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Role of Membranes of Mycelial *Mucor rouxii* in Synthesis and Secretion of Cell Wall Matrix Polymers

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Membrane fractions of the mycelial form of *Mucor rouxii* contained endogenous polyuronides and glycoproteins with sugar compositions similar to the cell wall polyuronide (mucoran) and extracellular (wall and filtrate) glycoproteins, respectively. The polyuronide pool was rapidly labeled with D-[*U*-^{14}C]glucose and on pulse-chase experiments appeared to turn over, suggesting that these polymers were precursors of the wall mucoran. In contrast, the glycoproteins appeared to accumulate. Although the membrane-associated glycoproteins had molecular weights similar to those of the extracellular glycoproteins, the bulk of the membrane-associated polyuronides were of a molecular weight lower than that of the wall polyuronide.

Considerable attention has been focused on the synthesis of chitin, the fibrillar polysaccharide of fungal walls, and its role in hyphal morphogenesis. Many studies have suggested that the plasmalemma is the site of chitin polymerization and that chitin synthetase activity is maximal at the hyphal apex (for reviews, see references 1, 9, and 10). However, with the exception of mannan proteins, comparatively little is known of the site(s) of synthesis of the nonfibrillar, matrix polymers of the wall. Autoradiographic evidence has established that the endomembrane system is the site of mannan polymerization in yeasts (16). This evidence complements studies on the localization of the biosynthetic enzymes (5, 17) and the isolation of mannan-containing vesicles from budding yeasts (6). In filamentous fungi, the apical vesicles and maturing face of the Golgi bodies appear by cytochemical staining to contain polysaccharide-positive material (11). The vesicles are thought to transport wall matrix polymers from the endoplasmic reticulum and Golgi bodies, the sites of synthesis, to the hyphal tip, the site of wall growth (12). These are analogous processes to those shown to be involved in the secretion of extracellular polysaccharides in higher plants (for reviews, see references 4 and 22). However, in fungi, evidence for the nature of the polymers transported on their degree of prefabrication before incorporation into the apical wall is lacking. Changes in the properties of the matrix polymers after incorporation (e.g., by cross-linking) could conceivably contribute to the differential properties of the lateral and apical walls required for hyphal morphogenesis.

The mycelial wall of *Mucor rouxii* contains as matrix polymers mucoran (a polyuronide), glycoproteins, and glycopeptides (2, 7). We have attempted by radiolabeling to identify and characterize membrane fractions of the cell involved in the synthesis and transport of these polymers. We have also compared the physical properties and compositions of these membrane-associated polymers with polymers from the mature cell wall and culture filtrate. Our studies show that, although membrane-associated glycoproteins are of the same molecular weight as the extracellular (wall and filtrate) glycoproteins, the bulk of polyuronides associated with membrane fractions have a molecular weight lower than those of the polyuronides extracted from the wall.

MATERIALS AND METHODS

Growth of the organism and radioactive labeling. *M. rouxii* NRRL 1894 was grown in 1 liter of yeast extract-peptone-glycerol medium (7) in 2-liter Erlenmeyer flasks. The medium was inoculated with a washed sporangiospore suspension to a final concentration of 10^5 spores per ml. The flasks were maintained in an orbitally shaking incubator at 28°C and 200 cycles per min. After 18 h, the myceiial mat was harvested by filtration, and 0.5 g of mycelium was suspended in 30 ml of yeast extract-peptone-glycerol medium supplemented with 20 μM D-glucose and 30 μCi of D-[*U*-^{14}C]glucose (New England Nuclear Corp.; specific activity, 200 Ci/mol). After the appropriate labeling period, the mycelial mat was harvested by filtration, washed with water, mixed with 6.5 g of unlabeled mycelium, and homogenized.

The time course of uptake of label into different wall fractions was done as described previously (8), except the uptake medium was yeast extract-peptone-glycerol containing 20 μM D-glucose and 5 μCi of D-[*U*-^{14}C]glucose (specific activity, 200 Ci/mol).

Homogenization and fractionation by differential centrifugation. All operations were carried

out at 4°C. Mycelium (7 g) was mixed with 14 g of acid-washed sea sand and homogenized in a pestle and mortar with the breakage buffer for 10 min. The breakage buffer contained 20 mM (2-*N*-morpholino)propanesulfonic acid-KOH (pH 7.5), 0.55 M sorbitol, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 1% bovine serum albumin. The homogenate was passed through Miracloth and centrifuged at 800 × *g* for 5 min to remove sand, cell walls, and unbroken cells. Subcellular fractions were then derived by centrifugation at 22,000 × *g* for 10 min and 80,000 × *g* for 1 h.

Release of polymers from subcellular fractions. Fractions were suspended in glass-distilled water and mixed with an equal volume of 1-butanol. After centrifugation to separate the phases, the aqueous layer was removed and dialyzed overnight against glass-distilled water. The butanol layer was discarded. There was no radioactivity associated with the insoluble interface material.

Sucrose density gradient centrifugation. Linear gradients of 20 to 60% (wt/vol) sucrose were prepared with a total volume of 12 ml. Pellets from differential centrifugation were resuspended (in a lossely fitting homogenizer) in breakage buffer without bovine serum albumin. One milliliter of suspension was layered on the top of each gradient. After centrifugation at 4°C in an SW41 rotor at 28,000 rpm for 1 h, the bottom of the tube was punctured and fractions (approximately 0.9 ml) were collected.

Marker enzyme assays. Cytochrome *c* oxidase was assayed as described by Haidle and Storck (13). 5'-Nucleotidase was assayed as described by Morré (20), except released P_i was estimated by the method of Taussky and Schorr (23). Mg^{2+}-dependent ATPase was assayed at pH 6.0 and 9.0 as described by Bowles and Kauss (3). The effects of K^+ stimulation were observed at a final concentration of 50 mM. NADPH-cytochrome *c* reductase and NADH-cytochrome *c* reductase were assayed at pH 7.4. The assays contained 100 μl of 0.2 M sodium phosphate buffer (pH 7.4), 100 μl of cytochrome *c* (5 mg/ml), 20 μl of 0.1 M NaCN, 500 μl of water, and 25 μl (for the NADPH enzyme) or 10 μl (for the NAD enzyme) of membrane suspension. The reaction was started with 150 μl of NADH or NADPH (3 mg/ml) and followed by the reduction of cytochrome *c* at 550 nm. The effects of antimycin A were observed by adding 10 μl of a solution of antimycin A in ethanol (2 mg/ml).

Analytical methods. High-voltage electrophoresis of polymers was performed on glass fiber paper on a Savant flat-bed apparatus at 1.5 kV for 40 min. The electrophoresis buffer was 0.05 M ammonium acetate (pH 6.5) containing 10 mM EDTA, and the voltage gradient was 40 V/cm. Gel chromatography was performed on Sepharose 6B. The column dimensions were 1.5 by 42 cm. The column was equilibrated and eluted with 0.05 M potassium phosphate buffer (pH 7.0), the flow rate was 18 ml/h, and 1.8-ml fractions were collected. Sugar analysis of labeled polymers by hydrolysis, paper chromatography, and electrophoresis was performed as described previously (7). The distribution of labeled polymeric materials on sucrose gradients was determined as follows. Fractions were precipitated with 10% trichloroacetic acid; after 1 h at 4°C, the precipitate was collected on GF/A disks, dried, extracted with 1-butanol, dried again, and counted. Radioactivity on chromatograms and electrophoretograms was determined by cutting the paper into strips (4 by 1 cm), with the long axis of the strip perpendicular to the direction of separation (7). Radioactivity on paper strips, on glass fiber disks, and in column eluates was determined as described previously (7, 8). Protein was measured by the method of Lowry et al. (18). For treatment with pronase, samples were incubated overnight in 0.05 M (2-*N*-morpholino)propanesulfonic acid-KOH (pH 7.5) at 37°C with 100 μg of pronase per ml.

RESULTS

Time course of cell wall labeling during radioactive pulse. The time course of incorporation of radioactivity from D-[*U*-^{14}C]glucose into the NaOH-insoluble and NaOH-soluble phases of the wall is shown in Fig. 1. Radioactivity was rapidly incorporated into both phases over the first 20 min; after 60 min, the rate of incorporation into both phases was almost negligible. This was probably due to a depletion of radioactive glucose in the medium although the organism continued to grow after the labeled glucose was depleted. Under the labeling conditions used, only carbohydrates were labeled from D-[*U*-^{14}C]glucose (7).

Subcellular distribution of labeled polymers. Cultures labeled for 5, 20, and 60 min were homogenized, and the homogenates were fractionated by differential centrifugation as described above. Water-soluble material associated with each fraction was released by treatment with 1-butanol. The polymeric materials in these extracts were obtained by dialysis for 18 h against glass-distilled water or by gel filtration on G-25 Sephadex. Both methods gave identical yields. Gel filtration resolved the released material into a high-molecular-weight peak elut-

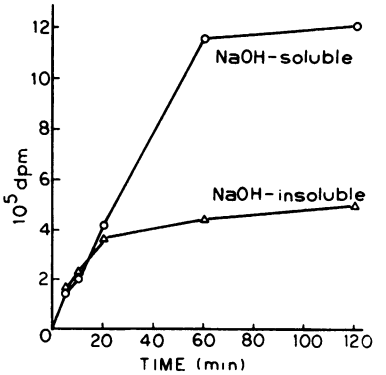

FIG. 1. *Time course of incorporation of radioactivity from* D-*[U-*^{14}*C]glucose into NaOH-insoluble and NaOH-soluble phases of the wall.*

ing in the blue dextran void volume and a peak eluting in the same volume as D-glucose. The low-molecular-weight material was not studied further. The distribution of radiolabeled polymers among the subcellular fractions at different labeling times is shown in Table 1.

Characterization of polymers in subcellular fractions after 5-min labeling. The polymeric material from each fraction was subjected to high-voltage electrophoresis on glass fiber paper at pH 6.5. The polymers from the 22,000 × *g* pellet were resolved into two discrete zones with different acidity (Fig. 2A), a strongly acidic zone (zone 1) and a weakly acidic or neutral zone (zone 2). The strongly acidic material was partially resolved into two peaks with different mobilities. Similar results were seen with the polymers of the 80,000 × *g* pellet and supernatant (Fig. 2B and C). Approximately 65% of the total applied label was in zone 1 for all fractions in this experiment. The materials in the strongly and weakly acidic zones were eluted and subjected to gel chromatography on Sepharose 6B. The results (Fig. 3) showed that the weakly acidic or neutral materials were of a molecular weight higher than those of most of the acidic materials. The acidic materials from the 22,000 × *g* pellet were of a molecular weight slightly higher than the molecular weights of those of the 80,000 × *g* fraction.

Weakly acidic and strongly acidic materials were digested separately with pronase and rechromatographed on Sepharose 6B. Although the strongly acidic material showed no change in elution position, the weakly acidic or neutral materials eluted in a larger volume, suggesting that they were glycoprotein in nature.

The sugar compositions of the polymers from each zone of acidity of each subcellular fraction are shown in Table 2. The strongly acidic materials contained D-glucuronic acid as the major sugar, with lesser amounts of L-fucose and D-galactose and small amounts of D-mannose. The weakly acidic or neutral materials contained D-mannose as the major sugar, with lesser amounts of L-fucose and D-galactose and small amounts of D-glucuronic acid.

TABLE 1. *Percent distribution of labeled polymers in subcellular fractions[a] after different labeling times with D-[U-^{14}C]glucose*

Fraction	Distribution (%) after labeling time (min) of:		
	5	20	60
22,000 × *g* pellet	19.6	21.7	25.3
80,000 × *g* pellet	54.2	52.9	42.2
80,000 × *g* supernatant	26.2	25.4	32.5

[a] Fractions were derived as described in the text.

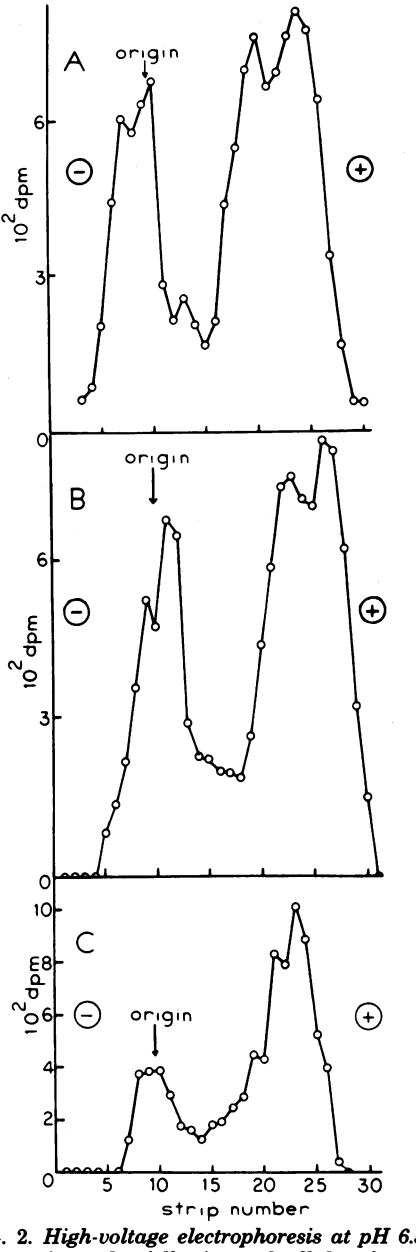

FIG. 2. *High-voltage electrophoresis at pH 6.5 of polymers from the following subcellular fractions after a 5-min labeling: A, 22,000 × g pellet; B, 80,000 × g pellet; C, 80,000 × g supernatant. Polymers were resolved into strongly acidic (zone 1, strips 15 through 31) and weakly acidic or neutral (zone 2, strips 5 through 12) fractions.*

The occurrence of labeled uronides in the supernatant fraction might be due to disruption of vesicles during the homogenization process. An alternative hypothesis in which soluble uronides were vesiculated during homogenization

was excluded by the following experiment. Mycelium was homogenized as before in buffer containing soluble labeled polyuronides. After differential centrifugation, less than 4% of the total label was found associated with membrane fractions.

Characterization of polymers in subcellular fractions after 60-min labeling. The polymeric materials from each fraction were subjected to high-voltage electrophoresis on glass fiber paper at pH 6.5. Polymers from the 22,000 × *g* and 80,000 × *g* pellets showed a single peak of radioactivity in the weakly acidic or neutral zone (zone 2), with only trace amounts of strongly acidic material (zone 1). Polymers from the supernatant fraction showed two peaks of radioactivity, a strongly acidic peak and a weakly acidic or neutral peak. The material in the strongly acidic peak was susceptible to degradation by both RNase and DNase, with sequential degradation by these enzymes causing >80% of the material to become dialyzable. This suggested that the acidic materials were largely RNA and DNA. Analysis of the sugar composition of this fraction showed largely D-ribose, again suggesting the presence of RNA. The zone 2 polymers from all three fractions were subjected to gel chromatography on Sepharose 6B (Fig. 4). The polymers from the 22,000 × *g* and 80,000 × *g* pellets had different elution profiles; the polymers of the 80,000 × *g* supernatant were of disperse molecular weight. Treatment of the polymers from the 22,000 × *g* and 80,000 × *g* pellets with pronase caused a shift in elution position to higher volumes, suggesting that the polymers in these fractions were glycoproteins.

Analysis of the sugar compositions of the zone 2 polymers of all three fractions are shown in Table 3. The glycoproteins from the 22,000 × *g* and 80,000 × *g* pellets had similar compositions although they differed in molecular weight. D-Mannose was again the predominant sugar of these polymers. A significant amount of D-glu-

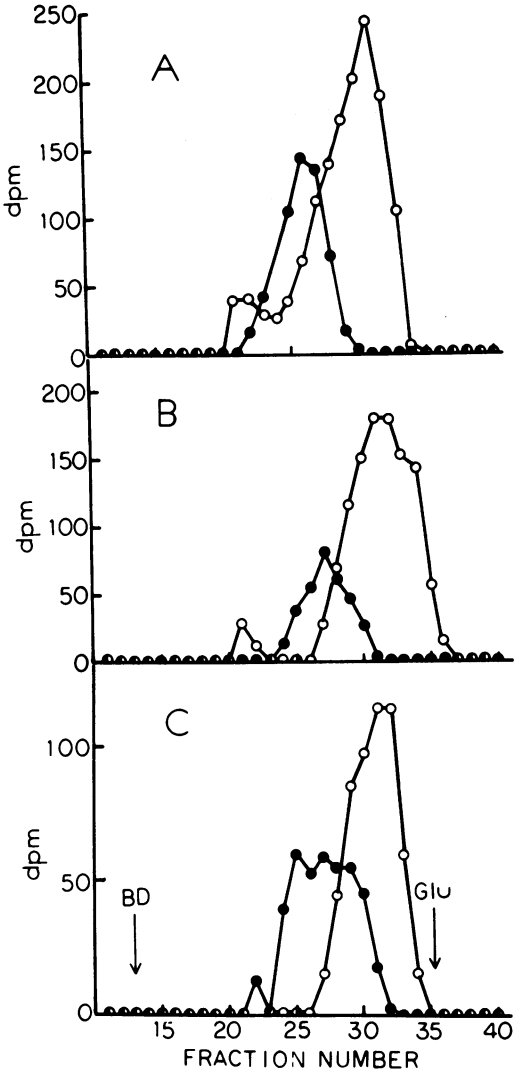

FIG. 3. *Sepharose 6B gel chromatography of strongly acidic* (○) *and weakly acidic or neutral* (●) *polymers from the following subcellular fractions after a 5-min labeling: A, 22,000 × g pellet; B, 80,000 × g pellet; C, 80,000 × g supernatant. The polymers were separated by high-voltage electrophoresis as shown in Fig. 2. The elution positions of blue dextran (BD) and D-glucose (Glu) are shown.*

TABLE 2. *Sugar composition of polymers of different electrophoretic mobility[a] from subcellular fractions after 5-min labeling[b]*

Sugar	Composition[c]					
	22,000 × *g* pellet		80,000 × *g* pellet		80,000 × *g* supernatant	
	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Glucuronic acid	45.2	5.6	54.2	9.6	44.0	4.3
Galactose	16.3	10.1	9.6	7.5	12.3	9.5
Mannose	7.2	62.2	1.7	63.6	1.3	58.5
Fucose	31.3	22.1	34.5	19.4	42.4	12.7
Glucose						15.0

[a] Polymers were fractionated into strongly acidic (zone 1) and weakly acidic or neutral (zone 2) polymers by electrophoresis on glass fiber paper at pH 6.5 as in Fig. 2.

[b] The total radioactivity (in disintegrations per minute) in the sugars in zones 1 and 2, respectively, was as follows: 22,000 × *g* pellet, 1,942 and 1,135; 80,000 × *g* pellet, 4,134 and 2,092; and 80,000 × *g* supernatant, 2,683 and 1,230.

[c] The amount of each sugar is given as a percentage of the total disintegrations per minute in the sugars.

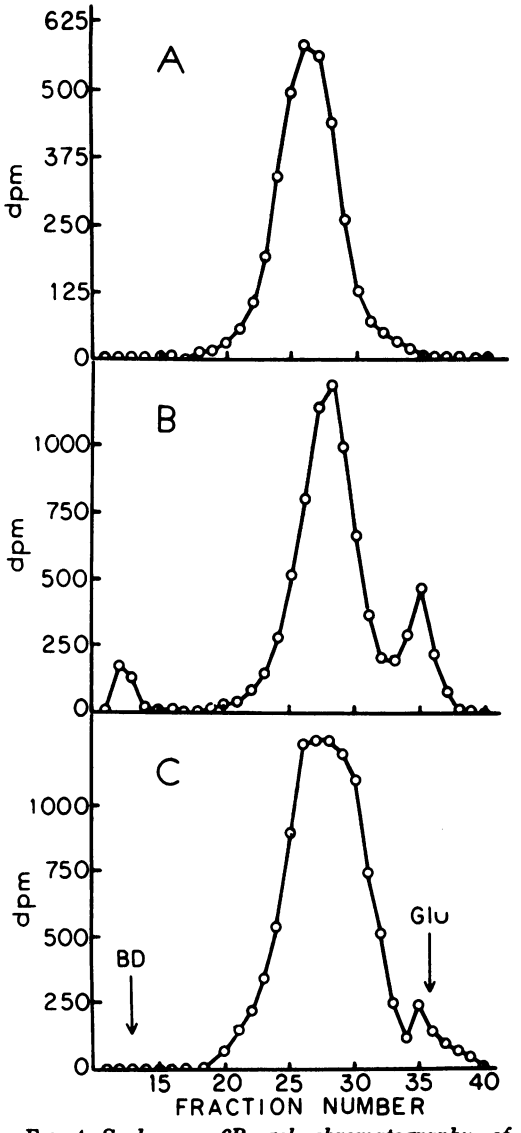

FIG. 4. *Sepharose 6B gel chromatography of weakly acidic or neutral polymers from the following subcellular fractions after a 60-min labeling: A, 22,000 × g pellet; B, 80,000 × g pellet; C, 80,000 × g supernatant. The elution positions of blue dextran (BD) and D-glucose (Glu) are shown.*

cose was found in the zone 2 polymers of the 80,000 × *g* supernatant after both 5- and 60-min labelings (Tables 2 and 3). This glucose could have come from a glycoprotein or perhaps from glycogen synthesized during the radioactive pulse.

Distribution of membrane-associated polymers on sucrose density gradients. The membrane fraction derived by centrifugation at 22,000 × *g* was resolved further by sucrose density gradient centrifugation. This fraction was chosen since it contained polyuronides of the highest molecular weight (the most complete?) and might thus represent this polymer in its stage of transport to the wall, perhaps in the apical vesicles. The 22,000 × *g* pellet was prepared after 5, 20, and 60 min of labeling, suspended, and layered onto three identical linear gradients of sucrose (20 to 60%, wt/vol). After centrifugation, the gradients were fractionated, and the distribution of polymeric material was determined as described above. The results (Fig. 5) showed that at 5 and 20 min of labeling, radioactivity was associated mainly with the lightest fractions (10 through 14), but that at 60 min of labeling, label was associated with two major regions. Parallel to this experiment, the extracted polymers of the homogenates were

TABLE 3. *Sugar composition of weakly acidic or neutral polymers in subcellular fractions after 60-min labeling*[a]

Sugar	Composition[b]		
	22,000 × *g* pellet	80,000 × *g* pellet	80,000 × *g* supernatant
Glucuronic acid	9.2	6.0	12.1
Galactose	7.7	8.2	17.4
Mannose	64.2	68.2	28.8
Fucose	18.9	17.6	9.7
Glucose			32.0

[a] The total radioactivity (in disintegrations per minute) in the sugars was as follows: 22,000 × *g* pellet, 7,348; 80,000 × *g* pellet, 4,265; and 80,000 × *g* supernatant, 8,331.

[b] The amount of each sugar is given as a percentage of the total disintegrations per minute in the sugars.

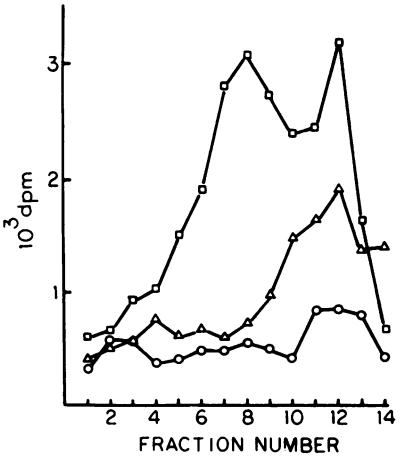

FIG. 5. *Distribution of labeled polymers on sucrose density gradients after the following labeling times with D-[U-^{14}C]glucose: ○, 5 min; △, 20 min; □, 60 min. The top of the gradient was fraction 14.*

subjected to high-voltage electrophoresis to separate acidic polyuronides from weakly acidic or neutral glycoproteins. The results (Table 4) again showed that polyuronides were absent from the fractions after 60 min of labeling, although the distribution of label between acidic and weakly acidic or neutral materials after 5 min of labeling was different from that seen previously. These results indicate that the distribution of label from cultures labeled for 60 min reflects the distribution of glycoproteins only. The distribution of glycoprotein and polyuronide on the gradient after 10 min of labeling was investigated to determine whether these polymers were associated with membranes of different density. The gradient was divided into three regions (A, fractions 1 through 4; B, fractions 5 through 9; C, fractions 10 through 14). The fractions in each region were pooled, treated with 1-butanol, dialyzed to remove low-molecular-weight material, and subjected to high-voltage electrophoresis on glass fiber paper to separate the strongly acidic polyuronides from the neutral or weakly acidic glycoproteins. The distribution of label between these polymers for each region is shown in Table 5. Polyuronides and glycoproteins showed a different distribution, with the uronides associated largely with the lightest membranes. The partial resolution of the acidic polymers by electrophoresis which was observed in the crude pellets was also observed on electrophoresis of all regions of the gradient.

TABLE 4. *Radioactivity in polyuronides and glycoproteins of the 22,000 × g pellet after different labeling times*[a]

Time of labeling (min)	Radioactivity (dpm) in:	
	Polyuronide	Glycoprotein
5	9,092	12,057
20	9,905	27,472
60	<300	55,407

[a] A similar pattern was observed with the 80,000 × *g* pellet.

TABLE 5. *Distribution of radioactivity*[a] *between polyuronides and glycoproteins in different regions*[b] *of sucrose density gradients*

Region of gradient	Radioactivity (dpm) in:	
	Polyuronide	Glycoprotein
A	980	1,620
B	2,144	7,556
C	5,141	6,786

[a] The fungus was labeled for 10 min. The distribution of label between polyuronides and glycoproteins was determined by high-voltage electrophoresis.

[b] See text.

Enzymatic characterization of membranes in sucrose gradients. We attempted to characterize membrane fractions on gradients by using the following enzymatic markers developed for higher plant systems (for a review, see reference 21): cytochrome *c* oxidase (inner mitochondrial membrane), antimycin A-insensitive NADPH-cytochrome *c* reductase (endoplasmic reticulum), antimycin A-insensitive NADH-cytochrome *c* reductase (endoplasmic reticulum and outer mitochondrial membrane), and K^+-stimulated Mg^{2+}-dependent ATPase and 5′-nucleotidase (plasmalemma). There is considerable doubt as to the usefulness of certain enzyme markers (particularly for plasmalemma) in higher plants (20) and in fungi (19). When the distributions of markers were compared with the distributions of labeled polymers, it was clear that label was not associated with mitochondria. However, the distribution of label could not be correlated with the distribution of any single marker enzyme, which could reflect that polymers were associated with a particular domain of the internal membranes.

Comparison of labeled polymers with cell wall and culture filtrate polymers. Mucoran and glycoproteins were extracted from walls labeled with D-[*U*-^{14}C]glucose as described previously (7). The electrophoresis pattern at pH 6.5 of extracted polymers is shown in Fig. 6. Separated mucoran and glycoprotein were subjected to gel chromatography on Sepharose 6B. The elution profiles (Fig. 7A) showed that both polymers had similar molecular weight distributions. The polymers of the culture filtrate were obtained by dialysis of filtrates after 18 h of labeling with D-[*U*-^{14}C]glucose. High-voltage electrophoresis of the retained fraction showed material in the weakly acidic or neutral zone of the electrophoretogram, with no strongly acidic polymers. The elution profile of the culture filtrate material on Sepharose 6B is shown in Fig. 7B. Treatment of this material with pronase caused a shift in the elution profile to higher volumes, suggesting that the filtrate polymers were glycoproteins. Sugar analysis of wall and filtrate polymers is shown in Table 6. These results show the similarity of the composition of the membrane-associated uronide to mucoran and of the membrane-associated glycoprotein to the wall and filtrate glycoproteins. Although membrane-associated glycoproteins were of a molecular weight similar to those of wall and filtrate glycoproteins, the bulk of the uronides associated with membrane fractions were of a molecular weight lower than that of the extractable wall mucoran (compare Fig. 3 and 7). A small percentage of membrane-associated uron-

ide (9%) had a molecular weight higher than that of the extractable wall mucoran.

DISCUSSION

Our results demonstrate that membrane fractions of *M. rouxii* contain polyuronides and glycoproteins with compositions similar to those of the extracellular polyuronides and glycoproteins of this organism. The absence of polyuronides from subcellular fractions after 60 min of labeling (Table 4) showed that these rapidly labeled polymers had been chased from the fractions. These data and their sugar composition suggest

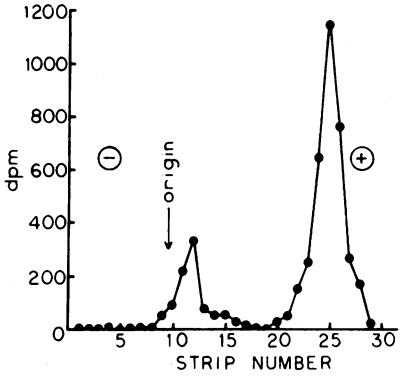

FIG. 6. *High-voltage electrophoresis of wall mucoran and glycoprotein, showing the strongly acidic mucoran (strips 21 through 29) and the weakly acidic glycoprotein (strips 9 through 14).*

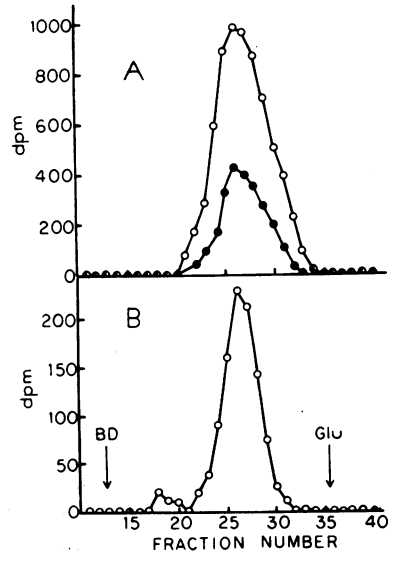

FIG. 7. *Sepharose 6B gel chromatography of extracted wall and culture filtrate polymers. A, Wall mucoran* (○) *and wall glycoprotein* (●); *B, filtrate glycoprotein. The elution positions of blue dextran (BD) and* D*-glucose (Glu) are shown.*

TABLE 6. *Sugar composition of cell wall and culture filtrate polymers*[a]

Sugar	Composition[b]		
	Wall		Filtrate glycoprotein
	Mucoran	Glycoprotein	
Glucuronic acid	41.1	3.4	8.2
Galactose	30.5	13.5	12.5
Mannose	4.0	62.6	60.1
Fucose	24.4	14.9	16.9
Glucose			2.3

[a] The total radioactivity (in disintegrations per minute) in the sugars was as follows: wall mucoran, 3,612; wall glycoprotein, 1,100; and filtrate glycoprotein, 1,530.

[b] The amount of each sugar is given as a percentage of the total radioactivity in the sugars.

that these polymers were precursors of the wall mucoran. Similar chase kinetics were not observed overall for the glycoprotein polymers (Table 4). Some of the labeled glycoproteins were perhaps chased into the wall; labeled glycoproteins could be extracted from wall preparations after 60 min of labeling, although no labeled glycoproteins were found in the filtrate. The association of the majority of the labeled polyuronides with membrane fractions suggests a role for the membranes in the synthesis and secretion of mucoran polymers. The cellular role of the accumulating glycoproteins is unknown.

Although our experiments have established a probable precursor-product relationship of the bulk of the membrane-associated polyuronides to the larger wall polyuronides, the mechanism of the transformation of the precursor to product is unknown, as is the significance of the multiple acidic peaks seen on electrophoresis of membrane-associated uronides. The formation of completed mucoran could involve cross-linking of the largely synthesized precursors or further stepwise addition of sugar residues.

Previous work in our laboratory has suggested that restricted lysis of polyuronides may also be required for wall growth and hyphal extension (8). This lysis and the putative cross-linking of wall matrix materials may, thus, be two elements of the balance of wall synthesis and lysis thought to be required to maintain apical wall plasticity during hyphal growth (1). The possible role of nonfibrillar wall components in hyphal morphogenesis has been suggested by the work of Katz and Rosenberger (14, 15). These workers isolated a temperature-sensitive mutant of *Aspergillus nidulans* which could not synthesize chitin at the restrictive temperature. Hyphae of this mutant maintained their shape when grown in medium containing osmoticum or when trans-

ferred to buffer. Thus, chitin microfibrils may not determine the pattern of hyphal morphogenesis in this mutant although they are undoubtedly important wall components. The relevance of our findings to hyphal morphogenesis will be better understood, however, when the pattern of synthesis in the yeastlike form of *M. rouxii* is determined, and work in this area is in progress.

ACKNOWLEDGMENTS

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