JOURNAL OF BACTERIOLOGY, Mar. 1977, p. 1584-1592

Vol. 129, No. 3
Printed in U.S.A.

Architecture and Chemistry of Microconidial Walls of *Trichophyton mentagrophytes*

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Received for publication 23 August 1976

The ultrastructure and chemical composition of the walls of *Trichophyton mentagrophytes* microconidia were investigated with particular emphasis on the localization of the major structural components within the walls. The walls consisted of carbohydrate (56.1% neutral polysaccharide, and 16.0% chitin), protein (22.6%), lipid (6.5%), ash (1.7%), and trace amounts of melanin (0.2%) and phosphorus (0.2%). In thin sections, three distinct layers were recognized. The electron-transparent pellicle (15 to 20 nm thick) covering the outermost surface of the wall consisted of a glycoprotein-lipid complex and was mostly extracted by sodium phosphate buffer (0.1 M, pH 6.5) containing 8 M urea, 1% (vol/vol) mercaptoethanol, and 1% (wt/vol) sodium dodecyl sulfate. The middle electron-dense layer (30 to 50 nm thick) represented the proteinaceous rodlet layer embedded in polysaccharides and could be completely solubilized by hot alkali extraction (1 N NaOH, 100°C, 1 h). The thick inner layer (200 to 300 nm thick) was relatively resistant to the above treatments and was found to consist of amorphous glucans and microfibrillar chitin. Approximately half of the inner wall glucans was susceptible to (1 → 3)-β-glucanase.

Trichophyton mentagrophytes is a dermatophyte that causes tinea pedis and other cutaneous lesions in humans. When grown on Sabouraud glucose agar medium, it produces numerous microconidia that are single celled, spherical, clavate, or pyriform in shape, and borne singly on the side of hyphae or in grapelike clusters (24).

Although the ultrastructure (5, 13, 21) and chemical composition (2, 9, 16, 17, 20) of conidial walls have been reported for a number of fungal species, very little is known about the morphology or chemical composition of the microconidial walls of this dermatophyte.

The purpose of this report is to describe the architecture and chemical composition of the walls of *T. mentagrophytes* microconidia with particular emphasis on the localization of the major structural components within the walls.

MATERIALS AND METHODS

Organism. *T. mentagrophytes* ATCC 26323 was used throughout this study. Stock cultures were maintained at room temperature on Sabouraud dextrose agar medium (Difco) with monthly transfer of the granular type colonies to prevent the pleomorphic transformation of the fungus.

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Preparation of microconidial walls. Microconidia were produced, harvested, and purified by a method described earlier (14). Microconidia suspended in sterile, distilled water were disintegrated in a Mickle cell disintegrator at 4°C using acid-cleaned glass beads (75 to 150 μm, Sigma Chemical Co.). Broken microconidia were allowed to stand for 10 to 15 min at 4°C to eliminate glass beads and unbroken microconidia. The microconidial walls remaining in the supernatant fluid were washed at least 10 times in distilled water by means of differential centrifugation (7,000 × *g* for 10 min alternating with 2,000 × *g* for 15 min). To eliminate cytoplasmic materials adhering to the walls, the washed conidial walls were further subjected to mild sonic oscillation (position 3 [2 A], model 75 sonifier, Branson Instruments Inc.) for 30 s (two to three times). The wall fragments recovered by centrifugation (7,000 × *g*) were washed further with distilled water (five times) until nucleic acids or proteins were no longer detectable spectrophotometrically in the washings. All procedures were carried out in a cold room (below 10°C) to minimize degradation of microconidial walls by endogenous enzymes. The microconidial walls prepared in this method were essentially free from cytoplasmic materials as evidenced by electron microscopy of the thin sections.

Electron microscopy. To prepare thin sections of microconidia and microcondial walls, samples were prefixed with 5% glutaraldehyde (Polyscience, Inc.) in veronal acetate buffer (pH 6.1) for 6 to 10 h at room temperature (23 to 26°C). After being washed in the buffer three times, these were fixed in 2%

osmium tetroxide at room temperature overnight. After dehydration through a graded acetone series, the walls were embedded in a mixture of Epon 812 and 815 and polymerized at 60°C for 22 h. Sections were cut with glass knives in an LKB 4800A ultratome (LKB Produkter AB) and mounted on Formvar-coated copper grids (300 mesh, Ernest F. Fullam, Inc.). Sections were stained with lead citrate (25). For shadowed preparations, samples were mounted on Formvar-coated copper grids and air-dried. The grids were shadowed at an angle of 35° with a platinum-carbon in a vacuum evaporator (type HUS-3B, Hitachi Ltd.). Freeze-etching preparations of various wall fractions were made in a Balzars freeze-etching device (model BA 360 M, Balzer High Vacuum Corp.) by the method of Friedman et al. (11) except that 4 to 6% of sodium hypochlorite (MCB Manufacturing Chemists) was used in place of Eau de Javelle to clean the replicas.

Chemical extraction and fractionation of microconidial walls. The purified microconidial walls were extracted sequentially with various chemicals and enzymes as outlined in Fig. 1.

Microconidial walls were first extracted with 0.1 M sodium phosphate buffer (pH 6.5) containing 8 M urea, 1% (vol/vol) mercaptoethanol, and 1% (wt/vol) sodium dodecyl sulfate at room temperature (23 to 26°C) with constant stirring for specified periods of time. This extraction will be referred to as UMS extraction in subsequent discussion. The UMS-soluble fractions were separated from the remaining walls by means of centrifugation (10,000 × *g*, 30 min). The extracted walls were washed 10 times with distilled water and stored at 4°C until use. The supernatant fluid and washings were combined and dialyzed against distilled water at 4°C for 5 days with frequent changes of water. The dialysate was then lyophilized and stored over P_2O_5 in a desiccator.

The UMS-extracted microconidial walls were further treated with 1 N NaOH (100°C, 1 h) with occasional agitation. The hot alkali extract was neutralized with 1 N HCl and then dialyzed against distilled water as before. A brownish precipitate formed in the dialysis sack was separated from the dialysate by means of centrifugation (12,000 × *g*). Both the precipitate and the dialysate were combined, lyophilized, and stored in a desiccator over P_2O_5. The alkali-insoluble wall residues were washed 10 times with distilled water, lyophilized, and stored.

The walls sequentially treated with UMS and hot alkali were further subjected to mild and strong acid treatments. For mild acid treatment, walls were boiled for 1 h in 1 N sulfuric acid. Some wall samples were subjected to more drastic acid treatment by heating in sealed ampoules in 1 N sulfuric acid for 16 h at 110°C.

Chemical and enzymatic methods. Protein was estimated by the method of Lowry et al. (19), with bovine serum albumin (Sigma Chemical Co.) as a standard. Since certain microconidial wall proteins were poorly soluble in cold 0.1 N NaOH (15), samples were first solubilized in hot alkali (1 N NaOH, 100°C, for 15 min) and then diluted appropriately prior to analyses. Protein content was also calculated by the total amino acids recovered from amino acid analysis. Samples (500-μg amounts) were hydrolyzed for 22 h in 6 N HCl at 105°C in a sealed ampoule, and the hydrolysate was analyzed with an automatic amino acid analyzer (Beckman model 120 C). Total neutral sugars were estimated by the anthrone method (23) with glucose as a standard. Glucosamine and chitin were quantitated by the modified method of Elson-Morgan (26) and the method of Blumenthal and Roseman (3), respectively. Total lipid was determined by method of Folch et al. (10), and phosphorus was determined by the method of Ames (1). For ash determination, walls were placed in predried, tared platinum crucibles and heated at 600°C in an oven until constant weights were obtained. Melanin or melanin-like

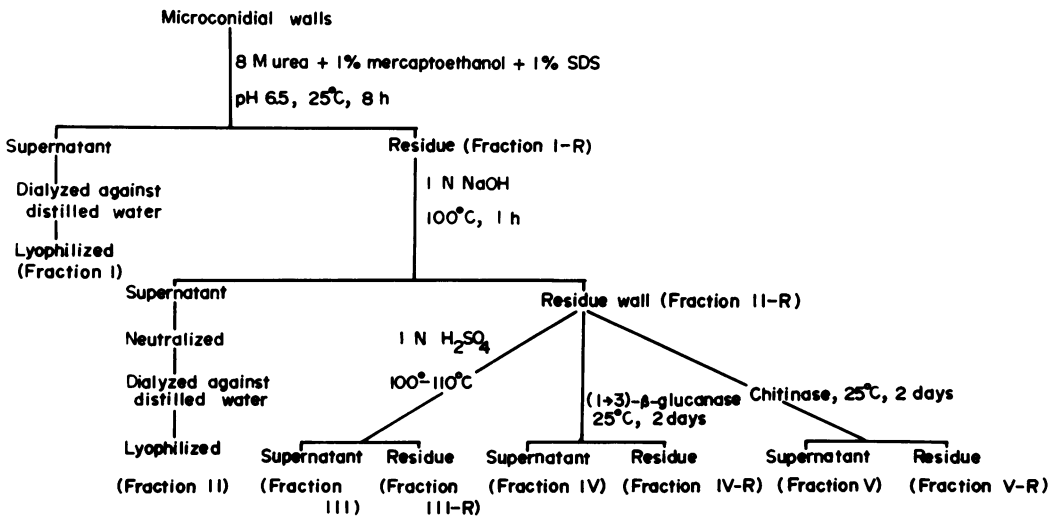


FIG. 1. *Fractionation procedure of T. mentagrophytes microconidial walls.*

pigment was extracted from the specimens by hot alkali (100°C, 1 h in 1 N NaOH), and the concentrations of the pigment in the extract were determined spectrophotometrically (540 nm, Spectronic 70), with synthetic melanin (Sigma Chemical Co.) as a standard. For analysis of monosaccharides in intact and chemically treated walls, samples were hydrolyzed with 1 N sulfuric acid in sealed ampoules for 16 h at 110°C. This hydrolytic condition was shown in our preliminary experiments to yield the maximum monosaccharides from the microconidial walls (C. D. Wu-Yuan, Ph.D. thesis, Loyola University of Chicago, Chicago, Ill. 1976). It was also shown that more than 90% of glucose, mannose, and galactose remained undestructed under this hydrolytic condition. Hydrolysates were neutralized with $Ba(OH)_2$, and the $BaSO_4$ was removed by centrifugation. The supernatant was concentrated in vacuo at room temperature prior to paper chromatography or gas chromatography. Individual monosaccharides in acid or enzyme hydrolysates were identified by descending paper chromatography using Whatman no. 1 chromatography paper. The solvent system used included *n*-butanol–acetic acid–water (4:1:1), ethyl acetate-pyridine-water (10:4:3), and *n*-butanol–pyridine–HCl (0.1 N) (5:3:2), all by volume. Spots containing neutral sugars and amino sugars were visualized by spraying with aniline oxalate and heating. Individual monosaccharides in given spots were determined quantitatively by the method of Chaykin (7). The undeveloped sugar spots, whose positions were determined by parallel chromatograms developed with aniline oxalate, were cut out and eluted with distilled water, and the sugar concentrations of the elutes were determined by the anthrone method using the proper standard sugar solution. Gas-liquid chromatography was also used to identify certain minor sugars in acid hydrolysates of microconidial walls. Lyophilized samples were treated with silation agent (pyridine-hexamethyldisilazane-trimethylsilylchloride, 5:1:1, by volume) at room temperature for 10 min by the method of Bolton et al. (4). The silylated derivatives were separated by gas-liquid chromatography (Varian Aerograph model 1200) using a glass column (3 mm by 1.8 m) packed with 5% SE-30 on 60 to 80 mesh Chromosorb W. The column temperature was maintained at 170°C, and nitrogen was used as a carrier gas. Authentic glucose, galactose, and mannose similarly silylated were used as known standards.

For disc gel electrophoresis, the method described by Davis (8) was used. Samples (200 to 250 μg) were placed on 7.5% polyacrylamide gel and subjected to electrophoresis at 4°C in tris(hydroxymethyl)aminomethane-glycine buffer (pH 7.5 and 8.3). The gels were stained with Coomassie blue (0.005% in 12.5% trichloroacetic acid), and excess dye was removed in 10% acid. The gels were also stained with periodic acid-Schiff reagent for carbohydrate, with alcian blue for glycoproteins and sudan black B for lipid.

In some instances, enzymes were used to elucidate the chemical composition and the location of certain components within the microconidial wall. The enzymes used in this study included chitinase (Sigma Chemical Co.) and (1 → 3)-β-D-glucanase (kindly provided by S. Nagasaki, Kochi University, Japan). Chitinase obtained from Sigma Chemical Co. was used without further purification. We confirmed, however, that it released *N*-acetylglucosamine from authentic chitin (Sigma Chemical Co.) and that no glucose or mannose was liberated from laminarin [(1 → 3)-β-glucan, Calbiochem] or from yeast mannan (Sigma Chemical Co.). (1 → 3)-β-glucanase was prepared and purified by S. Nagasaki from an imperfect fungus. It was highly specific for (1 → 3)-β-glucans and was almost totally inactive on other forms of glucans, chitin, and other polysaccharides (27). Aqueous solutions of chitinase and (1 → 3)-β-glucanase were used after being filtered through a membrane filter (0.45-μm pore size, Millipore Corp.) as described earlier (15). Cell wall specimens suspended in sterile distilled water were digested singly or in sequence with these enzymes at 25°C for 24 to 48 h. After digestion, the supernatant fluid was separated from the insoluble residue by centrifugation (12,000 × *g*, 1 h) and desiccated. The absence of bacterial contamination in hydrolysates was confirmed by means of phase-contrast microscopy at the end of the experiment. After thorough desiccation, samples were dissolved in 0.1 ml of pyridine. Sugars or amino sugars in the samples were identified by paper chromatography as described above.

RESULTS

Ultrastructure of the microconidial wall. Electron microscopy of the freeze-etched replica of the microconidia (Fig. 2 and 3) revealed that the wall surface was covered by a layer of rodlet patches. There was at least one abscission scar in each microconidium, and the center of an abscission scar was covered by concentrically oriented microfibrils (Fig. 2). The rodlet layer was coated by a thin filmy layer (Fig. 2) which tended to be removed during the freeze-etching process. In thin-sectioned preparations (Fig. 4 and 7a), three distinct layers could be distinguished in the wall of a *T. mentagrophytes* microconidium: the outermost electron-transparent layer, the middle electron-dense layer, and the inner layer of low electron density (Fig. 4 and 7a). The outermost layer and the middle layer measured 15 to 20 nm and 30 to 50 nm in thickness, respectively. The inner wall layer was 200 to 300 nm in thickness.

Examination of shadowed preparations of isolated microconidial walls showed that the outer surface appeared to be amorphous and somewhat granular, whereas the inner surface was smooth and almost featureless although the outline of underlying microfibrils (arrows) might sometimes be noticed (Fig. 5).

Ultrastructural changes of the microconidial wall associated with various chemical and enzymatic treatments. The extraction of isolated microconidial walls with UMS at room

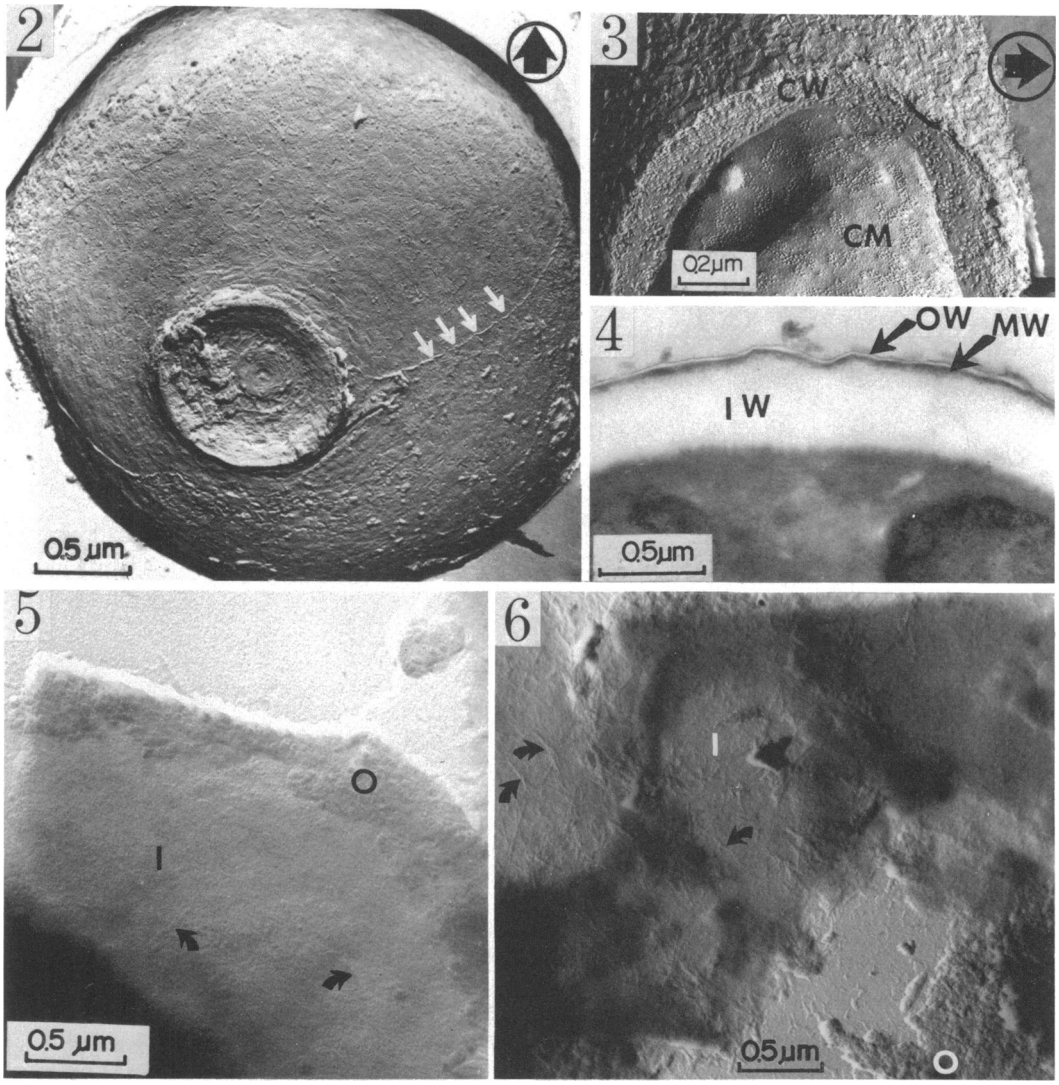


FIG. 2. *The freezed-etched surface of T. mentagrophytes microconidium showing an abscission scar and the rodlet layer. The outer wall covering the rodlets is believed to be partially removed during freeze fracture. White arrows indicate the fracture line of the outer wall. The large arrow indicates the direction of shadow.*

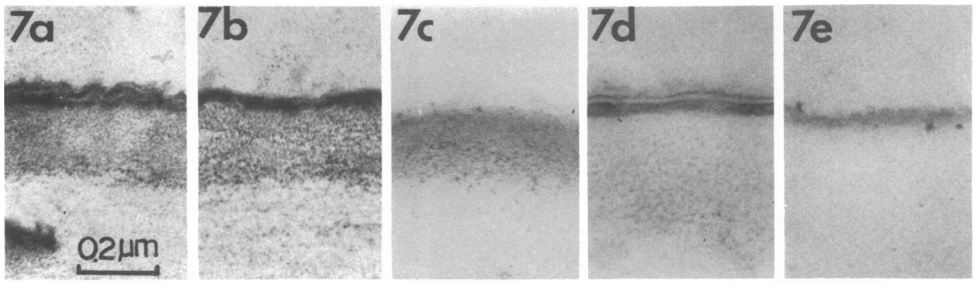
FIG. 3. *A portion of a freeze-etched T. mentagrophytes microconidium showing the obliquely fractured wall. Note that the rodlet layer is located at the near surface of the microconidial wall. The arrow indicates the direction of shadow. Abbreviations: CM, cytoplasmic membrane; CW, cell wall.*

FIG. 4. *A portion of a thin-sectioned microconidium of T. mentagrophytes illustrating three morphologically distinct layers of the wall. Abbreviations: OW, outer wall; MW, middle wall; and IW, inner wall.*

FIG. 5 and 6. *Changes in the profile of the T. mentagrophytes microconidial walls resulting from UMS treatment. (Fig. 5) Before UMS treatment; (Fig. 6) after UMS treatment (fraction I-R). Note that the microfibrils (arrows) in the inner surface became much more clearly discernible after UMS treatment. The walls were shadowed with platinum-carbon.*

temperature (23 to 26°C) for 8 h was found to cause the following ultrastructural alterations in the walls: (i) removal of material(s) from the inner wall resulting in the emergence of abundant microfibrils in the inner surface (Fig. 6), and (ii) disappearance of most of the outer wall (Fig. 7b). There was no visible change either in the rodlet pattern (data now shown) or in the middle layer after UMS treatment (Fig. 7b).

Further extraction of the UMS-treated microconidial walls with hot alkali (1 N NaOH,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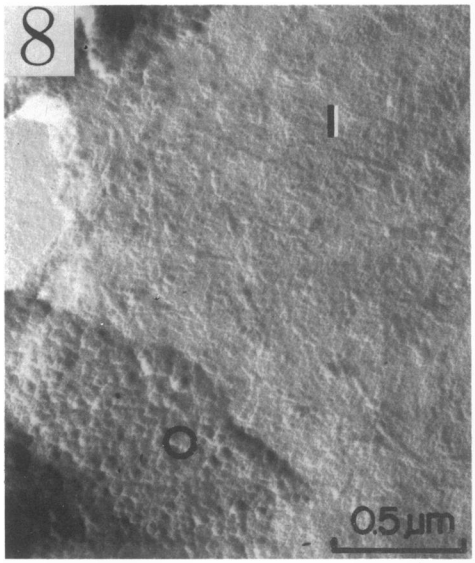
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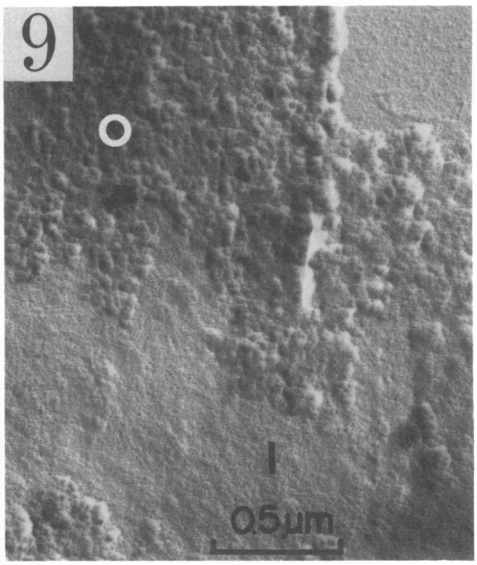
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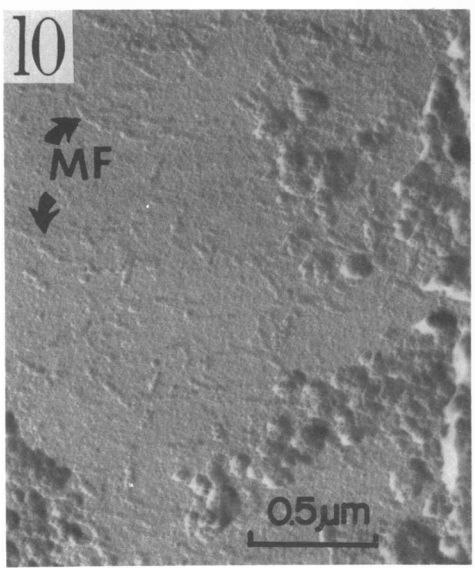
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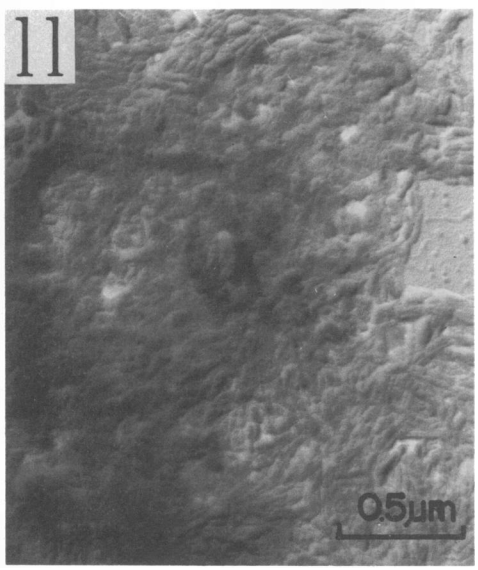
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0.5μm

100°C, 1 h) completely removed the middle electron-dense wall (Fig. 7c). Electron microscopy of freeze-etched preparations revealed that the rodlets had been almost completely removed (data not shown). In addition, hot alkali treatment seemed to slightly reduce the thickness of the inner wall and altered its stainability by lead citrate (Fig. 7b). However, such hot alkali treatment did not alter the profile of the microfibrils of the inner surface (Fig. 8). Electron micrographs of thin sections of the wall treated with (1 → 3)-β-glucanase and chitinase, and the isolated rodlet layer prepared by the method described earlier (15) are shown in Fig. 7d and 7e, respectively.

Extensive digestion of the UMS- and hot alkali-treated walls with chitinase (3 mg/ml of distilled water, 2 days at 25°C) resulted in the release of *N*-acetylglucosamine with the concomitant disappearance of the microfibrils previously seen in the inner surface of the walls (Fig. 9). Extensive digestion of the UMS- and hot alkali-treated walls (fraction II-R) by (1 → 3)-β-glucanase (2 mg/ml of distilled water, 2 days at 25°C) solubilized approximately 45 to 50% of the glucans present in the fraction II-R, releasing most of the microfibrils from the glucan matrices (Fig. 10). The walls previously treated with UMS and hot alkali underwent rapid disintegration when exposed to hot acid (1 N H_2SO_4, 100°C). Electron microscopy of partially hydrolyzed walls revealed the transformation of the glucan matrices into bundles of microfibers (Fig. 11) which were considerably thicker than the chitin fibrils. More extensive acid hydrolysis (1 N H_2SO_4, 110°C, 12 h) resulted in almost complete disintegration of UMS-hot alkali-treated walls, leaving only remnants of wall fragments and microfibrils (data not shown) which were predominantly chitinous in nature.

Chemical composition of the microconidial walls. Chemical analyses of the purified microconidial walls showed that they were composed predominantly of carbohydrate (56.1% neural polysaccharides and 16.0% chitin), protein (22.6%), and with smaller amounts of lipid (6.5%), melanin-like pigment, ash, and phosphorus (Table 1). The wall polysaccharides contained glucose and mannose at a ratio of 3:1. A trace amount of galactose could be demonstrated by gas-liquid chromatography (data not shown). The amino acid composition of the microconidial wall proteins is summarized in Table 2.

Chemical characterization of the major wall fractions. The UMS extraction solubilized approximately 25% of the intact wall as determined by dry weight measurement (Table 1). Dialysis of the UMS extract against distilled water and subsequent desiccation of the dialysate yielded a white powdery material which we referred to as fraction I (Fig. 1). Disc gel electrophoresis of fraction I yielded only one band when stained with Coomassie brilliant blue (Fig. 12). When several gels were run in parallel and stained with different reagents, a single band was observed consistently in each gel at mobilities corresponding to that of the protein band (Fig. 12) indicating that fraction I was a glycoprotein-lipid complex. The same band was also stained intensely with alcian blue, a stain for glycoprotein (data not shown).

Upon chemical analysis, fraction I was found to consist of protein (42.5%), carbohy-

FIG. 7. *Changes in the profiles of thin-sectioned T. mentagrophytes microconidial walls resulting from various chemical and enzymatic treatments. (a) Untreated wall. (b) Wall extracted with UMS (8 h at 25°C). Note that the outer wall is absent. (c) Wall extracted with UMS (8 h at 25°C) and then with 1 N NaOH (1 h at 100°C). Note that no outer and middle walls are present. (d) Wall treated with (1 → 3)-β-glucanase and chitinase (25°C, 48 h). Note that the inner wall is mostly digested off. (e) The rodlet layer prepared by the method described earlier (15). Both the outer and inner walls are completely removed.*

FIG. 8. *Microconidial wall of T. mentagrophytes sequentially treated with UMS and hot alkali (1 N NaOH, 1 h, 100°C) (fraction II-R). The wall was shadowed with platinum-carbon. Note that the wall fragment still retained the rigid original shape. The irregularly woven network of microfibrils can be clearly seen in the inner surface of the wall.*

FIG. 9. *Microconidial wall of T. mentagrophytes sequentially treated with UMS, hot alkali, and chitinase (fraction V-R). The wall was shadowed with platinum-carbon. The microfibrils were no longer visible in the inner surface indicating that the microfibrils were chitinous in nature.*

FIG. 10. *Microconidial wall of T. mentagrophytes sequentially treated with UMS, hot alkali (1 N NaOH, 1 h, 100°C), and (1 → 3)-β-glucanase (fraction IV-R). The wall was shadowed with platinum-carbon. Note that microfibrils (MF) originally embedded in the inner wall layer (Fig. 6 and 8) were rendered free as a result of the digestion of the glucan matrices.*

FIG. 11. *Microconidial wall of T. mentagrophytes sequentially treated with UMS, hot alkali, and hot acid (1 N H_2SO_4, 100°C, 1 h). The wall was shadowed with platinum-carbon. Note that the amorphous glucan matrices were transformed into bundles of microfibers.*

TABLE 1. *Chemical composition of the major fractions of T. mentagrophytes microconidial walls*

Component	Composition (%)[a]					
	Microconidial wall	I[b] (24.6 ± 3.2)[c]	I-R[b] (72.1 ± 8.6)	II[b] (20.3 ± 3.2)	II-R[b] (48.4 ± 6.7)	Rodlet layer[d] (9.6 ± 1.1)
Protein[e]	22.6 ± 1.2	42.5 ± 2.1	15.0 ± 0.9	38.1 ± 1.6	3.0 ± 0.2	83.0 ± 2.7
Carbohydrate:						
Neutral sugar (as glucose)	56.1 ± 2.6	29.2 ± 1.8	64.1 ± 3.4	56.4 ± 2.8	55.6 ± 3.1	7.5 ± 1.6
Chitin	16.0 ± 1.3	0	22.5 ± 1.6	0	39.7 ± 1.8	0
Lipid	6.5 ± 0.8	21.0 ± 2.6	0	0	0	0
Melanin-like pigment	0.2 ± 0.02	0	0.3 ± 0.02	1.6 ± 0.06	NT[f]	2.0 ± 0.3
Phosphorus	0.2 ± 0.01	NT	NT	NT	NT	0
Ash	1.7 ± 0.08	NT	NT	NT	NT	0

[a] Average (mean ± standard deviation) of three analyses. Phosphorus and ash were analyzed twice.
[b] See Fig. 1 for fraction designation.
[c] Figures in parentheses represent percent of dry weight (mean ± standard deviation) of intact microconidial walls in each fraction. Data based on five to seven experiments.
[d] Data from our earlier report (15).
[e] Determined by the method of Lowry et al. (19). All samples were solubilized in hot alkali (1 N, 100°C, 15 min), and then cooled to 25°C prior to analysis.
[f] Not tested.

TABLE 2. *Amino acid composition of selected fractions of T. mentagrophytes microconidial wall*

Amino acid	mol%		
	Microconidial wall	Fraction I	Rodlet layer[a]
Lysine	4.3	4.5	7.7
Histidine	Trace	Trace	1.3
Arginine	1.9	3.8	1.1
Cysteric acid	0.1	0.2	0.1
Aspartic acid	12.2	8.7	15.7
Threonine	5.4	5.5	5.1
Serine	5.9	7.8	4.0
Glutamic acid	9.5	9.5	8.3
Proline	3.8	5.3	3.4
Half-cystine	3.0	0.6	1.9
Glycine	6.1	8.0	9.0
Alanine	6.4	7.6	7.0
Valine	5.8	4.7	7.5
Methionine	0.7	0.9	0.1
Isoleucine	3.2	3.5	3.2
Leucine	6.0	6.0	7.4
Tyrosine	1.5	2.2	1.3
Phenylalanine	2.6	2.9	2.7
Amide NH_3	21.6	18.2	11.6

[a] Data cited from our earlier report (15).

drate (29.2%), and lipid (21.0%). Amino acid analysis of fraction I (Table 2) showed that the protein moiety of the glycoprotein-lipid complex contained high percentages of glutamic and aspartic acids but only trace amounts of sulfur-containing amino acids. The sugar moiety of this fraction contained glucose, mannose, and galactose at a ratio of 2:1:trace. The UMS-extracted microconidial wall (fraction I-R) was composed of 64.1% neutral sugars, 22.5% chitin, and 15.0% protein (Table 1).

The hot alkali treatment solubilized approximately 27 to 33% of the UMS-treated walls. This alkali-soluble fraction (fraction II) was darkly pigmented and consisted of 38.1% protein and 56.4% carbohydrate (Table 1).

The microconidial walls sequentially treated with UMS and hot alkali (fraction II-R) consisted of glucans (55.6%), chitin (39.7%), and a lesser amount of protein (3.0%).

DISCUSSION

In the present study, several major structural components of the microconidial wall of *T. mentagrophytes* were isolated and chemically characterized. We were also able to determine the approximate localization of these components within the wall by the use of sequential chemical and enzymatic digestion and electron microscopy. The fractionation procedure employed in the present study (Fig. 1) sequentially removed the individual wall layers of *T. mentagrophytes* microconidia, thus enabling us to characterize the chemical nature of each layer and to observe the ultrastructural changes resulting from each treatment.

A glycoprotein-lipid complex (fraction I) obtained by the UMS extraction is considered to represent the major component constituting the outermost wall as well as a portion of the inner wall of the microconidial walls. This speculation is based on the observations that most of the outermost wall and a portion of the inner layer were removed during the UMS

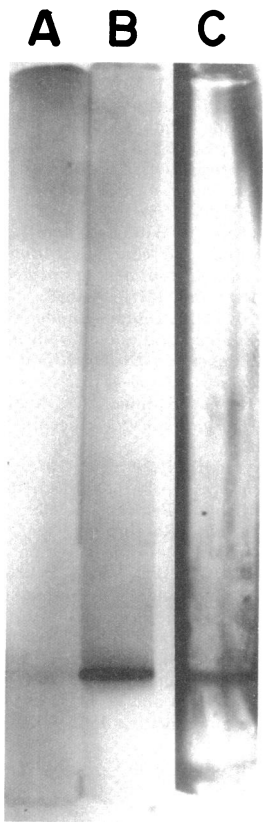

FIG. 12. *Disc gel electrophoresis of fraction I isolated from microconidial walls by the UMS treatment. (A) Gel stained with periodic acid-Schiff reagent for carbohydrate. (B) Gel stained with Coomassie brilliant blue for protein. (C) Gel stained with sudan black B for lipids.*

treatment (Fig. 6 and 7b) and that the material obtained by the dialysis of the UMS extract was electrophoretically homogeneous and reacted positively to protein, carbohydrate, and lipid stains (Fig. 12). This glycoprotein-lipid complex was partially removed from the wall surface by subjecting the whole microconidia to a less drastic extraction condition (4 M urea plus 1% mercaptoethanol plus 1% sodium dodecyl sulfate, for 1 h, at 25°C) under which no viability of the microconidia was lost (D. R. Wu, unpublished data). However, such microconidia underwent germination more slowly than the untreated spores when exposed to L-leucine, a known germination inducer of this microconidium (14). It is probable that the glycoprotein-lipid complex has some physiological or enzymatic function(s) in germination of the microconidium. Enzymatic activities of various glycoproteins associated with fungal cell walls have been known and reviewed recently by Gander (12).

Since the rodlet layer and the middle layer coincidentally disappeared as the result of hot alkali treatment (Fig. 7c), it is assumed that a portion of the middle layer corresponds to the rodlet layer. The bulk portion of protein (fraction II) solubilized from UMS-treated walls by hot alkali is believed to have been derived from the rodlets because the isolated rodlet layer of *T. mentagrophytes* microconidial wall has been shown (15) to consist predominantly of protein and was soluble only in hot alkali (1 N, NaOH, 100°C), but not in UMS.

The observations that the microconidial wall sequentially treated with UMS and hot alkali still retained its original wall shape (Fig. 8) and that it underwent partial disintegration upon treatment with (1 → 3)-β-glucanase (Fig. 10) or mild hot acid (Fig. 11) are compatible with the idea that the (1 → 3)-β-glucan is partially responsible for the shape and rigidity of *T. mentagrophytes* microconidial walls. The exact chemical nature of bundles of microfibers (Fig. 11) seen as a result of acid treatment remains to be elucidated. A similar transformation of an amorphous fungal (1 → 3)-β-glucan into bundles of microfibers by acid treatment has been reported (18).

Since the microfibrils present in the inner microconidial wall (Fig. 6 and 8) were selectively removed by chitinase (Fig. 9), with concomitant accumulation of *N*-acetylglucosamine in the supernatant (data not shown), we assume these microfibrils to be chitinous.

One interesting ultrastructural feature of the *T. mentagrophytes* microconidial wall is the presence of an abscission scar (Fig. 2) that strikingly resembles the bud scar of yeast (22). To our knowledge, this is the first demonstra-

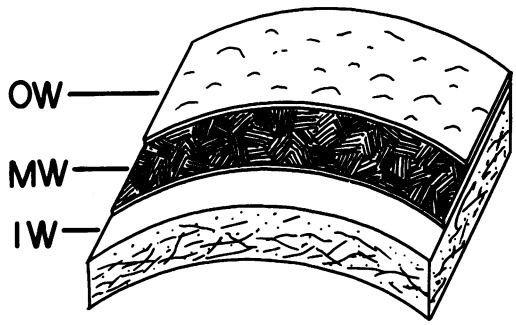

FIG. 13. *Diagrammatic representation of the microconidial wall of T. mentagrophytes. The outer wall (OW) consists of a glycoprotein-lipid complex. The middle electron-dense wall (MW) represents the proteinaceous rodlet layer embedded in polysaccharides. The inner wall (IW) consists of amorphous glucan and microfibrillar chitin.*

tion of a bud scar-like structural in fungal conidia. Although the yeast bud scars are rich in chitin microfibrils (6), we obtained no evidence for a similar enrichment of the abscission scars of this microconidium with chitinous microfibrils.

A diagrammatic model of the *T. mentagrophytes* microconidial walls based on the data presented in this paper is shown in Fig. 13.

ACKNOWLEDGMENTS

This investigation was supported by the Office of Naval Research.

Excellent technical assistance was provided by Jordan Pollack and Vivien Pacold. The cooperation of C. F. Lange for amino acid analysis and of S. Nagasaki for supplying purified (1 → 3)-β-glucanase is also acknowledged.

LITERATURE CITED

1. **Ames, B. N.** 1966. Assay of inorganic phosphate, total phosphate and phosphatase, p. 116–117. *In* E. F. Neufeld and V. Ginsburg (ed.), Methods in enzymology, vol. 8. Academic Press Inc., New York.
2. **Bartnicki-Garcia, S., and E. Reyes.** 1964. Chemistry of spore wall differentiation in *Mucor rouxii*. Arch. Biochem. Biophys. 108:125–133.
3. **Blumenthal, H. J., and S. Roseman.** 1957. Quantitative estimation of chitin in fungi. J. Bacteriol. 74:222–224.
4. **Bolton, C. H., J. R. Clamp, and L. Hou.** 1965. The use of gas liquid chromatography in investigations on glycoproteins. Biochem. J. 96:5c–6c.
5. **Buckley, P. M., V. E. Sjaholm, and N. F. Sommer.** 1966. Electron microscopy of *Botrytis cinerea* conidia. J. Bacteriol. 91:2037–2044.
6. **Cabib, E., and B. Bowers.** 1971. Chitin and yeast budding. Localization of chitin in yeast bud scars. J. Biol. Chem. 246:152–159.
7. **Chaykin, S.** 1966. Protein structure: aldolase, p. 64–66. *In* S. Chaykin (ed.), Biochemistry laboratory techniques. John Wiley and Sons, Inc., New York.
8. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404–427.
9. **Esouerré-Tugayé, M. T., and A. Touzé.** 1971. La paroi des conidies de *Collectotrichum lagenarium* (Pass.) Ell. et Halst. Phytochemistry 10:821–827.
10. **Folch, J., M. Lees, and G. H. Stanley.** 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.
11. **Friedman, B. A., P. R. Dugan, R. M. Pfister, and C. C. Remsen.** 1968. Fine structure and composition of the zoogloeal matrix surrounding *Zoogloea tamigeria*. J. Bacteriol. 96:2144–2153.
12. **Gander, J. E.** 1974. Fungal cell wall glycoproteins and peptidopolysaccharides. Annu. Rev. Microbiol. 28:103–119.
13. **Ghiorse, W. C., and M. R. Edwards.** 1973. Ultrastructure of *Aspergillus fumigatus* conidia development and maturation. Protoplasma 76:49–59.
14. **Hashimoto, T., C. D. R. Wu, and H. J. Blumenthal.** 1972. Characterization of L-leucine-induced germination of *Trichophyton mentagrophytes* microconidia. J. Bacteriol. 112:967–976.
15. **Hashimoto, T., C. D. Wu-Yuan, and H. J. Blumenthal.** 1976. Isolation and characterization of the rodlet layer of *Trichophyton mentagrophytes* microconidial walls. J. Bacteriol. 127:1543–1549.
16. **Hemmes, D. E., E. S. Kojima-Buddenhagen, and H. R. Hohl.** 1972. Structural and enzymatic analysis of the spore wall layers in *Dictyostellium discoideum*. J. Ultrastruct. Res. 41:406–417.
17. **Horikoshi, K., and S. Iida.** 1964. Studies of the spore coats of *Aspergillus oryzae*. Biochim. Biophys. Acta 83:197–203.
18. **Jelsma, J., and D. R. Kreger.** 1975. Ultrastructural observations on (1→3)-β-D-glucan from fungal cell walls. Carbohydr. Res. 43:200–203.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
20. **Mahadevan, P. R., and U. R. Mahadkar.** 1970. Major constituents of the conidial wall of *Neurospora crassa*. Indian J. Exp. Biol. 8:207–210.
21. **Mangenot, F., and O. Reisinger.** 1975. Form and function of conidia as related to their development, p. 789–846. *In* D. J. Weber and W. M. Hess (ed.), The fungal spore: form and function. John Wiley and Sons, New York.
22. **Matile, P., H. Moor, and C. F. Robinow.** 1969. Yeast cytology, p. 219–302. *In* A. H. Rose and J. S. Harrison (ed.), The yeast, vol. 1. Academic Press Inc., New York.
23. **Morris, D. L.** 1948. Quantitative determination of carbohydrate with Dreywood's anthrone reagent. Science 107:254–255.
24. **Rebell, G., and D. Taplin.** 1970. Dermatophytes, their recognition and identification. University of Miami Press, Coral Gables, Fla.
25. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208–212.
26. **Winzler, R. J.** 1955. Determination of serum glycoproteins, p. 279. *In* D. Glick (ed.), Methods of biochemical analysis, vol. 2. Interscience Publishing Co., New York.
27. **Yamamoto, S., J. Fukuyama, and S. Nagasaki.** 1974. Production, purification, crystallization, and some properties of yeast cell lytic enzyme from a species of fungi imperfecti. Agr. Biol. Chem. 38:329–337.