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Schizosaccharomyces pombe glycosylation mutant with altered cell surface properties

(galactomannoprotein/core oligosaccharides/proton NMR/invertase/chromatography)

CLINTON E. BALLOU*†, LUN BALLOU*, AND GRAHAM BALL‡

Departments of *Molecular and Cell Biology and ‡Chemistry, University of California, Berkeley, CA 94720

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ABSTRACT **Mutagenesis of *Schizosaccharomyces pombe* cells yielded a strain that made reduced amounts of invertase. A comparison of the O- and N-linked carbohydrate chains of the wild-type and mutant glycoproteins revealed that a single type of α1→2-linked mannose was missing in the mutant. Analysis of the wild-type galactomannoprotein showed that it contained a heterogeneous small "core" oligosaccharide fraction linked to asparagine with sugar compositions that ranged from $Man_9(GlcNAc)_2$- to $Gal_4Man_{10}(GlcNAc)_2$-. The galactose units are in terminal positions of a $Man_{10}(GlcNAc)_2$- unit that is similar to the mannoprotein core of *Saccharomyces cerevisiae*. Attached to this core in a larger oligosaccharide fraction is an α1→6-linked polymannose chain that is substituted at position 2 with α-linked mannose and galactose. The O-linked sugars consist of mannose, α1→2-linked mannosylmannose and α1→2-linked galactosylmannose, along with small amounts of tri- and tetrasaccharides. The glycosylation mutant lacks α1→2-linked mannose on both the O-linked chains and the outer chain of the large N-linked chains, suggesting that it may be defective in regulation of an α1,2-mannosyltransferase that adds mannose to glycoproteins in the Golgi.**

Schizosaccharomyces pombe is a fission yeast that has attracted interest as an experimental organism for studies on control of the cell cycle (1) and glycoprotein synthesis (2). The carbohydrate component of *Sch. pombe* glycoproteins is distinctive in that it contains galactose in addition to the mannose found in most fungal glycoproteins (3), which has led to the name *galactomannoprotein* (4) for this class of molecule. Although its carbohydrate structure has not been elucidated in the same detail as that of *Saccharomyces cerevisiae* mannoproteins (5), the *Sch. pombe* galactomannoproteins are known to contain both O- and N-linked carbohydrate chains.

Here we describe experiments to characterize the carbohydrate components of *Sch. pombe* glycoproteins by the methods applied to those from *Sac. cerevisiae* (5). We also report a glycosylation-defective mutant of *Sch. pombe* that fails to add a specific type of α1→2-linked mannose to both O- and N-linked carbohydrate chains. The mutant cells are flocculant and difficult to disperse, as though their surface properties have been altered by the change in carbohydrate structure.

MATERIALS AND METHODS

Materials. Two mating types of *Schizosaccharomyces pombe* were used, ATCC nos. 24969 and 24970, and were grown and maintained on standard medium (6). Mutagenesis with ethyl methanesulfonate was done as described (7), and the cells were allowed to grow overnight on a shaker at 30°C to express any mutant phenotypes. Unsuccessful attempts were made to enrich for cells that lacked galactose in the glycoproteins by agglutination with *Bandeiraea simplicifolia* lectin (8). During these manipulations, several flocculant isolates were obtained, and two of these made less glycosylated invertase (strains Sp331 and Sp421). Sp331 was used in the studies reported here. Genetic analysis followed standard procedures (7). Coffee bean α-galactosidase (9) was from Sigma.

Chromatography. Mannoprotein, obtained by Cetavlon (Sigma) precipitation, was fractionated on a DEAE-Sephacel column (5). The Dionex (Sunnyvale, CA) system, with a CarboPac PA1 column, and a pulsed amperometric detector (PAD) detector, was used for HPLC analysis of hexitols (solvent A, 5 mM NaOH), monosaccharides and the products of reductive β-elimination (solvent B, 25 mM NaOH), the acetolysis fragments (solvent C, 25 mM sodium acetate in 100 mM NaOH), and oligosaccharides with 10–20 hexose units (solvent D, 50 mM sodium acetate in 100 mM NaOH). Preparative separation of these small carbohydrates was done by gel filtration on a Bio-Gel P-2 or P-4 column (2 × 190 cm; Bio-Rad) by elution with water, and the peaks were detected with the phenol/sulfuric acid reagent. Small amounts of oligosaccharide, sufficient for proton NMR, were also recovered from the effluent of material fractionated on the Dionex system. *Sch. pombe* invertase (10) was extracted, electrophoresed, and detected as described (5).

Chemical Methods. Saccharides were hydrolyzed in 2 M trifluoroacetic acid (110 for 3 hr), and the hydrolysates were evaporated under vacuum to remove the acid. Partial acetolysis (5) of oligosaccharides was done in a mixture of acetic acid/acetic anhydride/sulfuric acid, 25:25:1 (vol/vol), at 40°C for 24 hr. The resulting acetylated material was deacetylated in dry methanol by adding sodium methoxide, and the residue was dissolved in water for analysis on the Dionex system. The reaction was scaled up for larger amounts of oligosaccharide, and the product was fractionated on a Bio-Gel P-2 column. For β-elimination, the glycoprotein was dissolved in 0.1 M NaOH, and $NaBH_4$ was added to make a 1 M solution. After several days at room temperature, Dowex 50W (hydrogen form) was added to acidify the reaction, and the solvent was evaporated under vacuum. The boric acid was removed by addition and evaporation of methanol, the residue was dissolved in water and centrifuged through an Amicon Centricon 10 filter to remove residual glycoprotein, and the filtrate was analyzed on the Dionex system. For large-scale reactions, the product was fractionated on a Bio-Gel P-2 column. Periodate oxidation was done in 10 mM $NaIO_4$ in the dark for 3 days, and NMR measurements were done as described (11).

RESULTS AND DISCUSSION

Many early studies on the soluble mannan extractable from yeast cells were done before the glycoprotein nature of such

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†To whom reprint requests should be addressed.

material was recognized. Generally, mannans were found to consist of α1→6-linked polymannose chains, usually with α1→2- and α1→3-linked mannose side chains (3). Gorin *et al.* (12) showed that the mannan from *Schizosaccharomyces* species had a similar structure, but that the side chains also contained α1→2-linked galactose. We have extended these studies by analyzing the structure of the bulk glycoprotein that is extractable from *Sch. pombe* cells with hot citrate buffer (5). The glycoprotein was purified by precipitation of the Cetavlon–borate complex followed by DEAE-Sephadex chromatography (Fig. 1). The yield of glycoprotein that bound to the column was 160 mg of carbohydrate from 22 g of wet cell paste. It contained 11% protein by weight.

Structure of the O-Linked Carbohydrate. Treatment of the glycoprotein under reductive β-elimination conditions released about 20% of the carbohydrate in the form of reduced mono- and disaccharides (Fig. 2*A*; in solvent B). The monosaccharide component (peak 1 in Fig. 2*A*) was mannitol (determined by Dionex HPLC with solvent A), as expected if mannose were attached to serine and threonine in the protein. Galactitol was not observed. The disaccharide was a mixture of two compounds, the minor one (peak 3) having the elution position of reduced α1→2-linked mannobiose (Fig. 2*B*; 3-O-α-D-mannopyranosyl-D-mannopyranose). The major disaccharide (peak 2 in Fig. 2*A*) was found to be α1→2-linked galactosylmannitol, which would be formed after release of the α1→2-linked galactosylmannose from its attachment to a hydroxyamino acid. The reduced disaccharide component was isolated by gel filtration on a Bio-Gel P-2 column, which does not separate the isomers, and a portion was hydrolyzed in acid. Analysis of this hydrolysate revealed the presence of mannose, galactose, and mannitol in the molar ratio 1:2:3.6, which is consistent with the disaccharide component being a 1:2 mixture of α1→2-linked mannosylmannitol and galactosylmannitol. The reduced trisaccharide (peak 4 in Fig. 2*A*) was converted to galactose and αMan→^{2}ManH$_2$ (short form, Manα2ManH$_2$) by digestion with α-galactosidase. The α1→2 linkage between mannose and mannitol in all of these compounds was confirmed by the release of 1 mol of formaldehyde upon periodate oxidation.

Peak 5, isolated by fractionation on a Bio-Gel P-2 column, was found by HPLC after acid hydrolysis to consist of mannitol, galactose, and mannose in the molar ratio 1:2:1, consistent with it being a reduced tetrasaccharide. The HPLC retention time was not affected by treatment with jackbean α-mannosidase, whereas digestion with α-galactosidase rapidly removed one galactose and slowly removed a second to produce αMan→^{2}ManH$_2$. Periodate oxidation destroyed all three hexoses and released 1 mol of formaldehyde per mol of tetrasaccharide, which is consistent with α1→2 linkages

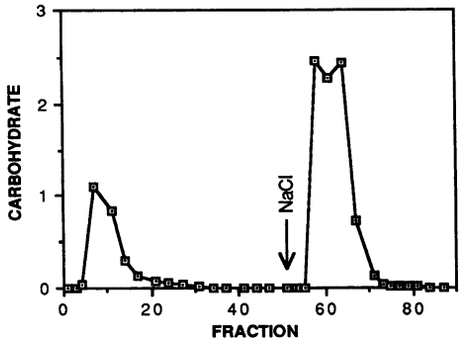

FIG. 1. Ion-exchange purification of galactomannoprotein. The material recovered from the Cetavlon–borate complex was fractionated on a DEAE-Sephadex column (5). The bound peak that was eluted by a salt gradient was dialyzed and lyophilized and used for further study.

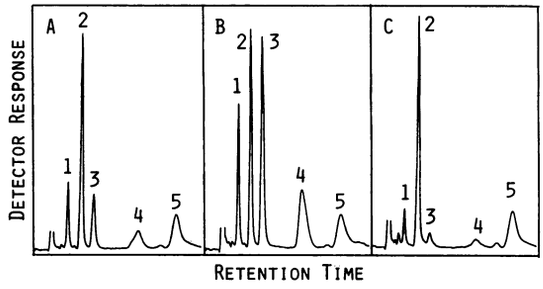

FIG. 2. Dionex pattern of reductive β-elimination fragments from *Sch. pombe* glycoprotein. Peaks: 1, mannitol; 2, α1→2-linked galactosylmannitol; 3, α1→2-linked mannosylmannitol; 4, reduced trisaccharides; 5, reduced tetrasaccharide. (*A*) Sp69 isolate. (*B*) Sp69 spiked with mannitol, α1→2-linked mannobiitol, and α1→2-linked mannotriitol. (*C*) Sp331 isolate. Solvent B was used.

between all four units. The proton NMR spectrum of peak 5 showed proton signals on C-1 (H1 signals) for three sugars, one at δ5.30 chemical shift and two at δ5.22. A correlated spectroscopy (COSY) spectrum showed that the first is coupled to an equatorial H2 signal at δ4.13, which identifies it as due to a mannose unit, whereas the other two signals are coupled to axial H2 signals at δ3.76 and δ3.83, as expected for galactose units. The probable structure is αGal→^{2}αGal→^{2}αMan→^{2}ManH$_2$.

Structure of the N-Linked Carbohydrate. Digestion of the glycoprotein with endoglucosaminidase H released about 17% of the carbohydrate in a form that was not retained when reapplied in low salt to the same DEAE column used in Fig. 1. This material was fractionated on a Bio-Gel P-4 column to give a major peak at the void (90% of the total carbohydrate) and a minor broad peak that was included (10% of the carbohydrate) (Fig. 3). Analysis of the latter (peaks B and C) on the Dionex system (solvent D) revealed several components with retention times comparable to the Man$_{8-15}$GlcNAc standards (13). This small-core material was partially resolved on a Bio-Gel P-4 column into a low-galactose fraction (Fig. 3, peak C) with the composition GlcN:Gal:Man of 1:0.5:8 and a high-galactose fraction (Fig. 3, peak B) with the composition GlcN:Gal:Man of 1:4.5:10. The low-galactose fraction, 15% of the small-core material, is separable on the

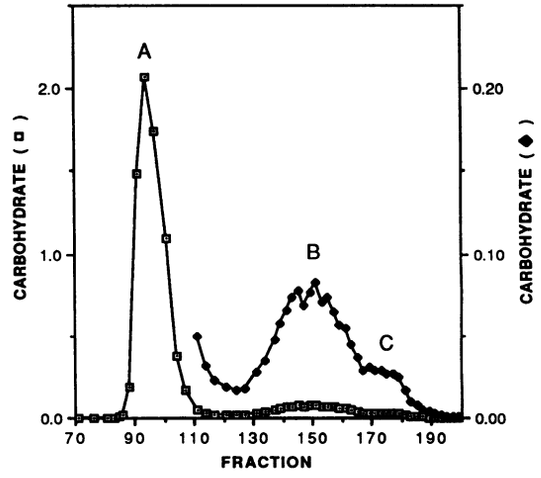

FIG. 3. Fractionation of N-linked oligosaccharides. The material, from digestion of the Sp69 galactomannoprotein with endoglucosaminidase H that failed to bind to DEAE-Sephadex was applied to a Bio-Gel P-4 (200–400 mesh) column (2 × 190 cm) that was eluted with water in 2-ml fractions. Carbohydrate was detected with the phenol/sulfuric acid reagent. Peaks: A, large oligosaccharides; B, small high-galactose oligosaccharides; C, small low-galactose oligosaccharides. ◆, Curve enlarged 10-fold.

Dionex system (solvent D) into six peaks with retention times of 6.0 min to 11.9 min. The high-galactose material, which represents 85% of the small core, is separable (solvent D) into at least seven peaks with retention times of 12.7 min to 33.1 min. For comparison, the retention times under these conditions for the *Sac. cerevisiae* core oligosaccharides $Man_{10}GlcNAc$ and $Man_{13}GlcNAc$ are 8.0 min and 21.1 min, respectively (13). None of the peaks in the low-galactose fraction corresponded to the *Sac. cerevisiae mnn9* $Man_{10}GlcNAc$, so this oligosaccharide does not appear to accumulate in *Sch. pombe*, but there was similarity to those found on the mannoproteins of other *Sac. cerevisiae* mutants (14).

The low-galactose core material was rechromatographed on a Bio-Gel P-4 column to give a galactose-free oligosaccharide with the composition $Man_{10}GlcNAc$ that had a proton NMR spectrum characteristic of the $Man_9GlcNAc$ initial protein glycosylation intermediate (15), along with a signal at δ4.93 for α1→6-linked mannose with the intensity of one proton (Fig. 4, spectrum A). The signal at δ5.05, intensity of three protons, demonstrates that none of the terminal α1→2-linked mannose is substituted, whereas the signal at δ4.93 shows that outer chain addition has been initiated, and the signal at δ5.39 indicates that most of the α1→2-linked mannose D_2 (15) has not been removed (compare with figure 3 in ref. 16). This mannose is also present in the galactose-containing oligosaccharides. These data are consistent with structure A in Table 1 (see *Note Added in Proof*).

The high-galactose core (Fig. 3, peak B) was fractionated by Dionex HPLC to give a homogeneous oligosaccharide with the composition $Gal_4Man_{10}GlcNAc$. The NMR spectrum (Fig. 4, spectrum B) shows H1 signals with unit intensities at δ4.77, δ4.87, δ5.12, and δ5.33, all being characteristic

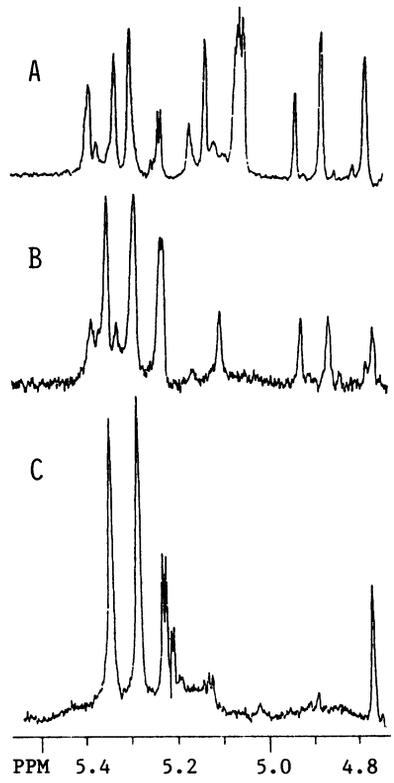

FIG. 4. Anomeric proton NMR signals for small-core oligosaccharides. Spectra: A, Spectrum of an oligosaccharide from the low-galactose fraction with the composition $Man_{10}GlcNAc$; B, spectrum of an oligosaccharide fraction with the composition $Gal_4Man_{10}GlcNAc$; C, spectrum of the product recovered after acetolysis of *B* oligosaccharide that had the composition $Gal_2Man_4GlcNAc$.

of mannose units in the *Sac. cerevisiae* core oligosaccharide. A signal at δ5.28 (intensity of four protons) indicates the presence of four α1→2-linked mannoses substituted at position 2. Completely lacking is any signal at δ5.05 for terminal mannose linked α1→2, which suggests that such terminal mannoses are substituted at position 2 by α-linked galactose. This is supported by the presence of a signal with the intensity of three protons at δ5.22 and one proton at δ5.36 as expected for such terminal galactose units. A signal at δ4.93 suggests the presence of an α1→6-linked mannose that is not substituted at position 2. In agreement with this structure, some of the galactose was rapidly released from the oligosaccharide by digestion with coffee bean α-galactosidase, and some was released more slowly. The resistance of α1→2- and α1→3-linked galactose to α-galactosidases has been noted (17). The recovered oligosaccharide had the composition $Gal_2Man_{10}GlcNAc$, and the H1 NMR spectrum showed a reduction in the signals at δ5.22, δ5.29, and δ5.33 and the appearance of a signal at δ5.05 with the intensity of one proton. Redigestion of this core fragment with the α-galactosidase released galactose at a rate less than 1/10th that of the first digestion.

Acetolysis (18) of the mixed high-galactose core oligosaccharide fraction yielded several fragments that contained mannose and galactose, along with one fragment that contained glucosamine as well. The latter fragment was eluted on a Bio-Gel P-2 column at the position of a heptasaccharide, and it had the composition Gal_2Man_4GlcN. This must represent the core portion that is linked to asparagine in the intact molecule. The proton NMR spectrum (Fig. 4, spectrum C) is consistent with the structure $\alpha Gal\rightarrow{}^2\alpha Gal\rightarrow{}^2\alpha Man\rightarrow{}^2\alpha Man\rightarrow{}^2\alpha Man\rightarrow{}^3\beta Man\rightarrow{}^4\alpha\beta GlcNAc$, where $\alpha\beta$ means α or β linkage. As confirmation of this structure, the $Gal_2Man_4GlcNAc$, which had retention time R_t = 13.9 min (solvent C), was slowly digested with α-galactosidase to yield galactose and a new peak with R_t = 11.1 min, which was more slowly converted to a second peak at R_t = 8.05 min. The R_t of authentic $\alpha Man\rightarrow{}^2\alpha Man\rightarrow{}^2\alpha Man\rightarrow{}^3\beta Man\rightarrow{}^4\alpha\beta GlcNAc$ was 8.07 min. This structure reveals the location of the fourth galactose in the $Gal_4Man_{10}(GlcNAc)_2$- core oligosaccharide and accounts for the observation that, although α-galactosidase digestion removed two galactose units from the core, it uncovered a single α1→2-linked mannose.

The structure most consistent with these data is shown as structure B in Table 1, although the presence of other closely related homologs is suggested by the heterogeneity in the Dionex elution profile and the presence of some unassigned H1 signals. Indicative of the structure of such homologs, acetolysis of the unfractionated high-galactose oligosaccharide yielded fragments with two to five hexose units. From a study of the HPLC retention times, the sugar compositions, and the susceptibility to α-galactosidase and α-mannosidase digestion, these were identified as $\alpha Man\rightarrow Man$, $\alpha Gal\rightarrow Man$, $\alpha Man\rightarrow\alpha Man\rightarrow\alpha Man$, $\alpha Gal\rightarrow\alpha Man\rightarrow Man$, $\alpha Gal\rightarrow\alpha Gal\rightarrow\alpha Man\rightarrow Man$, $\alpha Gal\rightarrow\alpha Man\rightarrow\alpha Man\rightarrow Man$, and $\alpha Gal\rightarrow\alpha Gal\rightarrow\alpha Man\rightarrow\alpha Man\rightarrow Man$. Thus, other side chains in the core may also be terminated by galactobiose as well as galactose units, with the consequence that the core could carry as many as six galactose units. We considered the possibility that the galactosidase-resistant galactose might have the furanose ring form, as reported for the galactomannan peptide from *Trichophyton* species (19). Such galactose is released by 0.01 M HCl at 80°C in 2 h but these conditions had no effect on the *Sch. pombe* core fragment. We conclude that all of the galactose is in the pyranose ring form. No NMR signal was observed that was assignable to a β-linked galactose.

The major oligosaccharide component released by endoglucosaminidase H digestion was too large for analysis on the Dionex system, so it was subjected to partial acetolysis to determine the chain composition. This reaction cleaves

Table 1. Anomeric proton chemical shifts and oligosaccharide structures

Residue and structure	H-1 chemical shift (δ)					
E D C B A	E	D	C	B	A_α	A_β
Structure A						
↓ ↓ ↓						
αM*			5.05			
↓2						
αM αM		5.05	5.39			
↓2 ↓3						
αM→^{6}αM→^{6}βM→^{4}αβGlcNAc		5.14	4.87	4.77	5.22	4.71
↑3						
αM→^{6}αM			4.93	5.34		
↑2						
αM				5.29		
↑2						
αM				5.05		
Structure B						
αM^2←αG†			5.29	5.24		
↓2						
†αG→^{2}αM αM	5.24	5.29	5.35			
↓2 ↓3						
αM→^{6}αM→^{6}βM→^{4}αβGlcNAc		5.12	4.87	4.77	5.21	4.72
↑3						
αM→^{6}αM			4.93	5.35		
↑2						
αM				5.29		
↑2						
αG→ ^{2}αG→^{2}αM		5.24	5.35	5.29		

M, mannose; G, galactose; GlcNAc, *N*-acetylglucosamine.
*In most organisms, this mannose is trimmed in the endoplasmic reticulum.
†In some homologs, additional galactose is attached at this position by α1→2 linkage.

mannosyl α1→6 linkages preferentially without appreciable breakdown of mannosyl α1→2 bonds (18), and we found a similar stability for the galactosyl-mannose α1→2 bond (see below). The Dionex pattern in Fig. 5*A* (solvent C) shows three major peaks, a monosaccharide (peak 1) and two disaccharides (peaks 2 and 3) in the ratio 1:1:1.65. Other minor components (peaks 4, 5, and 6) are presumed to come from a core oligosaccharide such as those discussed above. Peaks 1, 2, and 4 were enhanced by cochromatography with added mannose, α1→2-linked mannobiose, and α1→2-linked mannotriose (Fig. 5*B*), which suggests their identity. When the mixture was analyzed on the Dionex system with solvent B, however, the monosaccharide peak was found to consist of mannose and galactose in the molar ratio 8:1. The mannose is presumed to come from unsubstituted α1→6-linked mannosyl units in the backbone, whereas the galactose probably is formed by nonspecific acetolysis of the α1→2-linked sidechain units. To test this hypothesis, a pure sample of α1→2-linked galactosylmannose obtained from the partial acetolysis of Sp331 galactomannoprotein was subjected to reacetolysis, and the products were analyzed on the Dionex system (solvent C). About 5% of the disaccharide was cleaved, so we conclude that this nonspecific acetolysis accounts for the formation of the small amount of galactose and that no α1→6-linked galactose is present in the glycoprotein. The acetolysate also contained a small amount of a fragment with the composition Gal_2Man_4GlcN that was identical in properties to the fragment isolated from acetolysis of the small-core oligosaccharide.

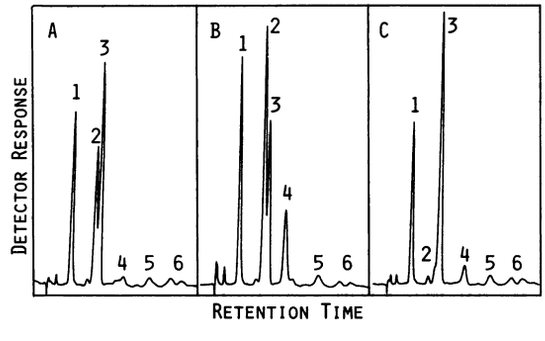

FIG. 5. Dionex pattern of acetolysis fragments from *Sch. pombe* large N-linked oligosaccharide. Peaks: 1, mannose and galactose; 2, α1→2-linked mannobiose; 3, α1→2-linked galactosylmannose; 4, trisaccharides. Other minor peaks are unidentified. (*A*) Sp69 isolate. (*B*) Sp69 spiked with mannose, α1→2-linked mannobiose, and α1→2-linked mannotriose; (*C*) Sp331. Solvent C was used.

Carbohydrate Structure of a Glycosylation Mutant. Several flocculant *Sch. pombe* mutants were isolated that made reduced amounts of invertase. Treatment of crude cell extracts with endoglucosaminidase H showed that the invertases from both the wild-type and mutant cells were converted to the same still more rapidly migrating unglycosylated enzyme. The bulk glycoprotein from one mutant (Sp331) was analyzed to assess any change in O- and N-linked carbohydrate. Fig. 2*C* shows that β-elimination yielded a single disaccharide peak with the retention time of α1→2-linked galactosyl mannitol. In confirmation, the compound gave 1.03 mol of formaldehyde per mol on periodate oxidation. The reduced α1→2-linked mannobiose and mannotriose (peaks 3 and 4) were absent. Surprisingly, the mutant made the same O-linked tetrasaccharide (peak 5) found in the wild type, which contains an α1→2-linked mannobiose unit. The small-core oligosaccharide fraction from endoglucosaminidase H digestion of Sp331 glycoprotein gave the same Dionex pattern as that from Sp69, indicating that the mutation had no effect on core synthesis. The acetolysate of Sp331 large oligosaccharide (Fig. 5*C*) also shows a loss in the α1→2-linked mannobiose and mannotriose peaks. Thus, the mutation in Sp331 selectively eliminates the unsubstituted α1→2-linked mannose on both O- and N-linked chains. Among the

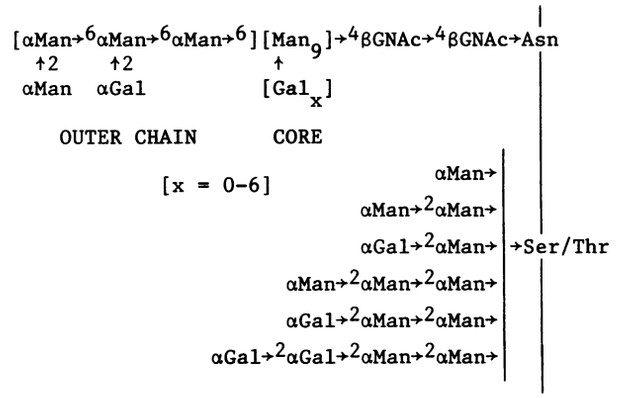

FIG. 6. Proposed structures for *Sch. pombe* galactomannoproteins. The O-linked mannose, mannobiose, and galactosylmannose are present in the ratio 1:1:2. Small amounts of tri- and tetrasaccharides are also present. The length of the outer chain of N-linked oligosaccharides is unknown, but the mannose, mannobiose, and galactosylmannose are present in the ratio 1:1:1.65. About 10% of the carbohydrate linked to asparagine is present as a heterogeneous mixture of core units without attached outer chains. Refer to Table 1 for the [Man_9] core structure.

glycosylation-defective mutants of *Sac. cerevisiae*, only the *mnn1* strain, which affects addition of terminal α1→3-linked mannose units, is altered on both the O- and N-linked chains (5).

A large-scale acetolysate of Sp331 large oligosaccharide was fractionated on a Bio-Gel P-4 column to give five peaks with retention times of mono- to pentasaccharide. The monosaccharide peak contained only mannose; the disaccharide peak on hydrolysis gave equimolar amounts of galactose and mannose as expected for α1→2-linked galactosylmannose. Analysis of the trisaccharide hydrolysate revealed galactose and mannose in a 2:1 ratio, indicative of αGal→^{2}αGal→^{2}Man, whereas the tetrasaccharide peak gave equimolar amounts of galactose and mannose that is consistent with the structure αGal→^{2}αGal→^{2}αMan→^{2}Man. Consistent with the assigned structures, the di- and trisaccharides were converted to monosaccharides through the action of α-galactosidase alone, whereas the tetrasaccharide yielded α1→2-linked mannobiose. Owing to the mutation in Sp331, the oligosaccharides that contain only mannose are absent.

The binding of alcian blue dye by whole yeast cells is a reflection of the amount of esterified phosphate in the wall mannoproteins (5), and some *Sac. cerevisiae* mutants (*mnn4* and *mnn6*) that bind the dye poorly are characterized by defects in the addition of mannosylphosphate to their glycoproteins (20). When grown on yeast extract (YE) medium, which contains about 2 mM phosphate, Sp69 cells bind the dye as well as *Sac. cerevisiae* X2180, whereas Sp331 binds it only weakly. When grown on YE medium supplemented with 7 mM phosphate, however, both Sp69 and Sp331 show strong binding. The results suggest that the glycosylation defect in Sp331 cells also interferes with the phosphorylation of cell wall macromolecules and that this effect can be reversed by increasing the concentration of orthophosphate in the medium.

Our results are consistent with the structure in Fig. 6 for the carbohydrate components of *Sch. pombe* glycoproteins. Gorin *et al.* (12) first demonstrated the presence of α1→2-linked mannobiose and galactosylmannose in the galactomannan from *Schizosaccharomyces* species, and the outer chain structure we propose for the galactomannoprotein of *Sch. pombe* is consistent with the earlier results (3). Our study provides detailed analysis of the O-linked and the small N-linked core oligosaccharides. It was reported (2) that the surface of an *Sch. pombe* mutant defective in synthesis of acid phosphatase, a glycosylated enzyme localized in the cell wall, shows a change in the surface appearance, and the binding of α-galactose-specific lectins to *Sch. pombe* cells shows that the galactomannoprotein is exposed on the cell surface. Perhaps the packing of the "hair-like structures" observed by electron microscopy on *Sch. pombe* cells (4) is also affected by this mutation.

Note Added in Proof. After submission of our paper, a report appeared that also noted the incomplete trimming of the *Sch. pombe* core oligosaccharide (21).

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