to serotype D; native GXMs of serotype A were slightly inhibitory. O deacetylation of the serotype D GXMs abrogated the inhibitory effect. O deacetylation alone abrogates the activity of antibody components 1, 2, 3, and 8. Reduction of glucuronic acid reduces the inhibitory activity of the GXM to antibody components 4, 5, and 6. Partial GXM structures and methyl glycosides did not effectively inhibit the activity of any of the factor sera.

Cryptococcus neoformans is a life-threatening opportunistic pathogen associated with immunocompromised individuals (12, 21). The incidence of cryptococcosis has been increasing steadily, particularly in patients with AIDS (19, 20). The yeast has a predilection for the central nervous system, where it causes cryptococcal meningoencephalitis (12). Disseminated forms of cryptococcosis are the fourth leading cause of death among patients with full-blown AIDS (24), and serotype A has been isolated from most of these victims (3, 22, 25). Anticapsular polysaccharide antibodies, produced by immunization of rabbits with killed whole cells, were used to describe five serotypes (A, B, C, D, and A/D) of *C. neoformans* (15, 17, 18, 31). *C. neoformans* has a major polysaccharide capsule, glucuronoxylomannan (GXM) (11), that is considered a significant virulence factor (4); acapsular mutants have decreased virulence (16). In addition, the *C. neoformans* capsular polysaccharide (GXM) may be responsible for enhancing the pathogenesis of human immunodeficiency virus type 1 in AIDS patients (23).

The typical GXM consists 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 (2, 11, 28). A simple structural relationship between the typical polysaccharides of the four serotypes exists. They are all composed 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. In this way, explicit typical molar ratios of Xyl/Man/GlcA in serotypes D, A, B, and C have been assigned at 1:3:1, 2:3:1, 3:3:1, and 4:3:1, respectively (2). GXMs from serotypes A and D are substituted mainly at *O*-2, whereas GXMs from serotypes B and C are substituted with Xyl*p* at *O*-2 and at *O*-4. Additional analytical data (obtained recently from the study of larger numbers of isolates of each serotype) show that the precise molar ratio and typical substitution patterns, as proposed in the original models of GXM structure, are an oversimplification (8, 9, 28) except for serotype B (29). In addition, substituent dispositions previously thought to be characteristic of one serotype have been identified in heterologous isolates (11, 28, 30).

Polyclonal *C. neoformans* antibodies have been produced in rabbits by immunization with killed whole cells of each serotype. The sera were selectively absorbed with whole cells, and the absorbed sera, factor sera, were used for the serotyping and identification of new isolates of *C. neoformans* (18). The presence of at least eight antigenic factors, distributed among the serotypes of *C. neoformans*, has been proposed on the basis of the reactivities of absorbed polyclonal antibodies in whole-cell agglutination tests. The distribution of the antigenic factors among the serotypes has been suggested to be as follows.

* Corresponding author. Mailing address: Department of Chemistry, Georgia State University, University Plaza, Atlanta, GA 30303. Phone: (404) 651-3868. Fax: (404) 651-1416. Electronic mail address: bobby@wolf.gsu.edu (Internet).

TABLE 1. Factor sera specific to *C. neoformans*

Factor serum	Antibody component(s)[a,b]	Serotype specificity[a]	Relative ELISA specificity[c]
1	1, 4, 6 (4)[d]	A, B, C, D	D > A > C > B
2	2, 3, 7 (7)	A, B, D	A = B = D
3	3, 7 (7)	A, D	A = D
4	4, 6 (4)	B, C	C > B
5	5 (5)	B	B
6	6	C	C >> B
7	7 (7)	A	A
8	8	D	D >> A > B

[a] Antibody component(s) remaining after adsorption. Antigenic components by serotype are as follows: A, 1, 2, 3, and 7; B, 1, 2, 4, and 6; C, 1, 4, and 6; D, 1, 2, 3, and 8; A/D, 1, 2, 3, 7, and 8. Data are taken from reference 18.

[b] Polyclonal antibodies to the indicated as-yet chemically undefined antigenic determinant(s) are present.

[c] Relative ELISA specificity of the factor serum as observed previously (1).

[d] The number in parentheses denotes a weak antibody component as determined in this study.

Factor 1 is common to all serotypes; factor 2 is common to serotypes A, B, and D; factor 3 is common to serotypes A and D; and factor 4 is common to serotypes B and C. Antigenic factors 7, 5, 6, and 8 are specific to serotypes A, B, C, and D, respectively. The A/D serotype contains antigenic factors 7 and 8 and factors 1, 2, and 3. Moreover, serological experiments revealed that GXM is the primary antigenic structure for the serotype specificities of *C. neoformans* (14). Chemical analyses used to explore the structure of GXM indicate the existence of antigenic multiplicity among the serotypes, particularly serotypes A and C (9, 30).

In an effort to develop reagents for mapping the epitopes responsible for serotype specificity, several monoclonal antibodies against the capsular polysaccharides of *C. neoformans* have been investigated for reactivities to GXM and GXM derivatives (5, 6, 13, 14, 27). The monoclonal antibodies isolated to date have binding activities and specificities similar to those of several factor sera, and it is generally assumed that monoclonal antibodies might correspond to some of the eight antigenic factors of the capsular polysaccharide. However, none of these monoclonal antibodies exhibit specificity for a single serotype and certainly not for a serotype-specific epitope. Though the importance of the eight factor sera in serotyping and identification of clinical isolates of *C. neoformans* has been shown (1, 18), an account of their use for epitope mapping of cryptococcal capsular polysaccharides has not been reported. Recently, a combination of an indirect enzyme-linked immunosorbent assay (ELISA) method and a semiquantitative dot enzyme assay was used to evaluate the activity and specificity of the eight factor sera against purified GXM antigens (1). In the present study, we attempted to define the epitopes responsible for the specific recognition of each factor serum by analyzing the competitive binding specificities of a large representative collection of GXMs and GXM derivatives by ELISA. GXMs purified from clinical isolates of *C. neoformans* from patients with confirmed AIDS infection were included in this study (10).

MATERIALS AND METHODS

Factor sera. Eight cryptococcal factor sera, lot 910610, were provided by Iatron Laboratories, Inc., Tokyo, Japan; they were stored at −20°C. The characteristics of the factor sera are given in Table 1. The data reported here are similar to those obtained with factor sera originally provided by T. Shinoda (18). Iatron Laboratories is marketing a new lot of factor sera (RM 30-K24). The new lot of factor sera (RM 30-K24) has not been as thoroughly tested as the factor serum lot used in these studies (lot 910610). When both lots were used, no statistical difference between the two sets of data was observed. However, we cannot say unequivocally that the two lots of sera will give identical results 100% of the time since complete repetition of the experiments was not performed.

TABLE 2. *C. neoformans* isolates used in the competitive binding study

Serotype[a]	Isolate	Source and/or reference[b]
A (I)	6	T. G. Mitchell (Duke University) (8, 10)
A (II)	98	T. G. Mitchell (Duke University) (8, 10)
A (II)	110	T. G. Mitchell (Duke University) (8, 10)
A (I)	118	T. G. Mitchell (Duke University) (8, 10)
A (III)	150	T. G. Mitchell (Duke University) (8, 10)
A (I)	194	T. G. Mitchell (Duke University) (8, 10)
A (IV)	271	K. J. Kwon-Chung (NIH) (30)
A (IV)	355	K. J. Kwon-Chung (NIH) (30)
A (III)	371	K. J. Kwon-Chung (NIH) (30)
A (I)	9759	E. Reiss (CDC)
A (I)	9104	E. Reiss (CDC)
A (I)	68	E. Reiss (CDC)
B	409	K. J. Kwon-Chung (NIH) (29)
B	444	K. J. Kwon-Chung (NIH) (29)
B	184	K. J. Kwon-Chung (NIH) (29)
B	3172	E. Reiss (CDC)
B	3939	E. Reiss (CDC)
C	34	K. J. Kwon-Chung (NIH) (9)
C	298	K. J. Kwon-Chung (NIH) (9)
C	401	K. J. Kwon-Chung (NIH) (9)
C	3183	E. Reiss (CDC)
D	9375	H. Jean Shadomy (Medical College of Virginia)
D	1254	E. Reiss (CDC)
D	3168	E. Reiss (CDC)
D	125	E. J. Jacobson (Medical College of Virginia)
D	127	E. J. Jacobson (Medical College of Virginia)
D	430	K. J. Kwon-Chung (NIH) (26)
A/D	132	T. Shinoda (Tokyo, Japan)

[a] The roman numeral given in parentheses refers to the serotype A GXM chemical group previously defined (10, 30).

[b] NIH, National Institutes of Health; CDC, Centers for Disease Control and Prevention.

Antigens. The origins of the *C. neoformans* isolates used in this study are listed in Table 2. Isolation and purification of GXMs were done in our laboratory as described previously (8). Structural derivatives of GXM (carboxyl-reduced, O-deacetylated, and O-deacetylated–carboxyl-reduced forms and xylomannan) were prepared as described previously (7–9). Methyl glycosides were purchased from Sigma Chemical Company, St. Louis, Mo. Reference GXMs of each serotype (reference GXMs are from isolates of *C. neoformans* whose GXMs have molar ratios of mannose, xylose, and glucuronic acid that most closely fit the classical chemical delineation of the type-specific polysaccharides suggested for serotypes A, B, C, and D by Bhattacharjee et al. [2]) were bound to microtitration plates for competitive ELISA analyses. Reference isolates of the four serotypes of *C. neoformans* used in this study were as follows: isolate 6, serotype A (8); isolate 34, serotype C (9); isolate 409, serotype B (29); and isolate 9375, serotype D (26).

ELISA. The optimal conditions for preparing the microtitration plates and for performing the ELISA analyses were described previously (1). The optimal dilution of each factor serum was determined from the linear part of the respective titration curve. Competitive binding ELISA was done by preincubating 200 μl of GXM (0.0 to 100 μg/ml in phosphate-buffered saline [PBS, pH 7.4]) with an equal volume of the predetermined dilution of the factor serum (1:1,000 to

1:2,500) in PBS-Tween 20 containing 2% fetal bovine serum (PBSTF) for 90 min at 23°C. Next, 100-μl portions of the incubated mixtures were added—in duplicate or triplicate—to microtiter plates that had been previously coated with GXM from the four reference isolates described above. A control consisting of antigen-coated microtitration wells containing factor sera preincubated with buffer containing the coating antigen (0.0 to 100 μg/ml) was included in each ELISA. Negative controls consisted of the complete system without factor serum or without GXM. ELISAs were done in duplicate or triplicate. Experimental protocols were repeated on two to three different occasions with plates prepared on different dates. The data in the figures are plotted with error bars showing ±1 standard deviation. All inhibitors listed in the figures are native GXMs unless stated otherwise.

RESULTS

A selection of GXMs, GXM derivatives, and related compounds were investigated by ELISA for their ability to competitively inhibit factor sera. The concentration of inhibitor that reduced the control absorbance (no inhibitor present) by 50% (IC_{50}) was determined in those experiments for which graphical representations of the data are not presented. The IC_{50} of the inhibitor for factor serum binding to the immobilized GXM was estimated by interpolation of the experimental curves. The compounds studied represent a broad spectrum of molecular species found in all the serotypes of *C. neoformans*. Microtitration plates were coated with native GXM obtained from reference isolates representing the four serotypes: A (isolate 6), B (isolate 409), C (isolate 34), and D (isolate 9375) (2, 11, 28). Residual antibody activity was quantitated following preincubation of factor serum with increasing concentrations of the sample being tested. No significant differences were observed when the experiments were repeated on different dates. The antibody components believed to compose each of the eight putative factor sera are given in Table 1.

Factor serum 1. Competitive binding analyses were done with the four reference GXMs bound individually to microtitration plates.

(i) Serotype A bound. GXMs from serotype A, B, C, and D isolates were studied for their inhibitory effect on the GXM of serotype A isolate 6. The GXMs from serotype A group I were equal to the homologous antigen in inhibitory potency (Fig. 1a). An exception was the GXM of isolate 194, which gave results similar to those observed for groups III and IV. Therefore, in Fig. 1 and in subsequent figures data pertaining to isolate 194 are plotted with data for those groups although on the basis of its chemical classification, this isolate was originally assigned to group I (30). A possible explanation is given in Discussion. The serotype A group II GXM (10, 30) and the GXM from the serotype A/D isolate, isolate CBS 132, were only slightly less effective as inhibitors. The GXMs from serotype A groups III and IV and the GXMs from serotype D were less effective competitive inhibitors (Fig. 1b and c, respectively). The GXMs from serotypes B and C were inhibitory only at elevated concentrations, IC_{50}s of 25 and 6.25 μg/ml, respectively. The GXM from serotype C isolate 401, whose xylose substitution closely resembles that of serotype B GXM, had an IC_{50} comparable to that of the GXM of serotype B isolates, 6.25 μg/ml. Serotype A GXMs that were O deacetylated or reduced (reduction of glucuronic acid residues under conditions whereby most of the *O*-acetyl substituents are retained) lost almost all their inhibitory properties in comparison with the activity of the native antigens (IC_{50} = 25 μg). The combination of carboxyl reduction and O deacetylation caused a decline in the inhibitory effect on factor serum 1 greater than that observed for O deacetylation alone ($IC_{50} \geq 100$ μg/ml). Selective chemical removal of glucuronic acid and *O*-acetyl substituents from GXMs produced xylomannan analogs. The xylomannans of isolates 194, 371, and 9104 (serotype A) and

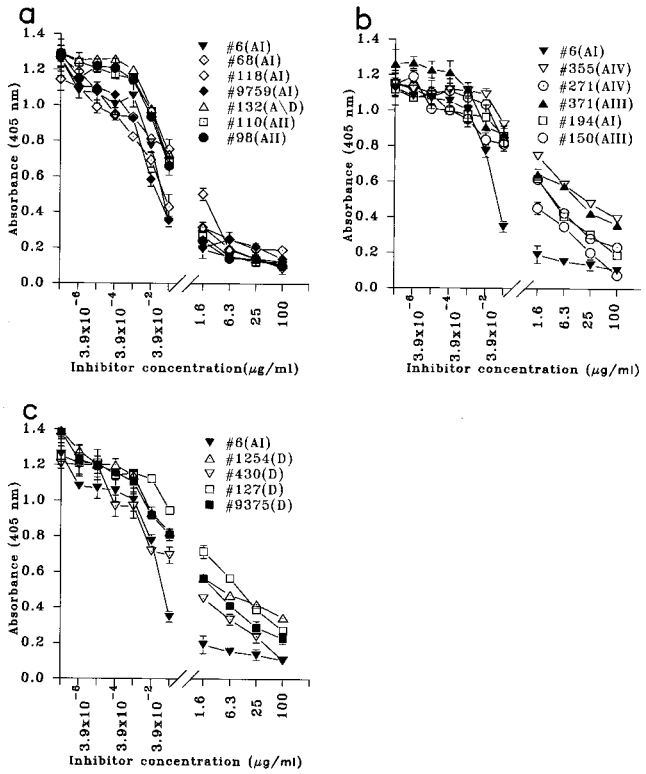


FIG. 1. Competitive inhibition of factor serum 1 binding to native GXM of serotype A isolate 6 by native GXMs. Different concentrations of each GXM in PBS were preincubated at 23°C for 90 min with an equal volume of factor serum 1 diluted at 1:2,500. The mixture was assayed by ELISA for residual binding to immobilized GXM of serotype A isolate 6. Native GXM from serotype A isolate 6, the coating antigen, was included in each microtitration plate. Activities are given for native GXMs from isolates of serotype A (a and b) and serotype D (c). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

isolates 34, 298, and 3183 (serotype C) had IC_{50}s for factor serum 1 of ≥100 μg/ml. Methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, and methyl β-D-glucopyranosyluronic acid were weak competitive inhibitors of factor serum 1; the inhibitory effect was greater than expected, an IC_{50} of 25 μg/ml.

(ii) Serotype D bound. The competitive inhibition of factor serum 1 was repeated with GXM from isolate 9375 (serotype D reference isolate) bound to the microtitration plate. The inhibition observed with this experimental variation in the ELISA protocol paralleled the results obtained when serotype A was used as the reference isolate. The GXMs from serotype A groups I and II and serotype A/D isolate 132 inhibited the binding at a concentration equivalent to that of the serotype D GXM used to coat the microtitration plates, an IC_{50} of ≤0.039 μg/ml. The GXMs from serotype A groups III and IV were less effective (Fig. 2a). The GXMs from the serotype D isolates were generally good inhibitors; however, this was not always the case (Fig. 2b). The serotype B and C GXMs consistently exhibited poor inhibitory effects on factor serum 1, with IC_{50}s of 6.25 and 25 μg/ml, respectively. O deacetylation caused a marked decrease in the inhibitory effects of the serotype A and D GXMs, with IC_{50}s from 8.2 to 75 μg/ml.

(iii) Serotype B bound. The inhibition of factor serum 1 with serotype B bound was best when the serotype B GXMs were used (Fig. 3a). The related GXMs from serotype C isolates

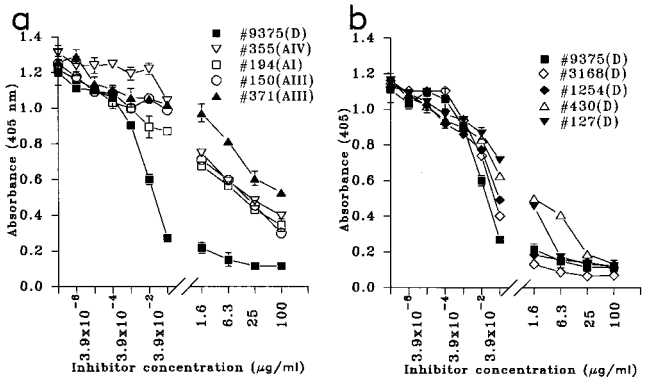


FIG. 2. Competitive inhibition of factor serum 1 binding to native GXM of serotype D isolate 9375 by various native GXMs. The inhibition ELISA was performed as described in the legend to Fig. 1. Native GXM from serotype D isolate 9375, the coating antigen, was included in each microtitration plate. Activities are given for native GXMs from isolates of serotype A (a) and serotype D (b). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

were not as effective as inhibitors (Fig. 3b), though serotype C was the immunizing serotype. Unexpectedly, the GXMs of serotype A groups I and II were effective inhibitors under these conditions (Fig. 3c). This may be due to a low titer of antibody

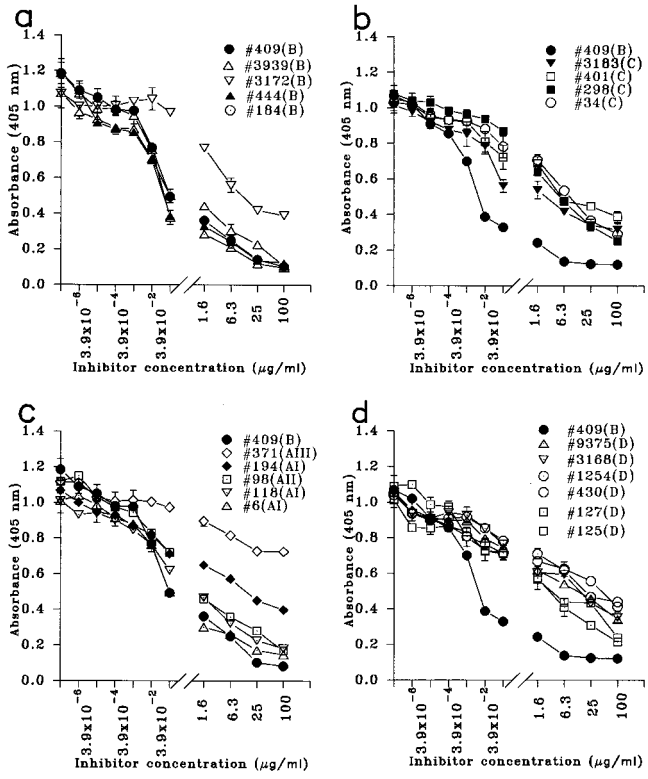


FIG. 3. Competitive inhibition of factor serum 1 binding to native GXM of serotype B isolate 409 by various native GXMs. The ELISA was performed as described in the legend to Fig. 1. Native GXM from serotype B isolate 409, the coating antigen, was included in each microtitration plate. Activities are given for GXMs from isolates of serotype B (a), serotype C (b), serotype A (c), and serotype D (d). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

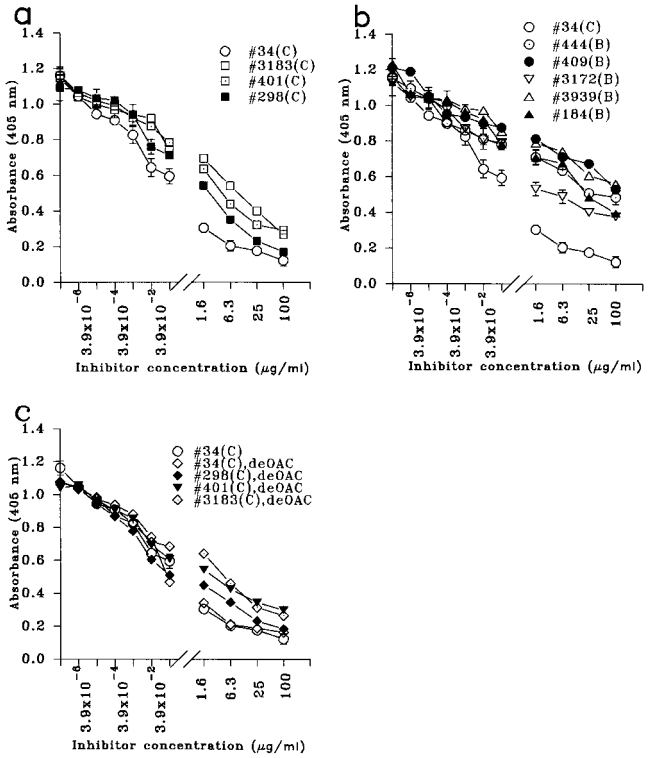


FIG. 4. Competitive inhibition of factor serum 1 binding to native GXM of serotype C isolate 34 by various native GXMs and by O-deacetylated GXMs. The ELISA was performed as described in the legend to Fig. 1. Native GXM from serotype C isolate 34, the coating antigen, was included in each microtitration plate. Activities are given for GXMs from isolates of serotype C (a) and serotype B (b) and for O-deacetylated GXMs from isolates of serotype C (c). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype. Abbreviation in panel c: deOAc, deacetylated GXM.

4 in factor serum 1 and the absence of antigen 6 in serotype A GXM. This leaves only antibody 1 and antigen 1 common to serotypes A and B. The GXMs from serotype D isolates were less effective in inhibiting the activity of factor serum 1 (Fig. 3d). O-deacetylated GXMs of the four serotypes were much less effective inhibitors of factor serum 1 than were the parent compounds when the GXM of serotype B was bound to the microtitration plates ($IC_{50} \geq 25$ μg/ml).

(iv) Serotype C bound. The serotype C GXMs, whose chemical composition and structure closely resemble those of the reference GXM (isolate 34), e.g., GXMs from isolates 298 and 401, were the most effective inhibitors of factor serum 1 activity (Fig. 4a). The GXMs from serotype B isolates were weak inhibitors (Fig. 4b). The GXMs from serotypes A and D exhibited no significant inhibitory effects ($IC_{50} \geq 100$ μg/ml). O deacetylation of serotype C GXMs (isolates 34 and 298) did not significantly alter their inhibitory effect on factor serum 1 when serotype C GXM was used to coat the microtitration plates (Fig. 4c).

Activity against factor serum 2. The activity of factor serum 2 was probed by competitive ELISA using the protocol described above. Factor serum 2 is specific for serotypes A, B, and D (1, 18).

(i) Serotype D bound. Groups I and II of the serotype A GXMs and the serotype D GXMs were generally good inhibitors of factor serum 2 activity, with an IC_{50} of 0.039 μg/ml (data not shown). GXMs from group III of serotype A (Fig. 5a,

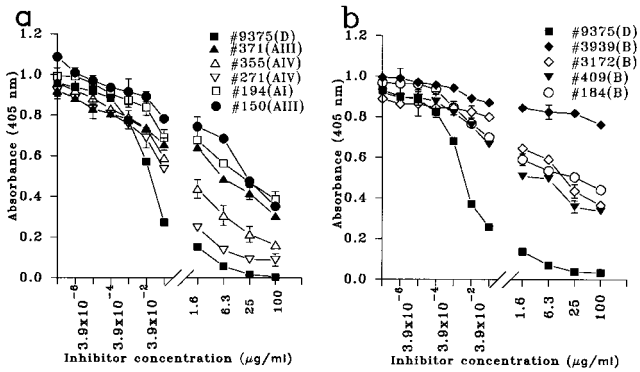


FIG. 5. Competitive inhibition of factor serum 2 binding to native GXM of serotype D isolate 9375 by native GXMs of *C. neoformans*. Different concentrations of each inhibitor in PBS were preincubated at 23°C for 90 min with an equal volume of factor serum 2 diluted at 1:2,500. The mixture was assayed by ELISA for residual binding to immobilized GXM of serotype D isolate 9375. Native GXM from serotype D isolate 9375, the coating antigen, was included in each microtitration plate. Activities are illustrated for GXMs from isolates of serotype A (a) and serotype B (b). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

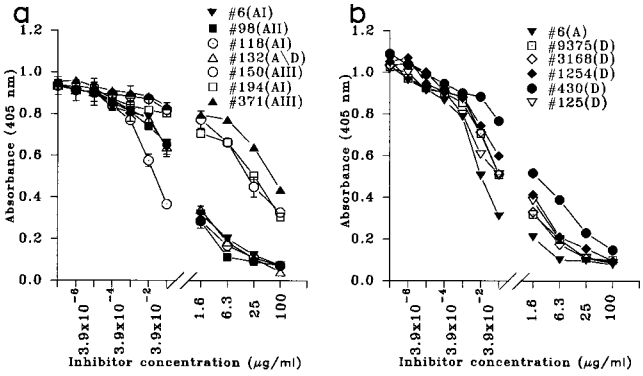


FIG. 6. Competitive inhibition of factor serum 3 binding to native GXM of serotype A isolate 6 by various native GXMs. Different concentrations of each inhibitor in PBS were preincubated at 23°C for 90 min with an equal volume of factor serum 3 diluted at 1:2,000. The mixture was assayed by ELISA for residual binding to immobilized GXM of serotype A isolate 6. Native GXM from serotype A isolate 6, the coating antigen, was included in each microtitration plate. Activities are given for native GXMs from serotype A (a) and serotype D (b) isolates. Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

GXMs 371 and 150) were poor inhibitors, but group IV GXMs were good inhibitors (GXMs 271 and 355). The serotype B GXMs were much less effective inhibitors (Fig. 5b). The least effective GXMs were those isolated from serotype C, with an IC_{50} of ≥100 μg/ml.

(ii) Serotype A bound. The GXMs from the serotype A isolates of groups I, II, and IV were the most effective inhibitors (IC_{50}s of 0.039 to 0.39 μg/ml), and the GXMs from serotype D isolates were the next most effective inhibitors (IC_{50} of 1.6 μg/ml). GXMs from serotype A group III were not effective ($IC_{50} \geq 25$ μg/ml). The GXMs of serotypes B and C did not significantly reduce the activity of factor serum 2 ($IC_{50} \geq 100$ μg/ml).

(iii) Serotype B bound. Serotype B isolates were less competitive than were serotypes A and D, which were excellent inhibitors of the binding of factor serum 2. This indicates that the GXMs from the serotype B isolates have a lower density of antigenic factor 2 epitopes. As expected, serotype C isolates did not inhibit factor serum 2 ($IC_{50} \geq 100$ μg/ml).

Structurally modified GXMs from typical well-characterized isolates of serotypes A, B, and C were examined for competitive inhibition of factor serum 2 binding activity. O deacetylation of GXMs resulted in a marked decrease in their inhibitory effects on factor serum 2 binding to immobilized GXM of serotype A. However, carboxyl-reduced derivatives of the same set of GXMs exhibited reduced inhibitory activity against factor serum 2 binding in an ELISA; the reduced GXMs lost all inhibitory activity after O deacetylation ($IC_{50} \geq 25$ μg/ml). Xylomannans had no inhibitory effect on factor serum 2 activity ($IC_{50} \geq 100$ μg/ml).

Factor serum 3. GXMs and GXM derivatives, obtained from isolates of serotypes A, B, C, D, and A/D, were examined for their inhibitory effects on the binding affinity of factor serum 3. Factor serum 3 is specific for serotypes A and D (18). The GXMs from serotype A isolate 6 and from serotype D isolate 9375 were bound to microtitration plates.

(i) Serotype A bound. The serotype A GXMs of group I and group II and the serotype A/D GXM (Fig. 6a) and serotype D GXMs (Fig. 6b) were excellent inhibitors of factor serum 3 activity. The serotype A GXMs of group III (Fig. 6a, isolates 371 and 150) and group IV (data not shown) were not inhibitors. O deacetylation resulted in a great diminution in the inhibitory effects of serotype A, D, and A/D GXMs ($IC_{50} \geq 25$ μg/ml). However, only relatively small decreases in the already weak inhibitory effects of the native GXMs of serotype A group III were observed after O deacetylation: the IC_{50} increased from 25 to ~100 μg/ml. When the carboxyl groups of Glc*p*A were reduced, under conditions whereby the *O*-acetyl residues are retained, no inhibitory effect was observed ($IC_{50} \geq 100$ μg/ml). The combination of carboxyl reduction and O deacetylation completely abolished the inhibitory effects of all the GXMs from the serotype A isolates ($IC_{50} > 100$ μg/ml).

(ii) Serotype D bound. The serotype D GXMs were all excellent inhibitors of factor serum 3 binding in an ELISA (Fig. 7a). O deacetylation of the serotype D GXMs almost completely abrogated the inhibition ($IC_{50} \geq 100$ μg/ml). Inhibition of factor 3 with serotype A GXMs was best with those obtained from the isolates within groups I and II (Fig. 7b,

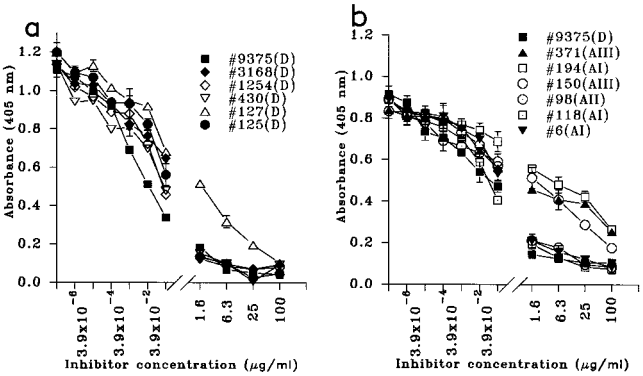


FIG. 7. Competitive inhibition of factor serum 3 binding to native GXM of serotype D isolate 9375 by native GXMs from serotype A and D isolates. The inhibition ELISA was performed as described in the legend to Fig. 6. Native GXM from serotype D isolate 9375, the coating antigen, was included in each microtitration plate. Activities are given for native GXMs from serotype D (a) and serotype A (b) isolates. Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

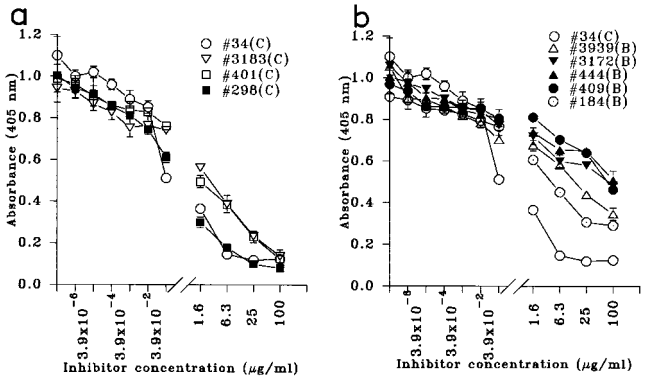


FIG. 8. Competitive inhibition of factor serum 4 binding to native GXM of serotype C isolate 34 by native GXMs. Native GXM from serotype C isolate 34, the coating antigen, was included in each microtitration plate. Activities are given for GXMs from isolates of serotype C (a) and serotype B (b). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Capital letters indicate the serotype.

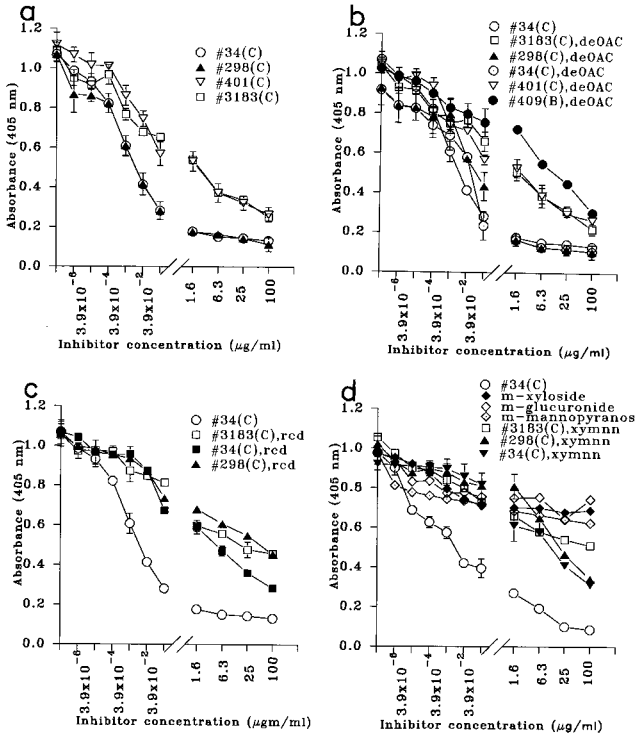


FIG. 9. Competitive inhibition of factor serum 6 binding to native GXM of serotype C isolate 34 by native GXM, GXM derivatives, and methylglycosides. Different concentrations of each inhibitor in PBS were preincubated at 23°C for 90 min with an equal volume of factor serum 6 at a dilution of 1:2,000. The mixture was assayed by ELISA for residual binding to immobilized GXM of serotype C isolate 34. Native GXM from serotype C isolate 34, the coating antigen, was included in each microtitration plate. Activities are given for native GXMs from serotype C isolates (a); O-deacetylated GXMs from serotype C isolates and serotype B isolate 409 (b); carboxyl-reduced GXMs from serotype C isolates (c); and xylomannans from serotype C isolates and the following methylglycosides: methyl-β-D-glucuronide, methyl-β-D-xylopyranoside, and methyl-α-D-mannopyranoside (d). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Abbreviations for panels (b through d): deOAC, deacetylated; rcd, reduced carboxyl group; xymnn, xylomannans; m, methyl; mannopyranos, mannopyranoside.

isolates 6, 98, and 118). O deacetylation of the serotype A GXMs reduced the inhibition to almost background levels ($IC_{50} \geq 50$ μg/ml). Carboxyl reduction and carboxyl reduction in combination with O deacetylation completely eliminated the ability of the serotype A GXMs to inhibit factor serum 3 ($IC_{50} \geq 100$ μg/ml). Little inhibition of factor serum 3 was observed when GXMs, or GXM derivatives, of serotypes B and C were used in the ELISAs (IC_{50}s ranged from 25 to >100 μg/ml).

Factor serum 4. ELISA plates coated with GXMs of serotype B isolates 409 and 444 and serotype C isolate 34 were used to study the inhibitory effects of various GXMs and GXM derivatives on the activity of factor serum 4. Antigenic factor 4 is common to serotypes B and C (18).

(i) Serotype B bound. Factor serum 4 gave titers by ELISA that were approximately twofold lower than the titers observed with other factor sera. The inhibitory effects of the GXMs from serotype B and of the GXMs from serotype C were not as strong as those observed in other ELISAs, with IC_{50}s between 1.6 and 6.25 μg/ml. The inhibitory effects of the GXMs from serotypes A and D were weak, but quantities were measurable ($IC_{50} = 6.25$ μg/ml).

(ii) Serotype C bound. The inhibitory effect of the GXMs from serotype C isolates was strong (Fig. 8a). GXMs from isolates 34 and 298 gave maximum inhibition. The observed inhibitory activity of the serotype C GXMs paralleled the presence of mannose residues that were disubstituted with Xyl*p* at O-2 and O-4 (activity of GXMs by isolate number was in the following order: 34 > 298 > 401 > 3183). GXMs from serotype B isolates were less effective inhibitors (Fig. 8b). However, GXMs from serotype B isolates 184 and 3939 were somewhat better inhibitors than the others tested (Fig. 8b). Apparently the antibody 6 component preponderates in factor serum 4. The GXMs from serotype A and D isolates were generally poor inhibitors, but the GXMs from serotype D isolates were marginally better inhibitors than were the GXMs from serotype A isolates at relatively high GXM concentrations, 25 and 100 μg/ml, serotypes D and A, respectively. The same level of inhibition was obtained for the corresponding O-deacetylated GXMs. Therefore, the *O*-acetyl substituent is not part of the factor 4 epitopes (probably antigenic factor 6), nor is it required for conformational stabilization of the epitopes. However, the carboxyl-reduced GXMs, the carboxyl-reduced–O-deacetylated GXMs, and the xylomannan derivatives of GXMs of serotype B and serotype C did not inhibit factor serum 4 ($IC_{50} \geq 100$ μg/ml).

Factor serum 5. Factor serum 5 is specific for a single antigenic determinant present in serotype B GXM (1, 18). The titer of factor serum 5 towards the GXM of serotype B isolate 409 was lower than that observed for the homologous reactions with factor sera 1 through 4 described above. Therefore, it was not possible to obtain reliable inhibition data with factor serum 5. However, it was possible to determine that O deacetylation did not reduce the inhibitory effect of GXMs from serotype B isolates (IC_{50} of 0.39 μg/ml). The carboxyl-reduced GXMs and the xylomannans lost their ability to inhibit factor 5 ($IC_{50} \geq 25$ μg/ml).

Factor serum 6. Factor serum 6 is specific for serotype C (1, 18). The inhibitory effects of various GXMs on the binding activity of factor serum 6 were studied by using microtitration plates coated with GXM from serotype C, isolate 34. The GXMs from serotype C isolates 34 and 298 were the best inhibitors of the binding of factor serum 6 (Fig. 9a). Serotype C isolates 401 and 3183 (Fig. 9a) and serotype B isolates (data not shown), e.g., 409, were less inhibitory. The inhibitory effectiveness of the serotype C GXMs paralleled the xylose-to-mannose ratios; the best inhibitors have the highest xylose/

mannose ratios. O deacetylation generally did not alter the inhibitory effect of the GXMs (Fig. 9b). However, a large decrease in binding of factor serum 6 was observed after reduction of the carboxyl groups of the best inhibitors, e.g., isolates 34 and 298 (Fig. 9c). Combined O deacetylation and carboxyl reduction of serotype C GXMs resulted in the same reaction pattern as obtained with those GXMs that had only been reduced. The inhibitory effects of xylomannans derived from serotype C isolates (isolates 34, 298, and 3183) were decidedly less than that of the GXM of isolate 34 (Fig. 9d). Furthermore, methyl β-D-xyloside, methyl α-D-mannopyranoside, methyl β-D-glucuronide, and GXMs from serotypes A and D showed no significant inhibitory effects. Generally, the inhibitory activity of the compounds tested with factor 6 decreased in the following order: GXM > O-deacetylated GXM > reduced GXM > xylomannans > methyl glycosides. The serotype C GXMs that have the highest concentration of mannose residues disubstituted with xylose at *O*-2 and *O*-4 (isolates 34 and 298) evoked the greatest inhibitory effects.

Factor serum 8. ELISA plates coated with serotype D GXM from the reference isolate 9375 were used to study the specificity of factor serum 8 (1, 18). Factor serum 8 was inhibited by all the serotype D GXMs tested (Fig. 10a). The inhibitory effect of the serotype D GXMs was equal to the effect of that of the reference isolate, 9375. Despite the apparent high degree of specificity of factor serum 8 for serotype D in a dot blot assay (1), several serotype A GXMs had strong inhibitory effects on factor serum 8 (Fig. 10b). The activity of factor serum 8 requires the *O*-acetyl group since O deacetylation of serotype A and D GXMs abrogates the previously observed inhibition (Fig. 10a and c, respectively). Carboxyl reduction resulted in a partial reduction in the inhibitory effects; this may be due to the partial unavoidable loss of *O*-acetyl that occurs during the reduction of the carboxyl groups (Fig. 10d). However, carboxyl reduction in conjunction with O deacetylation resulted in the complete loss of the inhibitory effects (IC_{50} > 100 μg/ml). Serotype A xylomannans, methyl β-D-xyloside, methyl α-D-mannopyranoside, and methyl β-D-glucuronide did not show inhibitory effects (IC_{50} > 100 μg/ml). Serotype A GXMs from isolates 98 and 110 and serotype A/D GXMs, which have molar ratios of mannose/xylose/glucuronic acid similar to that of the serotype D reference (isolate 9375), were good inhibitors of factor serum 8. Serotype A GXMs from atypical isolates 271 and 355 were poor inhibitors. Inhibitory effects of serotype B and C GXMs were nil. Generally, the inhibitory effects of test compounds on factor 8 decreased in the order of GXM > reduced GXM > O-deacetylated GXM > xylomannans > methyl glycosides.

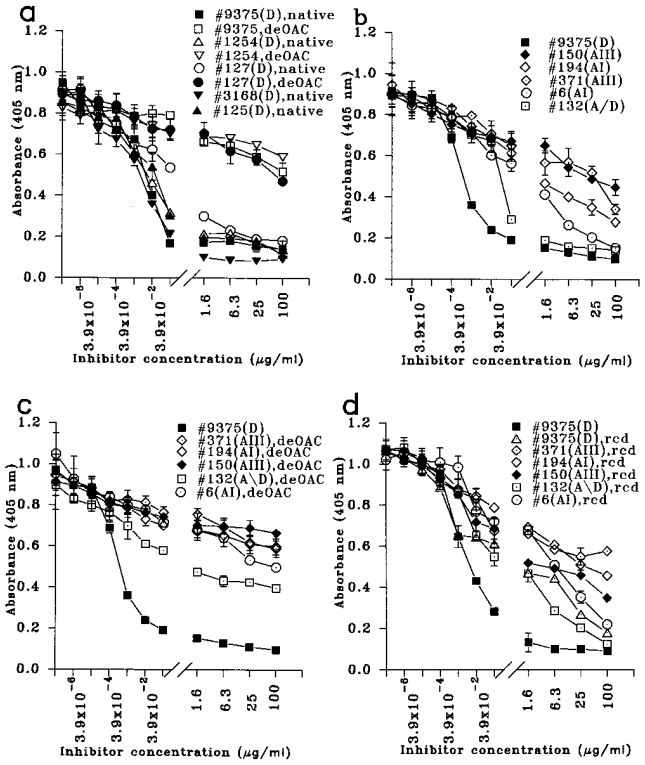


FIG. 10. Competitive inhibition of factor serum 8 binding to native GXM of serotype D isolate 9375 by native GXM and GXM derivatives. Different concentrations of each inhibitor in PBS were preincubated at 23°C for 90 min with an equal volume of factor serum 8 at a dilution of 1:2,000. The mixture was assayed by ELISA for residual binding to immobilized GXM of serotype D isolate 9375. Native GXM from isolate 9375, the coating antigen, was included in each microtitration plate. Activities are given for native and O-deacetylated (deOAc) GXMs from serotype D isolates (a), native GXMs from serotype A isolates (b), O-deacetylated GXMs from serotype A isolates (c), and carboxyl-reduced GXMs from serotype A isolates and serotype D isolate 9375 (d). Each datum point represents a mean absorbance value for two or more wells ± 1 standard deviation (error bars). Abbreviations in panels c and d: deOAC, deacetylated; rcd, reduced carboxyl group. Capital letters indicate the serotype, and roman numerals refer to the serotype A GXM chemical group.

DISCUSSION

Polyclonal sera, produced by differential reciprocal absorption of immune sera obtained from rabbits immunized with formalinized whole cells of serotypes A, B, C, and D, have been used to identify the presence of eight antigenic factors in *C. neoformans* (18). The absorbed sera, factor sera, were used in a slide agglutination assay to serotype isolates of cryptococcus. Although the presence of eight antigenic factors of *C. neoformans*, either unique to a serotype or shared by two or more serotypes, has been reported with whole cells used as antigens, the use of factor sera as probes of epitope structure with chemically defined polysaccharides has not been reported. In this study, the competitive binding specificities of GXMs, O-deacetylated GXMs, reduced GXMs, O-deacetylated and reduced GXMs, xylomannans, and methyl glycosides were surveyed by ELISA in an attempt to determine the epitope specificity of the factor sera.

Factor 1 is common to all serotypes, but the titers obtained by ELISA versus typical GXMs of the four serotypes decrease in the following order: D > A > C > B (1). Variable inhibition patterns for GXMs from the reference GXM of each serotype were observed. In addition, individual isolates within each serotype gave variable responses in inhibition studies. These differences in inhibitory ability indicate that the determinant for antigenic factor 1 is unequally shared among the four serotypes and among isolates of an individual serotype. It is also likely that the activity level of the factor 4 antibody component of factor serum 1 is low (Table 1). The major activities appear to be antibody components 1 and 6. The competitive binding data support data in previous reports concerning the existence of extensive variation in composition and structure within an individual serotype. This variation was most common among GXMs of serotypes A (30) and C (9). Another possible explanation for the variable response of factor serum 1 is that antibody component 1 is a composite of subpopulations of panspecific antibodies specific for common determinants that are unequally expressed on the various isolates of *C. neoformans*.

The GXMs of serotypes A and D lack antigenic factor 6 and do not significantly inhibit factor serum 1 binding to GXM of serotype C because of the presence of antibody component 6 in factor serum 1. The activity of the antibody 1 component of factor serum 1 is *O*-acetyl dependent, and the antibody 6 component is not. The poor inhibitory effect on factor serum 1 by GXMs of serotypes A and D when serotype C is bound to the microtitration plates is due to the presence of antibodies specific for antigenic factors 4 and 6. The GXMs from serotypes A and D are without a demonstrable inhibitory effect because both lack antigenic factors 4 and 6.

Factor 2 is common to serotypes A, B, and D and absent from serotype C (1, 18). The inhibitory effect of GXMs of serotype A isolates on factor serum 2 binding activity was variable. This observation is consistent with chemical heterogeneity reported previously for this serotype (30). GXMs from serotype A groups I and II, which are most like the reference serotype A GXM in structure, exhibited the greatest inhibitory effect versus factor serum 2 binding, while GXMs from serotype A group III isolates exhibited much smaller inhibitory effects. The GXMs of serotypes A and D inhibited factor serum 2 when the reference GXM of serotype A, B, or D was bound to the microtitration plates. The GXMs of serotypes B and C were partially inhibitory and not inhibitory at all, respectively. These results are consistent with the postulated antibody composition of factor serum 2, antibodies to antigenic factors 2, 3, and 7 (18). The reason for the low-level inhibitory effect of GXM of serotype B is the presence of antibody component 3 in factor serum 2 and the fact that its antigenic factor is not present in serotype B GXM. The *O*-acetyl substituent in serotype A, B, and D GXMs appears to play a prominent role in the activity of factor serum 2. However, the *O*-acetyl cannot be sterically equivalent in serotype B GXM since its inhibitory activity is much less than the inhibitory activities of the GXMs of serotypes A and D. This may be due to steric hindrance induced by the *O*-4 linked Xyl*p* residues. Antigenic factors 2 and 3 are common to serotypes A and D. However, all serotype A GXMs were not equally effective in inhibiting factor serum 2. The differences in the observed inhibitory effects could not be correlated directly with the dissimilarities in the chemical structures of the GXMs examined. However, the presence of some Xyl*p* linked *O*-4 to Man*p* may be responsible for the decreased inhibitory effect of the GXM from serotype A isolate 371. The GXM of isolate 194, which has group I structure (30), responded in most cases as a group III structure. A possible explanation is that the 6-*O*-acetyl disposition on the mannopyranan backbone plays a critical role in determining antigenic activity. The data presented herein show the importance of the *O*-acetyl substituents in maintaining the antigenicity of the GXMs. The linkage sequence of the *O*-acetyl groups is currently under investigation.

Factor serum 3 exhibits activity against antigenic factors 3 and 7. As expected, the inhibitory effects were excellent with serotype A and D GXMs and the GXMs from serotypes B and C were essentially without inhibitory effects. However, again, the group III and IV GXMs of serotype A showed reduced inhibitory activity. Reduction of the Glc*p*A residue resulted in a decrease in inhibitory activity. O deacetylation of GXMs, with or without prior reduction of the glucuronic acid substituents, resulted in the complete loss of inhibitory activity.

Factor serum 4 exhibits activity against antigenic factor 4 (specific to serotypes B and C) and antigenic factor 6 (serotype C specific). However, the ELISA absorbances were not as high as those observed with other factor sera when GXM of serotype B was bound to the plate. Therefore, the inhibitory effects of the GXMs from serotype B and C isolates could not be quantitated confidently. The specificity of factor serum 4 by dot enzyme immunoassay was consistent with the proposed antibody composition of the serum (1). We do not know whether the low level of activity of factor serum 4 is caused by (i) poor adsorption of serotype B GXM to ELISA plates, (ii) the masking of the epitopes when bound to the plate, or (iii) the low antibody titer for antigenic factor 4. The activity of antibody component 4 present in factor serum 1 was also of low titer. When the reference GXM for serotype C (isolate 34) was bound to the microtitration plate, the serotype B GXMs were modest inhibitors and the serotype C GXMs were excellent inhibitors. GXMs from isolates 34 and 298, having the highest disubstitution of mannose with xylose at *O*-2 and *O*-4, showed the best inhibitory effect on factor serum 4. The O-deacetylated and reduced derivatives of serotype B and serotype C GXMs did not have altered inhibitory effectiveness versus factor serum 4. The activity of factor 4 is consistent with data published previously (Fig. 1d of reference 1; C > B). However, the data in the text and in Table 1 of the same report incorrectly show the reverse order of activity (the correct order of activity is that factor serum 4 reacted strongly with the GXM of serotype C and reacted less strongly with the GXM of serotype B).

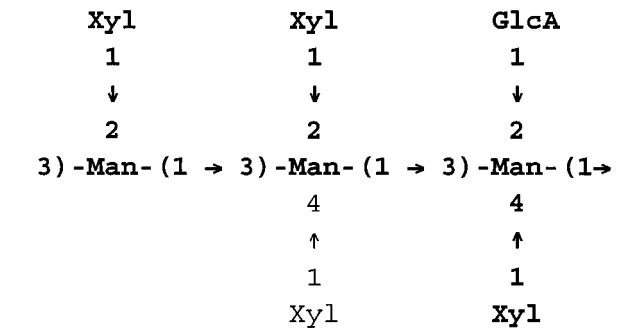


FIG. 11. GXM of serotype B (boldface structure) and GXM of serotype C (entire structure).

Factor serum 5 is specific for a single antigenic factor of serotype B GXM (1, 18). The GXMs derived from the serotype B isolates and the GXM obtained from the serotype C isolate related in structure to serotype B (isolate 3183) were inhibitory (Fig. 11). O deacetylation of the GXMs from serotype B isolates did not substantially affect the inhibitory activity versus factor 5. Factor serum 6 is specific for a single antigenic factor of serotype C GXM (1, 18). The best inhibitory effects were observed with GXMs from serotype C isolates having the highest frequency of Man*p* disubstituted with Xyl*p* at *O*-2 and *O*-4 (isolates 34 and 298) (Fig. 11). Factor 6 was inhibited poorly by serotype B isolates. The GXM obtained from isolate 3183 has the antigenic character of GXMs of serotypes B and C. Therefore, the lack of the *O*-4 linked xylose does not completely eliminate the activity of factor serum 6 for GXM of isolate 3183. The structural basis for this reaction is not evident, but one possible explanation for this observation is the difference in the molar ratio of the 6-*O*-acetyl substituent and variation in their relative disposition. Both factor sera 5 and 6 appear to have specificity only for the carbohydrate components of GXM since O deacetylation of the homologous GXMs did not affect their inhibitory efficiency. The carboxyl reduction of GXM from isolate 34 substantially reduced its inhibitory effect. Xylomannans and the various glycosides were not inhibitory to factor 6.

Factor serum 8 is specific for a single antigenic factor specific for serotype D (1, 18). The GXMs obtained from serotype D isolates were all excellent inhibitors. The inhibitory effect of all the serotype D GXMs was essentially nil after O deacetylation. Therefore, the *O*-acetyl substituent is the major determinant for factor 8. Serotype A GXMs were generally only marginally inhibitory towards the activity of factor 8. However, several GXMs, those of serotype A isolates 6 and 194 and serotype A/D isolate 132, did show substantial inhibition of factor 8. This agrees with the data from the direct titration of factor 8 by ELISA when immobilized serotype A GXMs were used (1). Although the precise structure of the factor 8 epitope is not known, it does appear that *O*-acetyl is a major component. However, 6-*O*-acetyl mannan does not inhibit factor serum 8.

It appears that antigenic factors 1, 2, 3, and 8 require the *O*-acetyl substituents to bind the corresponding factor serum antibody components. Alternatively, the *O*-acetyl may be required for fixing the correct conformation of the epitopes. The *O*-acetyl group does not play a crucial role in the specificity of factor sera 4, 5, and 6. The spatial relationships of the Xyl*p* and Glc*p*A residues in serotype B and serotype C GXMs may be sterically more restricted because of the increased substitution of the mannan backbone (9). The Glc*p*A residues appear to be of marginal significance in the presence of acetyl substitution, although previous findings indicated the importance of both *O*-acetyl and Glc*p*A residues for monoclonal antibody activity (5, 13, 14). The preeminent role of the combination of *O*-acetyl and Glc*p*A residues is illustrated by the total lack of inhibition of any of the factor sera by any of the xylomannan derivatives. The GXMs from isolate 132 (serotype A/D), isolate 6 (serotype A), and isolate 9375 (serotype D) exhibited similar, but quantitatively different, inhibitory effects against factor sera 1, 3, and 8. These observations provide experimental evidence for the presence of common or overlapping determinants among isolates of these three serotypes. Although factor sera 5, 6, 7, and 8 are called type specific, absolute specificity was not observed with any of the factor sera; this is probably due to incomplete absorption and the presence of determinants of heterologous serotypes at trace levels. Some sera were very weak, e.g., factor 7, because of the extensive absorption required or the poor immunogenicity of the GXM. Factor serum 7, serotype A specific, was not studied since its low titer by ELISA prohibited the collection of reliable data. There is something unique about antigenic factor 7, since neither rabbit polyclonal antibodies nor mouse monoclonal antibodies have provided significant serotype A-specific activities. Coincidentally, serotype A preponderates in cryptococcal infections associated with AIDS. The factor sera have certain limitations, but they have produced important preliminary mapping information concerning the composition of *C. neoformans* determinant groups within the major serogroups. Perhaps improved epitope-specific antibody reagents can be obtained by affinity chromatography with immobilized GXMs of defined purity and structure. With this technique it is theoretically possible to individually obtain all eight factors. The specificities of monoclonal antibodies with GXMs, GXM derivatives, and representative synthetic oligosaccharides will be used to further delineate the epitope structure of the eight antigenic factors of *C. neoformans*.

ACKNOWLEDGMENT

This research was supported in part by Public Health Service grant AI-31769 from the National Institutes of Health.

REFERENCES

1. **Belay, T., R. Cherniak, and T. Shinoda.** 1993. Specificity of *Cryptococcus neoformans* factor sera determined by enzyme-linked immunosorbent assay and dot enzyme assay. Infect. Immun. **61:**2879–2885.
2. **Bhattacharjee, A. K., J. E. Bennett, and P. J. Glaudemans.** 1984. Capsular polysaccharides of *Cryptococcus neoformans*. Rev. Infect. Dis. **6:**619–624.
3. **Bottone, E. J., I. F. Salkin, N. J. Hurd, and G. P. Wormser.** 1987. Serogroup distribution of Cryptococcus neoformans in patients with AIDS. J. Infect. Dis. **156:**242.
4. **Bulmer, G. S., and M. D. Sans.** 1968. *Cryptococcus neoformans*. III. Inhibition of phagocytosis. J. Bacteriol. **95:**5–8.
5. **Casadevall, A., J. Mukherjee, S. J. Devi, R. Schneerson, J. B. Robbins, and M. D. Scharff.** 1992. Antibodies elicited by a *Cryptococcus neoformans*-tetanus toxoid conjugate vaccine have the same specificity as those elicited in infection. J. Infect. Dis. **165:**1086–1093.
6. **Cherniak, R., M. M. Cheeseman, G. H. Reyes, E. Reiss, and F. Todaro.** 1988. Enhanced binding of capsular polysaccharides of *Cryptococcus neoformans* to polystyrene microtitration plates for enzyme-linked immunosorbent assay. Diagn. Immunol. **5:**344–348.
7. **Cherniak, R., R. G. Jones, and M. E. Slodki.** 1988. Type-specific polysaccharides of *Cryptococcus neoformans*. N.M.R.-spectral study of a glucuronomannan chemically derived from a *Tremella mesenterica* exopolysaccharide. Carbohydr. Res. **182:**227–239.
8. **Cherniak, R., L. C. Morris, R. C. Anderson, and S. A. Meyer.** 1991. Facilitated isolation, purification, and analysis of glucuronoxylomannan of *Cryptococcus neoformans*. Infect. Immun. **59:**59–64.
9. **Cherniak, R., L. C. Morris, and S. A. Meyer.** 1992. Glucuronoxylomannan of *Cryptococcus neoformans* serotype C: structural analysis by gas-chromatography-mass spectrometry and ^{13}C-nuclear magnetic resonance spectroscopy. Carbohydr. Res. **225:**331–337.
10. **Cherniak, R., L. C. Morris, S. A. Meyer, and T. G. Mitchell.** 1993. Glucuronoxylomannan of *Cryptococcus neoformans* obtained from patients with AIDS. Carbohydr. Res. **249:**405–413.
11. **Cherniak, R., and J. B. Sundstrom.** 1994. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. Infect. Immun. **62:**1507–1512.
12. **Diamond, R. D., G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.).** 1990. Principles of infectious diseases, p. 1980–1989. Churchill Livingstone, New York.
13. **Dromer, F., J. Salamero, A. Contrepois, C. Carbon, and P. Yeni.** 1987. Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. Infect. Immun. **55:**742–748.
14. **Eckert, F. T., and T. R. Kozel.** 1987. Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. Infect. Immun. **55:**1895–1899.
15. **Evans, E. E.** 1950. The antigenic composition of *Cryptococcus neoformans*. I. A. serologic classification by means of the capsular reactions. J. Immunol. **64:**423–430.
16. **Fromtling, R. A., H. J. Shadomy, and E. J. Jacobson.** 1982. Decreased virulence in stable acapsular mutants of *Cryptococcus neoformans*. Mycopathologia **79:**23–29.
17. **Ikeda, R., A. Nishikawa, T. Shinoda, and Y. Fukazawa.** 1985. Chemical characterization of capsular polysaccharide from *Cryptococcus neoformans*. Microbiol. Immunol. **29:**981–991.
18. **Ikeda, R., T. Shinoda, Y. Fukazawa, and L. Kaufman.** 1982. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. J. Clin. Microbiol. **16:**22–29.
19. **Kaplan, M. H., P. P. Rosen, and D. Armstrong.** 1977. Cryptococcosis in a cancer hospital. Cancer **39:**2265–2274.
20. **Kovacs, A. J., A. A. Kovacs, M. Polis, C. Wright, V. J. Gill, C. U. Tuazon, E. P. Gelmann, H. C. Lane, R. Longfield, G. Overturf, A. M. Macher, A. S. Fauci, J. E. Parrillo, J. E. Bennett, and H. Masur.** 1985. Cryptococcosis in the acquired immunodeficiency syndrome. Ann. Intern. Med. **103:**533–538.
21. **Kwon-Chung, K. J., and J. E. Bennett.** 1992. Medical mycology, p. 397–446. Lea and Febiger, Malvern, Pa.
22. **Ordonez, N., and E. Castaneda.** 1990. Serotyping of *Cryptococcus neoformans* clinical isolates in Columbia, abstr. F66, p. 80. *In* Abstracts of the 90th Annual Meeting of the American Society for Microbiology 1990. American Society for Microbiology, Washington, D.C.
23. **Pettoello-Mantovani, M. A., A. Casadevall, T. R. Kolloman, A. Rubinstein, and H. Goldstein.** 1992. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans* Lancet **339:**21–23.
24. **Powderly, W. G.** 1992. Therapy for cryptococcal meningitis in patients with AIDS. Clin. Infect. Dis. **14**(Suppl.1)**:**S54–S59.
25. **Shimizu, R. Y., D. H. Howard, and M. N. Clancy.** 1986. The variety of Cryptococcus neoformans in patients with AIDS. J. Infect. Dis. **154:**1042.
26. **Skelton, M. A., R. Cherniak, and H. van Halbeek.** 1991. Structure of the de-*O*-acetylated glucuronoxylomannan from *Cryptococcus neoformans* serotype D, as determined by 2-D NMR spectroscopy. Magn. Reson. Chem. **29:**786–793.
27. **Todaro-Luck, F., E. Reiss, R. Cherniak, and L. Kaufman.** 1989. Character-

ization of *Cryptococcus neoformans* capsular glucuronoxylomannan polysaccharide with monoclonal antibodies. Infect. Immun. **57:**3882–3887.

28. **Turner, S. H., and R. Cherniak.** 1991. Multiplicity in the structure of the glucuronoxylomannan of *Cryptococcus neoformans*, p. 123–142. In J. P. Latagé and D. Boucias (ed.), Fungal cell walls and immune response. NATO ASI series, vol. H53. Springer-Verlag, New York.
29. **Turner, S. H., and R. Cherniak.** 1991. Glucuronoxylomannan of *Cryptococcus neoformans* serotype B: structural analysis by gas-liquid chromatography-mass spectrometry and ^{13}C-nuclear magnetic resonance spectroscopy. Carbohydr. Res. **211:**103–116.
30. **Turner, S. H., R. Cherniak, E. Reiss, and K. J. Kwon-Chung.** 1992. Structural variability in the glucuronoxylomannan of *Cryptococcus neoformans* serotype A isolates determined by ^{13}C NMR spectroscopy. Carbohydr. Res. **233:**205–218.
31. **Wilson, D. E., J. E. Bennett, and J. W. Bailey.** 1968. Serologic grouping of *Cryptococcus neoformans*. Proc. Soc. Exp. Biol. Med. **127:**820–823.