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Altered Immunochemical Reactivity of *Saccharomyces cerevisiae* a-Cells After α-Factor-Induced Morphogenesis

PETER N. LIPKE[1] AND CLINTON E. BALLOU[2]*

Department of Biological Sciences, Hunter College, New York, New York 10021,[1] and Department of Biochemistry, University of California, Berkeley, California 94720[2]

Antibodies were raised against *Saccharomyces cerevisiae* a-cells that had been exposed to the sex pheromone, α-factor. After adsorption of the antiserum with diploid cells, antibodies remained that reacted specifically with the mannan from haploid cells. The characteristic determinant was observed in mannan from pheromone-treated a-cells, in mannan from untreated α-cells, and at a much lower concentration, in mannan from control a-cells. The antigens from these three mannans appeared to be identical. The determinant was destroyed by mild-acid hydrolysis or periodate oxidation, but not by proteolysis or digestion with exo-α-mannanase. Mutants with altered mannan were unable to express the antigen. Complete acid hydrolysates of mannan from α-factor-treated a-cells contained mannose, glucose, and *N*-acetylglucosamine. Partial acid hydrolysis, under conditions that destroyed the antigenic determinant, released only mannose and mannobiose. The mannose fraction was labeled to high specific activity during response of a-cells to α-factor if radioactive glucose was the carbon source. Neither α- nor β-D-mannopyranosyl phosphate was a hapten. The results are consistent with the presence of a haploid-specific antigen containing an acid-labile mannose determinant and show that the amount of this antigen in a-cell mannan is increased in response to α-factor.

α-Factor, a peptide sex pheromone of the yeast *Saccharomyces cerevisiae*, is produced by cells of the α-mating type and induces premating behavior in a-mating type cells. The affected cells stop dividing, enlarge, and elongate (3). Initiation of DNA synthesis is inhibited, and the cells arrest in G1 of the cell cycle. α-Factor also induces sexual agglutinability of the a-cells (5). a-Cells produce a pheromone that similarly affects α-cells (for a review, see reference 13). Cells of opposite mating type fuse at the tips of their extensions, and the intervening cell walls are digested away before nuclear fusion occurs.

The morphogenesis induced by α-factor is accompanied by changes in cell wall composition and structure (11, 24). The cells make more glucan and less mannan than they do during vegetative growth, and the mannan has shorter side chains than normal. There is no observable destruction of cell wall during morphogenesis, but the cells become more susceptible to lysis by β1→3-glucanases (11).

This paper reports immunochemical and structural changes induced in a-cell mannan by α-factor. A previously unreported antigen has been detected on cells of both mating types, and α-factor appears to modulate its expression in the mannan of a-cells.

MATERIALS AND METHODS

Organisms and growth conditions. The *S. cerevisiae* strains used were X2180-1A (a), X2180-1B (α), and X2180 (a/α), all wild-type strains; and the mannan mutants *mnn1-2* (a) (LB1-10B), *mnn1-2* (α) (LB1-22D), *mnn2-1* (a) (LB1-3B), *mnn2-1* (α) (LB1-16A), *mnn3-1* (a) (LB54-2C), *mnn3-1* (α) (LB54-6B), and *mnn4-1*(a) (LB6-5D). All strains are available from the Yeast Genetics Stock Center, University of California, Berkeley.

They were grown in a minimal salts medium containing 2.2 g of yeast nitrogen base without amino acids and ammonium sulfate (Difco Laboratories), 4.5 g of $(NH_4)SO_4$, and 20 g of D-glucose per liter of distilled water. Liquid cultures were incubated at 30°C on a rotary shaker. Slants and plates were made from the same medium containing 2% agar. Cells in liquid suspension were counted in a hemocytometer to calibrate cell density against absorbance at 660 nm, and cell density was subsequently determined spectrophotometrically.

Methods for determination of carbohydrate, reducing sugar, amines, and phosphate were as previously described (10, 11).

α-Factor preparation. α-Factor was determined by the serial dilution morphogenesis assay of Duntze et al. (4), modified as previously described (11). α-Factor used for this study was purified 4,000-fold by the following modification of the published procedure (4). Chilled supernatant solutions from stationary-

phase cultures of X2180-1B cells were absorbed onto Amberlite CG-50 (Mallinckrodt, 2.5 g/liter of culture) by passage over a column at 100 ml/min at 4°C. The column was washed sequentially with 0.1 M acetic acid and 0.5 M pyridinium acetate, pH 4.2. The pheromone was quantitatively eluted with 3.5 M pyridinium acetate, pH 5.5, and the combined active fractions were diluted into ice water and lyophilized. The specific activity of the peak tubes was 800 to 1,000 U/mg (dry weight), representing a 4,000- to 5,000-fold purification. α-Factor used as a hapten was purified further by extraction with 50 mM methanolic ammonium acetate, followed by gel filtration on Sephadex LH-20 (Pharmacia Fine Chemicals) in 10 mM ammonium acetate in methanol and thin-layer chromatography on silica gel in *n*-butanol-acetic acid-water (4:1:5, vol/vol) by the method of Duntze et al. (4). This purified material had a specific activity of 16 U/nmol and contained 4% impurities by amino acid analysis.

Mannan isolation. Log-phase cultures of a-cells (3×10^6 to 4×10^6 cells/ml) were treated with α-factor (1 U/ml) for 4 h at 30°C with rotary shaking. The cultures were then centrifuged, and the cells were washed twice with distilled water. They were suspended in an equal volume of 20 mM sodium citrate buffer, pH 7.0, and autoclaved 90 min. The insoluble residue was separated by centrifugation and reextracted in 1.5 volumes of the same buffer. The extracts were combined, dialyzed against water, and lyophilized. The mannan was precipitated from the redissolved material by the method of Lloyd (12). Cetyl trimethyl ammonium bromide (Cetavlon; Sigma Chemical Corp.) was added in aqueous solution to a final concentration of 1 g/g (dry weight) of extract. Mannan was precipitated as its borate complex by addition of H_3BO_3 and titration to pH 8.8. The precipitate was washed with ethanol and acetone, dissolved in water, dialyzed against distilled water, and lyophilized.

Analyses of mannan. Total hydrolysis of polysaccharides was carried out in 1 M trifluoroacetic acid at 120°C for 90 min. The acid was removed by repeated evaporation from water under reduced pressure. Treatment in 0.01 N HCl at 100°C for 30 min was used to hydrolyze especially labile glycosidic bonds. Sodium periodate oxidation of X2180-1A mannan was done in water in the dark at 0°C, using 2 mol of $NaIO_4$ per mol of mannose, and consumption of the oxidant was followed by the method of Avigad (1). Gel filtration was carried out in water on Bio-Gel P-2 (200 to 400 mesh) or P-4 (400 mesh).

Descending paper chromatography of carbohydrates was done on Whatman no. 1 paper in one of the following solvents: (i) *n*-butanol-pyridine-water (10:3:3); (ii) ethyl acetate-pyridine-water (5:3:2); (iii) ethyl acetate-acetic acid-water (3:3:1); or (iv) methanol–28% ammonia–water (6:1:3). Samples for gas-liquid chromatography were first reduced with a fivefold excess of $NaBH_4$ in water for several hours. A drop of glacial acetic acid then destroyed the excess borohydride, and the mixture was evaporated under reduced pressure to dryness and repeatedly evaporated from methanol to remove borate. A 50-μl amount of acetic anhydride was added, and the alditols were acetylated at 110°C for 30 min. A Varian series of 1600 gas chromatograph was run at 190°C with an 8-foot column of 3% OV-210 on Supelcoport or with a 5-foot (ca. 150-cm) column of SE-30 on 100/110-mesh ABS (Varian Associates). Mass spectra were taken on a DuPont model 21-491 mass spectrometer interfaced with the gas chromatograph.

Bacterial exo-α-mannanase was purified through the $(NH_4)_2SO_4$ step of Jones and Ballou (8). The enzyme was incubated with mannan in 50 mM sodium phosphate buffer, pH 6.8, containing 0.1 mM Ca^{2+}. Trypsin (Sigma type XII) and pronase (Calbiochem, B grade) digestions were done in 50 mM Tris-chloride (pH 8.5)–0.1 mM Ca^{2+} at 30°C overnight in 1 mM NaN_3.

Immunochemical methods. Rabbit antibodies to yeast mannan were raised by intravenous injection of 10^9 heat-killed cells in 1 ml of 0.15 M NaCl three times weekly for 4 weeks. The rabbits were subsequently bled and given an intravenous booster at weekly intervals. Sera used for complement fixation or quantitative precipitin analyses were obtained on or after week 10 of immunization. Antisera were absorbed by incubation with heat-killed cells (100 mg, dry weight, per ml of serum) or by passage over a mannan affinity column prepared by the method of March et al. (14). Sepharose 2B (Pharmacia) was activated with CNBr for 2 min, and purified mannan from X2180 was coupled to the beads. Undiluted antiserum raised against X2180 diploid cells was absorbed onto a mannan column at the pH of the serum and eluted with 0.1 M acetic acid. Purified immunoglobulin was digested with pepsin to yield $F(ab)_2$ fragments that were coupled to fluorescein by published procedures (6).

Quantitative microprecipitin analyses were done, using 50 μl of serum per tube (2). Microcomplement fixation tests followed the procedure of Wasserman and Levine (25), using 1/10 the stated volumes in test tubes (10 by 75 mm) with Isotris buffer as diluent. For hapten inhibition of complement fixation, the hapten was mixed with the antibody in 0.4 ml of buffer and incubated at 25°C for 1 h. The complement and antigen were then added (0.2 ml), and the reaction was carried out. It was necessary to do hapten controls in the presence of antibody and complement, because several potential haptens possessed anti-complementary activity. These haptens were assayed by precipitin reactions.

Cellular agglutination titers were done in microtiter plates, each well containing 50 μl of 0.9% NaCl diluent and 50 μg (dry weight) of cells. Ouchterlony double diffusion was carried out in petri plates containing 1% agar in Isotris buffer (16). Table 1 lists haptens and mannans used as immunochemical reagents. The mannosyl phosphates were free of contaminants as judged by paper chromatography in systems C and D. The β-D-mannopyranosyl-1-phosphate (17), a gift from E. J. Behrman, exhibited the expected proton magnetic resonance for the anomeric proton of a β-glycosyl phosphate at $\tau = 5.11$.

Immunofluorescence. Cells, incubated with rabbit anti-mannan sera for 20 min at 0°C in Isotris buffer, were centrifuged, washed twice in the same buffer, and then incubated under the same conditions with fluorescent goat antibody to rabbit immunoglobulin (Antibodies Inc., Davis, Calif.). The cells were then washed three times with buffer before they were examined for fluorescence. The goat antibody was pread-

TABLE 1. *Materials used for immunochemical studies*

Reagent	Structure/immunodominant determinant	Reference
Hapten		
Mannotrioses	αMan→^{2}αMan→^{2}Man[a]	9
	αMan→^{3}αMan→^{2}Man[a]	9
	αMan→^{6}αMan→^{6}Man	15
Mannotetraoses	αMan→^{2}αMan→^{2}αMan→^{2}Man	10
	αMan→^{3}αMan→^{2}αMan→^{2}Man	9
	βMan→^{4}βMan→^{4}βMan→^{4}Man	22
Mannopentaose	αMan→^{6}αMan→^{6}αMan→^{6}αMan→^{6}Man	15
Yeast mannan		
S. cerevisiae X2180	αMan→^{3}αMan→	21
Kloeckera brevis	αMan-PO$_4$→	18
Kluyveromyces lactis	αGlcNAc→	19
Hansenula polymorpha	αGlc-PO$_4$→	10

[a] These two isomers occur together in a mixture.

sorbed with α-factor-treated a-cells to lower the background fluorescence. When the fluorescein-F(ab)$_2$ preparation was used, the treatment with fluorescent goat antibody was eliminated.

RESULTS

Acid hydrolysis of mannan. Total acid hydrolysis of mannans from control a-cells and pheromone-treated a-cells yielded mannose and trace amounts of glucose and *N*-acetylglucosamine (paper chromatography in solvent A). Hydrolysis of yeast mannan in 10 mM HCl releases the sugars in phosphodiester linkage, and with *S. cerevisiae* mannan the saccharide products are α1→3-linked mannobiose and mannose (21). When mannan from α-factor-treated a-cells was hydrolyzed and the oligosaccharides were resolved on Bio-Gel P-2, the ratio of mannose to mannobiose was not significantly different from that of untreated a-cell controls. However, when mannan from cells labeled with D-[6-^{3}H]glucose during α-factor treatment (11) was hydrolyzed in 10 mM acid, the mannose fraction was much more highly labeled than the mannobiose fraction (Table 2). Paper chromatography showed that 85% of the radioactivity in the monosaccharide fraction had the mobility of mannose and that all of the radioactivity in the disaccharide cochromatographed with mannobiose (Fig. 1). Furthermore, there was no ninhydrin-positive material in these fractions. Gas-liquid chromatography of the alditol acetate of the monosaccharide peak showed that mannose was the only carbohydrate present (Fig. 2), and mass spectrometry confirmed the major peak as an alditol hexaacetate. The minor peaks gave fragments with even *m/e* ratios and were not identifiable as carbohydrate derivatives. Chromatography of mild-acid-hydrolyzed mannans on Bio-Gel P-4 revealed no consistent difference between control and experimental mannans, whether the columns were assayed for carbohydrate, reducing sugar, phosphate, protein, or amines.

TABLE 2. *Release of mannose and mannobiose by mild-acid hydrolysis of mannans*

Source of mannan	Mannose/mannobiose ratio	
	Carbohydrate[a]	Radioactivity[b]
Control a-cells	0.72	0.60
	0.60	0.32
	0.39	0.46
		0.15
		0.53
		0.87
	0.57 ± 0.16[c]	0.49 ± 0.22[c]
α-Factor-treated a-cells	0.72	2.38
	0.82	1.56
	0.79	2.59
		2.51
		4.72
	0.78 ± 0.06[c]	2.75 ± 1.17[c]

[a] By phenol-sulfuric assay.

[b] By scintillation counting of the products from mannan formed during growth in D-[6-^{3}H]glucose.

[c] Value represents mean ± standard deviation.

Immunochemistry of mannan from α-factor-treated a-cells. Rabbit antisera raised against α-factor-treated a-cells reacted with homologous cells and with control cells, agglutination titers being higher with the former cell type (Fig. 3). The antisera also precipitated the mannans isolated from X2180 diploid or from α-factor-treated or control a-cells. The reaction with mannan from X2180 was due to the presence of antibodies directed against the α1→3 mannosyl determinant, because the reaction was completely inhibited by the mannotetraose αMan→^{3}αMan→^{2}αMan→^{2}Man isolated from X2180, but not by other haptens. In contrast, the precipitin reaction with mannan from α-factor-treated a-cells was inhibited only 50% by the tetrasaccharide (Fig. 4). This result showed that another determinant was present in this man-

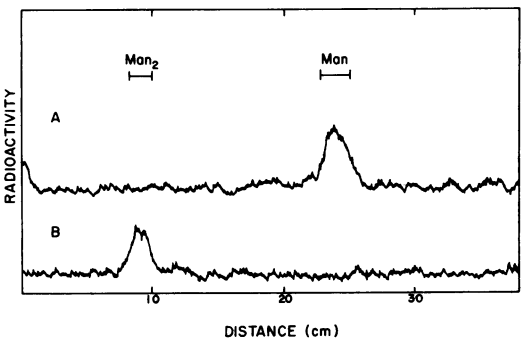

FIG. 1. *Paper chromatography (solvent B) of saccharides released from mannan of α-factor-treated* **a***-cells by hydrolysis in 10 mM HCl. (A) Monosaccharide fraction; (B) disaccharide fraction; the standards are mannose (Man) and α(1→2)-mannobiose (Man_2) (9).*

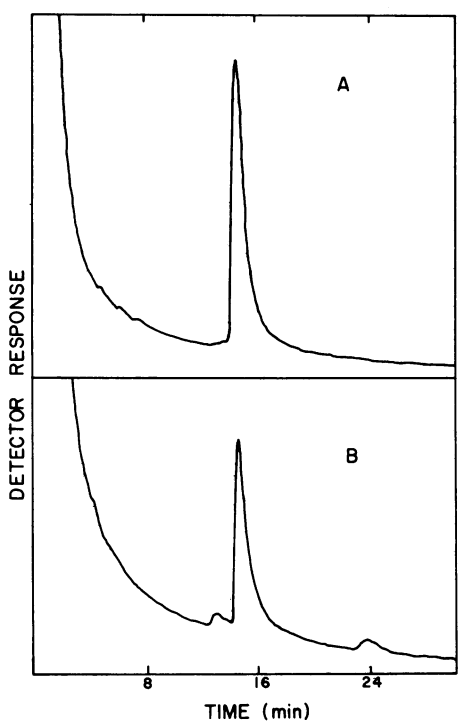

FIG. 2. *Gas-liquid chromatograms of monosaccharide alditol acetates released from mannan by hydrolysis in 10 mM HCl. The monosaccharides were isolated from the hydrolyzed mannan by gel filtration on Bio-Gel P-2, then reduced and acetylated and subjected to chromatography on OV-210 at 180°C. Mannans were from (A) control* **a***-cells, and (B)* **a***-cells treated with α-factor. The major peak had the retention time of mannitol hexaacetate.*

nan. Double diffusion analysis showed that the sera reacted with a mannan antigen not present in mannan from diploid cells.

The antisera lost reactivity toward the αMan→^{3}αMan→determinant if they were adsorbed with X2180 cells or mannan. Such sera no longer agglutinated diploid cells, although they retained activity against haploid cells. Although the adsorbed sera did not precipitate mannan from diploid cells, it did react with haploid cell mannans, confirming the presence of a haploid-specific antigenic determinant. The serum agglutinated α-cells and α-factor-treated **a**-cells at agglutination titers 2^3 to 2^4 higher than for control **a**-cells. Double diffusion analyses showed that only the mannans from α-cells and α-factor-treated **a**-cells reacted with serum. These two mannans reacted to give a single faint precipitin line that appeared fully fused across the diffusion front.

The diploid-adsorbed sera showed similar

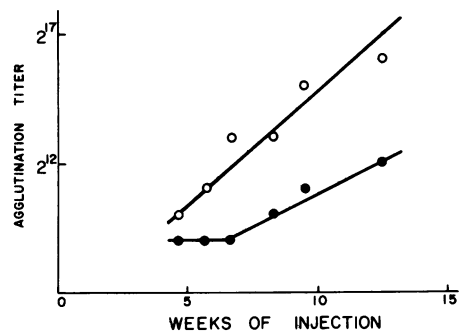

FIG. 3. *Agglutination titers of rabbit sera during immunization with α-factor-treated* **a***-cells. Titers were measured on unadsorbed sera with control* **a***-cells (●) and α-factor-treated* **a***-cells (○). Details are given in the text.*

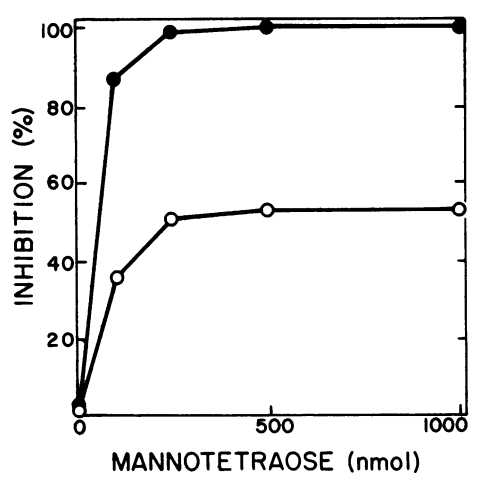

FIG. 4. *Inhibition of mannan immunoprecipitation by the mannotetraose from S. cerevisiae mannan. The mannans from α-factor-treated* **a***-cells (○) and X2180 diploid cells (●) were reacted with unadsorbed serum against α-factor-treated* **a***-cells.*

specificity in quantitative precipitin and microcomplement fixation analyses. The serum was equally reactive toward mannans from α-cells and α-factor-treated **a**-cells (Fig. 5). However, control **a**-cell mannan reacted only at much higher mannan concentration. This result suggested that the determinant was present in control **a**-cell mannan, but at lower concentration. To test this hypothesis, we adsorbed several antisera with control **a**-cells. Such sera lost all activity toward mannan from α-cells and α-factor-treated **a**-cells. Therefore, the antibody to the haploid-specific determinant was adsorbed onto the control **a**-cells. Such sera retained antihaploid activity if adsorbed with diploid cells.

Properties of the antigen. The structure of the haploid-specific determinant was investigated by using mannan from α-factor-treated **a**-cells as antigen. Several yeasts have determinants other than the αMan→^{3}αMan→ structure of *S. cerevisiae* X2180, and the reaction of such antigen-antibody systems with that from *S. cerevisiae* haploids would demonstrate the presence of immunologically related structures. Mannan from treated **a**-cells was not precipitated by wheat germ agglutinin (specific for *N*-acetylglucosamine residues) or by antiserum to *S. cerevisiae mnn1-2* (specific for mannopyranosyl phosphate). Adsorbed anti-haploid serum did not cross-react by immunodiffusion or microprecipitation with mannan from *Hansenula polymorpha* or *Kluyveromyces lactis*. Unadsorbed anti-haploid serum reacted with the latter mannan, but the tetrasaccharide αMan→^{3}αMan→^{2}αMan→^{2}Man was an effective hapten, suggesting that the reactive antibody was that directed against the αMan→^{3}αMan→ determinant. Haptenic structures for these yeasts are shown in Table 1.

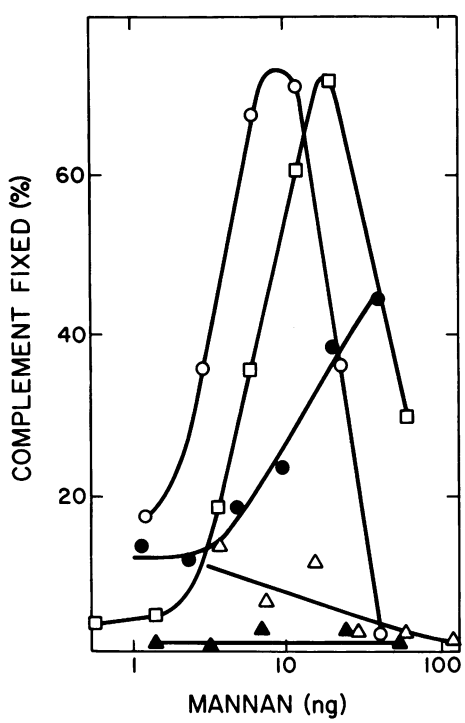

FIG. 5. *Complement fixation by diploid-absorbed antiserum with mannan antigens. Mannans were from α-factor-treated* **a**-*cells* (○), *control* **a**-*cells* (●), *α-cells* (□), *X2180 diploid cells* (△), *or mannan from α-factor-treated* **a**-*cells heated at 100° C with 10 mM HCl for 30 min* (▲).

Lability of the haploid-specific antigen. The determinant was destroyed by periodate oxidation of the mannan for 2 h in the dark at 0°C, during which 0.34 mol of periodate was consumed per mol of hexose. Antigenic activity was not altered by extensive digestion with pronase or trypsin, or by release of 25% of the mannosyl residues by exo-α-mannanase. This study suggests that, although the determinant contains carbohydrate, it is not an ordinary pyranoside because 30-min hydrolysis (100°C in 0.01 M HCl) of mannan from pheromone-treated **a**-cells destroyed the haploid-specific activity (Fig. 5). That the antigen was destroyed and not rendered monovalent was shown by assaying untreated mannan in the presence of a 100-fold excess of acid-treated mannan. No inhibition of complement fixation was observed. Acetolysis of the mannan also destroyed the antigen.

Hapten inhibition. Glycosyl phosphates are common mannan antigens that are destroyed by mild acid hydrolysis (10, 18). Because mannose was the only monosaccharide released by this treatment, we tested the two anomeric D-mannopyranosyl phosphates as haptens. Neither was active. Of the other saccharides listed in Table 1, none except the acetolysis tetrasaccharide mentioned above inhibited the precipitin reaction of unadsorbed sera with mannan from pheromone-treated **a**-cells. These results were confirmed by hapten inhibition of complement fixation with antiserum adsorbed by diploid cells. None of the sugars inhibited the reaction at concentrations up to 20 μM, although the reaction of anti-diploid sera with diploid mannan was completely inhibited by 7 μM mannotetraose. The reaction was not inhibited by 10 μM α-factor, implying that the antigen is not surface-bound pheromone. Neither D-glucose nor *N*-acetyl-D-glucosamine had haptenic activity at concentrations up to 20 mM.

Immunofluorescence patterns. Control **a**-cells reacted only weakly with diploid-adsorbed antisera by this test, whereas α-cells fluoresced brightly under similar conditions. Pheromone-

treated **a**-cells fluoresced brightly in the region of cell extension, and dimly in the rest of the cell. The same staining pattern is observed in the metamorphosed cells with fluorescent antibody to X2180 cells (Fig. 6).

Response of mannan biosynthesis mutants to α-factor. Cells carrying mutations in the *MNN1*, *MNN2*, *MNN3*, and *MNN4* genes have cell wall mannans of altered structure (Fig. 7). The **a** strains of these mutants all responded normally to α-factor and are all competent to mate (20). Furthermore, all the *mnn* mutants agglutinated with wild-type cells of the opposite mating type after a 1-h induction with culture fluid from wild-type cells of the opposite mating type.

The presence of an *mnn* mutation does, however, prevent expression of the haploid-specific antigen. Cetavlon-precipitated mannans from **a** mating type *mnn* mutants that had been treated with α-factor failed to react with haploid-specific antiserum. Furthermore, mild-acid hydrolysis released no oligosaccharide from the *mnn2* and *mnn4* strains, and it released only mannose from a *mnn1* mutant. These are results expected from previous studies of mannans from untreated *mnn* mutants.

DISCUSSION

Yeast mannoproteins contain two types of saccharide chains (Fig. 7), the manno-oligosaccharides linked to seryl and threonyl residues that are released by alkali under mild conditions, and the polysaccharide chain attached to asparaginyl residues in the peptide through di-*N*-acetyl-chitobiose units. The latter consists of a core with 15 mannose residues composed of an α1→6-linked backbone with short side chains attached in α1→2 and α1→3 linkage, and an outer chain of similar structure. This portion contains the phosphodiester units, which in *S. cerevisiae* X2180 consist of α1→3-mannobiosyl phosphate and α-mannosyl phosphate linked to position 6 of sugars in the side chains. The relative amounts of the various mannan side chains can be determined by gel filtration of the products of acetolysis and are similar in **a** and α-cell mannan. Mannan is the major antigen in yeast, and hapten inhibition studies indicate that the bulk of the antibody activity is directed against

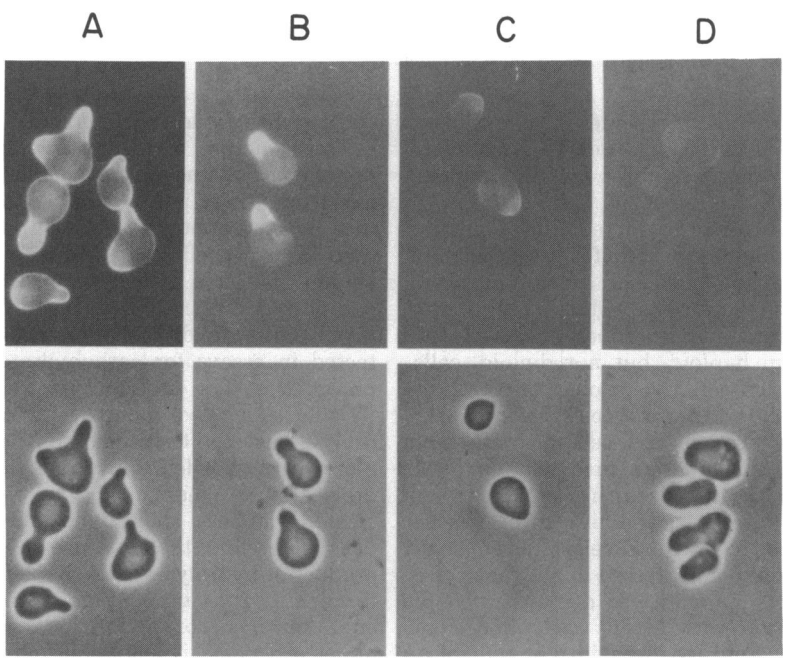

FIG. 6. *Direct immunofluorescence of S. cerevisiae* **a**- *cells with serum specific for α1→3-mannosyl units. The cells were incubated with fluorescein-conjugated $F(ab)_2$ fragments prepared from antibodies isolated on an affinity column of Sepharose-coupled mnn4 mannan. The top row shows the fluorescence patterns, and the bottom row shows phase-contrast pictures of the same fields. The* **a**-*cells were exposed to α-factor for (A) 6 h, (B) 3 h, and (C) 1 h. In D, cells exposed for 6 h were treated with antibody in the presence of the manno-tetraose hapten side chain from S. cerevisiae mannan (Table 1). Similar results were obtained by using antiserum that was specific for α-factor-treated* **a**-*cells, except that the labeling of the cells was not reversed by the addition of the manno-tetraose.*

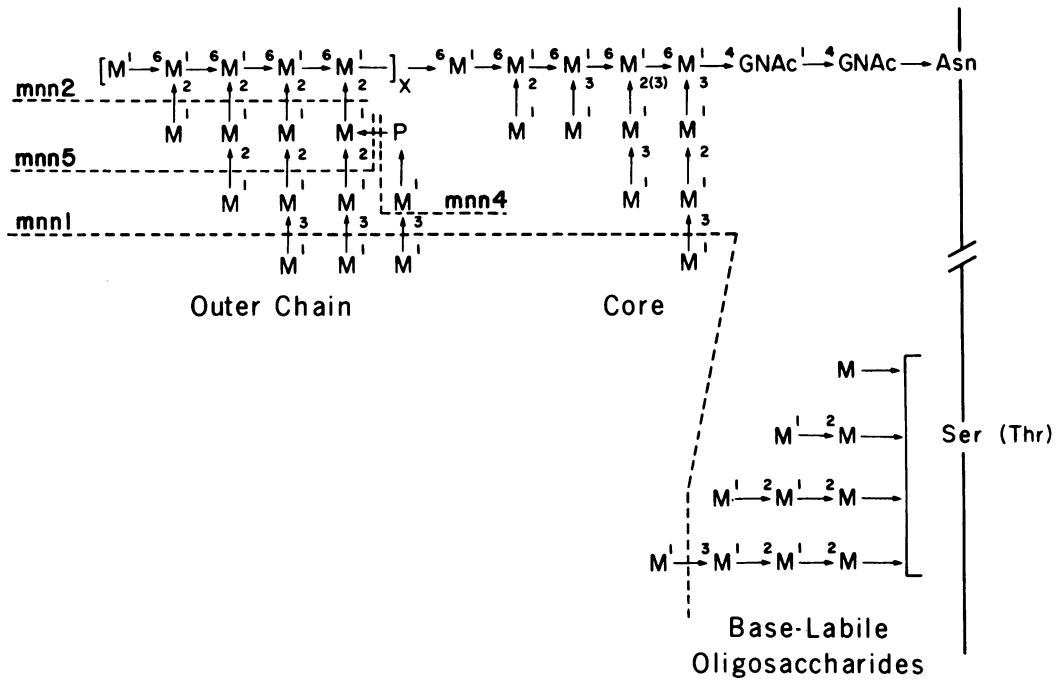

FIG. 7. *Structure of S. cerevisiae X2180 mannan. M represents α-D-mannopyranosyl residues and P is phosphate. That portion of the structure eliminated in each of the mnn mutants is indicated by the broken lines. The mnn5 mutant is a new isolate (R. E. Cohen and D. L. Ballou, unpublished data) that is similar to the mnn3 mutant but has a more clearly defined chemotype.*

α1→3-linked mannosyl residues in *S. cerevisiae* X2180 (2). In yeast lacking the α1→3-mannosyl determinant, other structures are immunodominant (Table 1).

Antiserum to α-factor-treated **a**-cells contains a class of antibodies specific for a determinant not present on diploid cells. After removal of antibodies specific for the αMan→^{3}Man determinant, the remaining antibodies reacted with mannans from haploid, but not diploid, cells. Complement fixation and whole-cell agglutination studies showed the haploid-specific determinant to be present in α-cell mannans at a higher concentration than in **a**-cell mannans. Treatment of the **a**-cells with α-factor for 4 h caused them to express the antigen in concentrations similar to that in α-cells. That a single class of antigen was expressed in all three types of haploid cell mannan was demonstrated by immunodiffusion with α-cells and pheromone treated **a**-cells, and by the ability of control **a**-cells to absorb all antibodies directed against haploid cells. Although expression of the haploid-specific antigen is modulated by α-factor, the antigen is not required for mating, because the mannan mutants fail to express it yet mate normally (20). The absence of the determinant in the *mnn* mutants also implies that its presence in the mannan is dependent on some structural feature of the wild-type mannan.

The structure of the haploid-specific determinant is not known. It is labile to periodate oxidation and to mild-acid hydrolysis, implying that it could contain a glycosyl phosphate, furanoside, or acyclic acetal. The only small products of mild acid hydrolysis we identified were mannose and mannobiose. If another structure is present, its hydrolysis products were not detected in assays for carbohydrate, amines, or phosphates. The products released from D-[6-^{3}H]glucose-labeled mannan by mild-acid hydrolysis revealed that the mannose fraction was more highly labeled than the mannobiose. Hydrolysates of mannans from *mnn* mutants treated similarly showed no labeled mannose peak. Therefore, the labeled mannose may be a product of hydrolysis of the haploid-specific determinant. It is unlikely that this reflects an enhanced incorporation of mannosylphosphate units; otherwise it should be observed in the *mnn1* mutant.

The lack of reactions of the haploid cell mannans and their homologous antisera with other yeast antisera and mannans rules out several structures as the determinant. For example, α-mannopyranosyl phosphate is a common-acid labile yeast antigen. Such a structure can be excluded because haploid cell mannan does not

react with antiserum raised against *S. cerevisiae mnn1* cells, which has this structure as its primary determinant; nor does antiserum raised against pheromone-treated a-cells react with the α-mannopyranosyl phosphate determinant in *Kloeckera brevis* mannan. Hapten inhibition studies also ruled out this structure as well as α-glucopyranosyl phosphate and other mannan determinants.

It is unlikely that the determinant is an acyclic acetal (7), because such a component, if periodate labile, should react with alkaline silver nitrate on paper chromatograms. Furthermore, the acetal should be labeled during morphogenesis in the presence of radioactive glucose and would appear as a radioactive component on chromatograms of acid hydrolysates. No such compound was observed. The determinant is not adsorbed α-factor, because the pheromone is not a hapten. A determinant containing mannofuranoside, on the other hand, would be consistent with the data.

Mannan from haploid cells is clearly different from that of diploid cells. The ratio of side chains to backbone is reduced in the haploids (23), and an antigen is expressed that is absent from diploid cell mannans. Both the side chain ratio and the concentration of the antigen are further altered in a-cells after they respond to the sex pheromone α-factor.

The results of the immunofluorescence experiments have implications for the structure of the cell wall in general. Antibodies to both haploid and diploid cells preferentially stain the tips of metamorphosed a-cells. Staining with fluorescent concanavalin A gives a similar pattern (24). Control cells reacted poorly with each reagent. Therefore, mannan must be more exposed to these reagents at the tips of the metamorphosed cells but is relatively inaccessible in untreated cells.

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