JOURNAL OF BACTERIOLOGY, Dec. 1979, p. 1063-1070
0021-9193/79/12-1063/08$02.00/0

Vol. 140, No. 3

Purification and Chemical Characterization of the Rodlet Layer of *Neurospora crassa* Conidia

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Received for publication 19 September 1979

The rodlet layer of *Neurospora crassa* macroconidia has been purified and chemically characterized. Sheets of rodlets were released from the conidial surface by vigorously shaking conidia in water. Conidia were removed by filtration and low-speed centrifugation, and the rodlets were recovered from the supernatant by high-speed centrifugation. The rodlet pellet comprised 1.9% of the initial dry weight. Chemical analysis was hampered by the insolubility of the rodlets. They were not solubilized by heating in various protein-denaturing buffers and were only partially dissolved by heating in 1 M NaOH at 100°C for 5 min. Nevertheless, they were found to be largely composed of protein (91%, based on total nitrogen). The major amino acids in acid hydrolysates were aspartic acid, glycine, serine, alanine, half-cystine, and valine. Glucosamine was not detected in acid hydrolysates. The sulfur content was 2.5%, and this could be accounted for in half-cystine and methionine. Carbohydrate comprised just over 2%. The phosphorus content was 0.21%, of which less than one-third was accounted for in phospholipid. The total fatty acid content was 1.0%, most of which could be accounted for by the fatty acids of the phospholipids.

Neurospora crassa conidia have been shown to be covered with a layer of regularly arranged fibers, or rodlets, which play a role in conferring water repellency to the conidia and in making them readily dispersed in the air (2, 9). Studies on the chemical composition of rodlets from other fungi have been hampered by difficulties in isolation (12), although Hashimoto et al. (14) have recently succeeded in isolating rodlet layers from microconidia of *Trichophyton mentagrophytes* by preparing cell walls and then chemically and enzymatically digesting away all of the wall except the rodlet layer.

We have shown that when *N. crassa* conidia are shaken in water, most of the rodlet layer is lost from the surface and appears as sheets in the surrounding liquid (9). We have devised a simple centrifugation method, based on this observation, to recover the rodlets from this species. Chemical analysis of the rodlets showed them to be composed largely of protein along with small, but significant, quantities of carbohydrate and lipid. The water repellency of the conidium can reasonably be attributed to the presence of a small amount of surface lipid coupled with the rough topography of the layer.

MATERIALS AND METHODS

The fungal strains, the growth medium, the growth conditions, and the electron microscopy techniques have been described in the preceding paper (9). Dry weights were determined by drying to constant weight under vacuum (<100 Pa) over P_2O_5 after freeze drying. Unless specified, the wild-type strain STA4 (St. Lawrence) was used.

Rodlet preparation. Our standard procedure was as follows. The aerial mycelium and macroconidia (referred to as conidia elsewhere in this paper) were scraped from the surface of 150 6- to 8-day-old petri dish cultures, dried in a freeze dryer without initial freezing, and then stored for up to 4 weeks under vacuum over P_2O_5. The mass of mycelium and conidia of about 12 g (dry weight) was dispersed by shaking it in 500 ml of chilled glass-distilled water. A thick curd formed, which was partially dispersed by ultrasonication (MSE Ultrasonic Disintegrator with a 1-cm-diameter titanium probe operated for three to six bursts of 15 to 30 s in duration). The mixture was then filtered through cotton mesh (holes about 0.25 by 0.25 mm). Most conidia passed through, but the mycelium was retained. This mycelium was ultrasonicated in 150 ml of water and filtered again through cotton mesh, and the filtrate was combined with the initial filtrate. The pooled filtrates were centrifuged at low speed (700 × *g* for 5 min), the supernatant was retained, and the pellet was washed twice by suspending it in 200 ml of water and centrifuging. The supernatant and washings were combined, ultrasonicated, and centrifuged at low speed. The supernatant, of just over 1 liter, was then centrifuged at high speed (48,000 × *g* for 1 h or 100,000 × *g* for 45 min). The orange, waxy-textured, crude rodlet pellets so obtained were combined and washed five times by suspending in 100 ml of water and centrifuging at high speed (100,000 × *g* for 45 min). A low-speed spin was interposed before the high-speed

spins of washes 1, 3, and 5, and the small pellets so obtained were discarded. The final rodlet pellet, of about 250 mg, was dispersed in 20 ml of water, frozen in liquid nitrogen, and freeze dried. This freeze-dried material was stored at −20°C under vacuum over P_2O_5. Samples for analysis were weighed and, if appropriate, dispersed in water. For chemical analysis two bulk preparations of about 500 mg of rodlets were prepared and analyzed separately.

Chemical analyses. (i) Acid hydrolysis and amino acid analysis. Rodlets (about 2 mg) were acid hydrolyzed by heating at 105°C in 6 M HCl (2.0 ml) with hydrolysis tubes (Pierce Chem. Co., Rockford, Ill.) that had been evacuated under vacuum for 5 min after foaming ceased. The hydrolysate was dried on a rotary evaporator at 45°C and redissolved in 0.1 M HCl (1.0 ml). Samples were analyzed quantitatively for amino acids, except tryptophan, with a Technicon TSM automatic analyzer. Tryptophan was estimated as follows. A rodlet sample was subject to alkaline hydrolysis [5 M $Ba(OH)_2$ at 110°C in evacuated tubes, 20 h]. Carbon dioxide was bubbled through the hydrolysate to remove barium ions, and the amino acids were separated by thin-layer electrophoresis at pH 2.0 (4). Tryptophan was detected with Ehrlich reagent and estimated visually by comparison with a range of standards. Semi-quantitative amino acid analysis was carried out by two-dimensional thin-layer electrophoresis and chromatography (4).

(ii) Protein estimation. Protein was estimated by four methods: (i) the Biuret procedure after heating samples in 1.0 M NaOH at 100°C for 5 min (15); (ii) measuring the yield of amino acids after acid hydrolysis with a method essentially similar to that of Yemm and Cocking (27); (iii) summation of the amounts of each amino acid as measured by an autoanalyzer; (iv) measuring it from the total nitrogen analysis, assuming nitrogen is present only in protein, and a conversion factor for total nitrogen to protein of 6.65 (based on the amino acid composition of rodlet protein, Table 2). Bovine albumin (fraction V, fatty acid free, Miles Laboratories, Inc., Elkhart, Ind.; dried under vacuum over P_2O_5) was used as a standard for the first two procedures. The purity of this standard was found to be 95% when checked against protein standard solution (Sigma Chemical Co., St. Louis, Mo.) by using the Biuret test. This figure is close to the manufacturer's claim for the preparation and was used in calculations of the protein present.

(iii) Carbohydrate estimation. Carbohydrate was estimated by the anthrone procedure (22) and also by the phenol method, with the absorbance read at 488 nm (15). Glucose was used as a standard.

(iv) Fatty acids. Samples (ca. 10 mg) were saponified by adding 10% (wt/vol) KOH in methanol (1 ml) followed by heating at 80°C for 1.5 h in a sealed tube. The solutions were acidified with dilute H_2SO_4, the fatty acids were extracted into petroleum spirit (40 to 60°C fraction), and the solvent was then removed. The acids were methylated with diazomethane and separated by gas-liquid chromatography (180°C on 2-m by 2-mm columns of Chromosorb W coated with 15% ethylene glycol succinate). Acids were identified by their retention time.

(v) Phosphorus and phospholipids. Total phosphorus was estimated by two methods: (i) colorimetric estimation of the phosphate after acid digestion (19) and (ii) uniformly labeling rodlets with ^{32}P by incorporating $^{32}P_i$ (specific activity, ca. 200,000 cpm/μmol) into the initial growth medium. Rodlets were prepared from the radioactive conidia in the standard manner, and the ^{32}P present in the final preparation was determined by liquid scintillation spectrometry (7). Radioactive phospholipids were separated by two-dimensional thin-layer chromatography (3). Phosphatidylcholine and phosphatidylethanolamine were identified by their migration in comparison with a standard phospholipid preparation made from *Spirodela oligorrhiza* (3). The identity of phosphatidylethanolamine was confirmed by reaction with ninhydrin.

(vi) Total nitrogen and sulfur. Total nitrogen was estimated by the Kjeldahl procedure. Total sulfur was estimated by the methylene blue method (17); inorganic sulfate was estimated by the same method, except that the acid digestion step was omitted.

(vii) Water. Residual water in rodlets was determined by drying to constant weight at 90°C under vacuum.

RESULTS

Purification and electron microscopy of the rodlet layer. The purification procedure was monitored by removing samples and examining them by light microscopy and by negative staining under an electron microscope. The crude pellet was composed largely of rodlets together with a few conidia. These few conidia were removed during the subsequent differential centrifugation steps, and no whole cells were present at the final suspension stage immediately before freeze-drying. Shadowing the discarded conidia (9) showed that almost all rodlets had been removed. When examined by phase-contrast microscopy, the preparation consisted of small particles showing Brownian movement, along with occasional much larger clumps. When viewed with a polarizing microscope, the clumps showed optical activity. When examined by negative staining, all visible material was composed of rodlets usually occurring in small sheets (Fig. 1; cf. Fig. 7 and 8, reference 9).

When examined under an electron microscope by thin sectioning, the purified preparation consisted of a mass of elongate structures and amorphous masses corresponding to the rodlet layer sheets cut in cross section and in face view, respectively (Fig. 2). The structures are mainly dark staining, although in cross section a lightly staining core bounded by two darker-staining bands could sometimes be distinguished. The width of this layer is about 8 nm, similar to that of the rodlet layer of intact spores cut in section (9). In some sections the sheets of rodlets seem to have packed together in doublets to give

structures bounded by two dark layers and with the central region divided by a further dark layer. Freeze-fractured rodlet preparations showed many small structures clearly identifiable as rodlets (Fig. 3), but in dry shadowed rodlet preparations the rodlet structure was not so obvious (Fig. 4) and resembled the inner rather than the outer rodlet surface (9). We suggest that during preparation, the rodlet layers became aligned with the outer faces opposite each other. Thus, when shadowed, only the inner rodlet face is visible. In freeze-fracturing, on the other hand, there is a tendency for fractures to pass between the aligned outer faces and thus reveal the hydrophobic outer-face surfaces. The orientation of stacked rodlet layers presumably confers optical activity to the large rodlet clumps.

When the final rodlet suspension was dried it formed a light orange powder which was difficult to wet but which could be evenly dispersed in water by vigorous shaking and ultrasonication. When the suspension was not frozen in liquid nitrogen before freeze drying, the rodlets formed large granules which were very difficult to disperse in water. The dry weight of the rodlet layer obtained comprised 1.90 ± 0.14% (mean ± standard error, four determinations) of the total dry weight of the cells.

Chemical analysis. Chemical analysis was hampered by the insolubility of the rodlet preparations. We show later that rodlets are largely composed of protein, but they were not dissolved to any major extent by warming at 37°C in 0.1 M pH 10.5 borate buffer containing dithiothreitol (0.05 M) and sodium dodecyl sulfate (1%, wt/vol), a buffer used to dissolve resistant protein in bacterial coat layers (1). Nor were the rodlets dissolved by boiling for up to 10 min in 0.05 M pH 6.5 phosphate buffer containing urea (4 M), mercaptoethanol (1%, wt/vol), and sodium dodecyl sulfate (1%, wt/vol), a denaturating buffer that will solubilize most viral coat proteins.

Rodlets were only partially dissolved by heating in 1 M NaOH at 100°C for 5 min. When the alkali-resistant residue was then subjected to our standard acid hydrolysis procedure for 16 h, it yielded amino acids (assessed semi-quantitatively), but again a residue remained. This alkali- and acid-resistant residue was completely solubilized by heating in concentrated HCl at 100°C for 1 h, again yielding amino acids. When rodlets were subjected directly to our standard hydrolysis procedure, some variation was found in behavior between different samples, even including replicates of the same bulk preparation. In some instances the solution had cleared by 20 h, but in other instances a residue remained, although all solutions became clear after 70 h. This resistance of rodlets to alkali and acid is attributed to the presence of the large clumps of rodlets, visible in the preparations under a light microscope, which provide a relatively low surface area available for chemical attack.

(i) Protein. The major component of rodlets is protein (Table 1), although different analytical methods gave slightly different estimates of the exact proportion present. Both the Biuret and the Yemm and Cocking procedures are limited in their accuracy by the assumption that the rodlet protein behaves in the assay exactly as does serum albumin. The Biuret procedure is further limited by our inability to completely solubilize the rodlets in alkali before assay. When the procedure was modified by using stronger alkali (5 M), or longer heating at 100°C (15 min), yield decreased rather than increased. Analysis of the insoluble residue by acid hydrolysis followed by Yemm and Cocking quantitation showed that the Biuret values of Table 1 are a minimum of 6% too low. The estimate of the protein present based on summing the amounts of the individual amino acids present after hydrolysis could be in error because of the destruction of some amino acids during hydrolysis and because of incomplete hydrolysis of some peptides. To reduce such errors, acid hydrolyses were carried out for 20, 40, and 70 h, and the estimates of yield and composition were based on the maximum yields of each of the individual amino acids (Table 2). Tryptophan is destroyed by acid hydrolysis and was estimated separately. For most of the amino acids, prolonged acid hydrolysis led to slight variation in yield (70-h values within 15% of those at 20 h). However, for valine and isoleucine, increases of 34 and 29%, respectively, were found, suggesting the presence in the 20-h hydrolysates of acid-resistant peptides involving these two amino acids (5). The figure of 79% protein in rodlets, based on amino acid analysis (Table 2), is limited by being based on one series of hydrolysates prepared from one preparation of rodlets. Furthermore, because of the initial insolubility, hydrolysis may not have been complete even by 70 h. Preliminary analyses based on 20-h hydrolyses, and not corrected for maximum yield, gave figures from 70 to 82% (four determinations). The amino acid composition of the rodlets (Table 2) shows relatively high proportions of aspartic acid (including asparagine), glycine, serine, alanine, half-cystine, and valine. Cysteic acid and hydroxyproline were not detected in the hydrolysates. Glucosamine was also absent, indicating the absence of chitin and chitosan from rodlets.

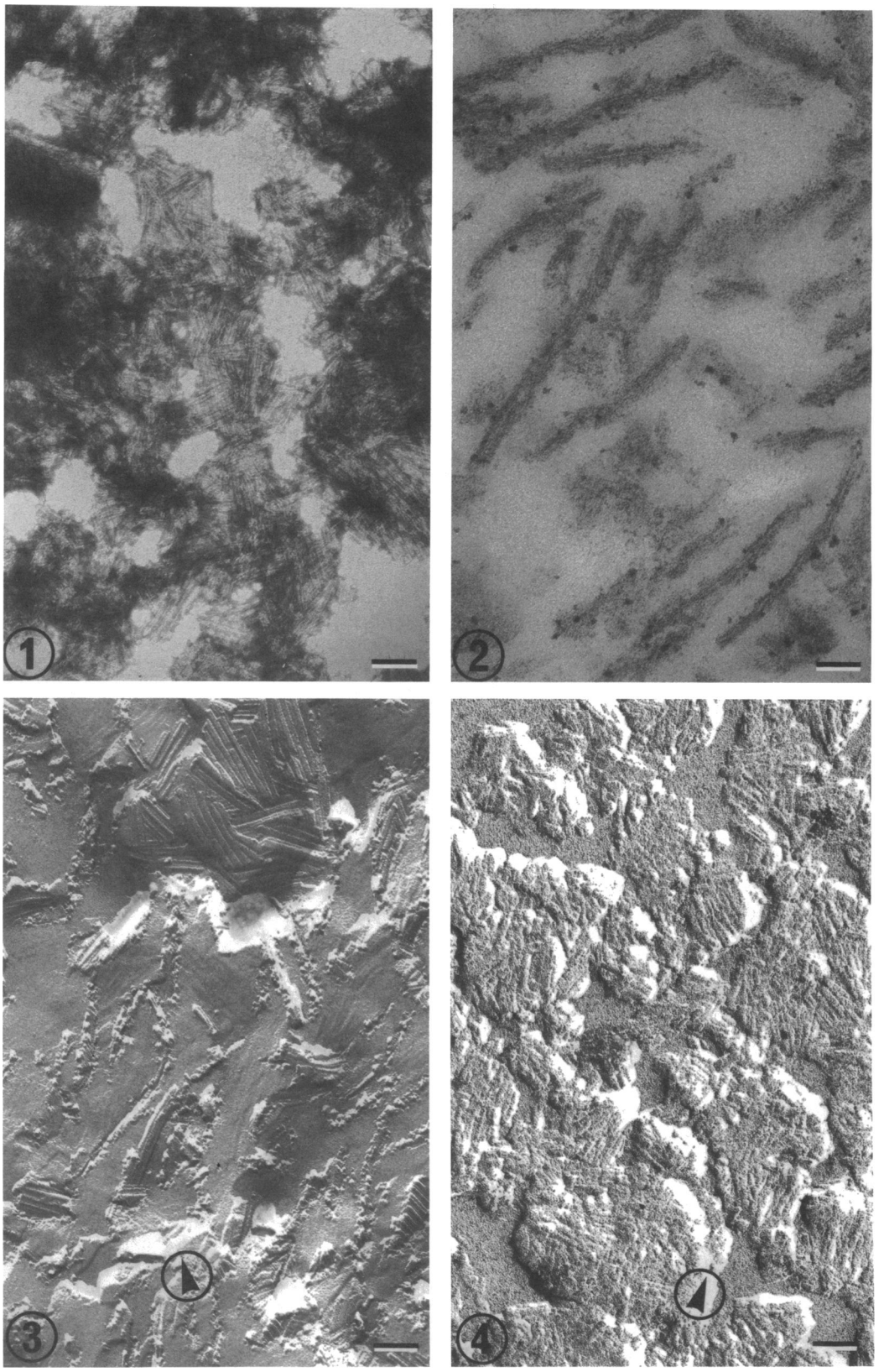
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TABLE 1. *Chemical composition of N. crassa rodlets*[a]

Component	% by wt
Protein	
Estimated by the Biuret procedure	85 ± 2
Estimated by the Yemm and Cocking procedure	98 ± 2
Sum of amino acids	79
Total nitrogen	91 ± 1
Carbohydrate (glucose equivalents)	
Estimated by the anthrone procedure	2.1 ± 0.3
Estimated by the phenol method	2.5 ± 0.3
Fatty acids	1.0 ± 0.2
Phospholipids	0.9
Phosphorus (total)	
Estimated by acid digestion	0.21 ± 0.01
Estimated by radioactivity	0.20 ± 0.05
Nitrogen (total)	13.7 ± 0.1
Sulfur (total)	2.5 ± 0.2
Water	0.9 ± 1.7

[a] Data are mean values of duplicate or triplicate determinations on each of the two standard rodlet preparations except for protein by method iii (see Table 2) and phosphorus by method ii, which is based on uniform labeling of two separate preparations with ^{32}P. The range indicated covers the extreme values obtained for all replicates. Phospholipid was calculated as described in the text.

TABLE 2. *Amino acid composition of N. crassa rodlets*

Amino acid	Residue yield[a] (mg g^{-1}) at hydrolysis time:			mol%[b]
	20 h	40 h	70 h	
Lysine	43.6	46.6	49.5	4.98
Histidine	3.6	4.1	4.1	0.39
Arginine	6.5	6.4	6.9	0.57
Aspartic acid	117.2	124.2	124.8	13.96
Threonine	36.8	36.8	37.4	4.76
Serine	59.3	61.3	62.1	9.19
Glutamic acid	38.4	39.0	42.3	4.22
Proline	43.7	43.0	42.5	5.81
Glycine	45.3	47.0	47.3	10.68
Alanine	46.7	46.1	48.8	8.85
Half-cystine	59.8	60.7	64.1	8.09
Valine	46.1	55.7	61.8	8.03
Methionine	16.6	15.7	16.3	1.64
Isoleucine	43.5	51.5	56.1	6.39
Leucine	61.9	63.0	64.8	7.38
Tyrosine	38.2	37.2	36.1	3.01
Phenylalanine	20.8	21.0	19.2	1.84
Tryptophan	3.0			0.21

[a] Mean of duplicate determinations on single hydrolyses from one bulk preparation.

[b] Based on maximum yield values after acid hydrolysis, except tryptophan, which was estimated after alkaline hydrolysis. Total yield based on summing maximum yield values is 792.5 mg g^{-1}.

The protein present in rodlets was also estimated by determining the total nitrogen content and by assuming that all the nitrogen present was as amino acids, the nitrogen content in phospholipids being relatively very small. Although the estimate is dependent on this assumption, we believe that the value of 91% so obtained is our most reliable estimate of the protein fraction of rodlets. This figure is the same as that obtained by the Biuret procedure corrected for insoluble residue (91%). The very high figure of 98% protein obtained with the Yemm and Cocking procedure may reflect relatively greater destruction of the amino acids of serum albumin during acid hydrolysis or a relatively lower color yield of serum albumin amino acids with ninhydrin or both.

(ii) Carbohydrates and fatty acids. Carbohydrates and fatty acids comprised only a small fraction of the rodlet weight (Table 1). The nature of the carbohydrates present was not investigated further. The major fatty acids present (Table 3) are the same as those reported for *N. crassa* cellular lipids, with the percent distribution being closer to that of the phospholipids than to that of the neutral lipids (6). Extracting conidia with a lipid solvent caused no obvious change in rodlet structure (9).

(iii) Phosphorus. The phosphorus contents of rodlets estimated by acid digestion and by uniformly labeling with $^{32}P_i$ were in close agreement. The distribution of phosphorus was studied with radioactively labeled samples. About 10% of the radioactivity was recovered in the supernatant when the rodlets were suspended in 0.05 M KH_2PO_4 at 40°C for 1 h. This fraction probably represents inorganic phosphate adsorbed onto the rodlets during the initial stages of the preparation. (Rodlets prepared from unlabeled conidia that had been suspended in the radioactive supernatant from a preparation of labeled conidia contained an amount of ^{32}P corresponding to about 5% of their total phosphorus.) When the damp rodlet pellet obtained after extraction with KH_2PO_4 was further extracted with chloroform-methanol (2:1, vol/vol), 16.5% (mean of two determinations) of the radioactivity was extracted into the organic phase. This

FIG. 1 to 4. *Electron micrographs of purified rodlet preparation immediately before the final freeze-drying step. Bars, 0.1 μm.*

FIG. 1. *Negatively stained with phosphotungstic acid.*

FIG. 2. *Thin section of rodlets pelleted, fixed in glutaraldehyde, postfixed in osmium tetroxide, embedded, sectioned, and stained with uranyl acetate and lead citrate.*

FIG. 3. *Freeze-fracture replica of rodlets pelleted and fixed in glutaraldehyde. Arrowhead indicates direction of shadowing.*

FIG. 4. *Replica of a drop of rodlet suspension spread over the surface of mica and then dried before shadowing. Arrowhead indicates direction of shadowing.*

TABLE 3. *Distribution of acids in the fatty acid fraction after saponification of N. crassa rodlets*[a]

Acid	mol%
Palmitic	24.1 ± 1.4
Stearic	2.7 ± 0.2
Oleic	13.3 ± 1.3
Linoleic	50.2 ± 0.9
Linolenic	9.7 ± 0.7

[a] Data are the mean of duplicate determinations on each of the two standard rodlet preparations. The range indicated covers the extreme values obtained for all replicates.

fraction was assumed to be phospholipid because less than 1% of the radioactivity remained at the origin after the components were separated by the phospholipid thin-layer chromatography procedure (water-soluble components that might have been extracted by the lipid solvents remain at origin in this system). Over half the radioactivity was present in phosphatidylcholine (31%) and phosphatidylethanolamine (21%). The remainder was distributed among seven unidentified phospholipids. The weight of extractable phospholipid present in our standard rodlet preparations was calculated to be 0.9% (Table 1), by assuming that 16.5% of the total phosphorus was present in phospholipid and by assuming a ratio of phospholipid to phosphorus of 26. The amount of fatty acid corresponding to this amount of phospholipid is about 0.6%. This is reasonably close to the fatty acid value of 1.0% obtained by direct analysis and suggests that most of the fatty acids in rodlets can be accounted for in phospholipid, particularly since our direct phospholipid estimate is likely to be low because of difficulties in obtaining complete extraction from the rodlet clumps. Even if we assume that only half of the phospholipid was extracted, this fraction accounts for only about 30% of the rodlet phosphorus. Thus, we have accounted for less than 50% of the total phosphorus in the readily exchangeable fraction and phospholipid. The nature of the remaining phosphorus was not further investigated.

(iv) Sulfur. The sulfur content of rodlets (Table 1) can be completely accounted for by the sulfur present in half-cystine and methionine (Table 2). With the maximum yield values, the sulfur in these two amino acids accounts for 2.4% of the total rodlet weight. Negligible inorganic sulfate was detected.

(v) Other components. Only a trace of water was found when rodlets were dried at high temperature (Table 1). The only other component found to be present in rodlets was an orange pigment. Rodlet preparations were orange like *N. crassa* conidia. The pigment was insoluble in 1 M NaOH at 100°C for 5 min, was soluble in chloroform, and showed absorbance between 400 and 500 nm. It is tentatively concluded that the pigment is a carotenoid. Based on the assumption that it has the same extinction coefficient as β-carotene, the pigment accounts for less than 0.1% of the rodlet dry weight.

In summary, our data suggest that the major components of *N. crassa* rodlets are protein (91%), carbohydrate (2.5%), and phospholipid (0.9%). We have not determined whether any of the carbohydrate is covalently bound to the protein.

It is possible that traces of contaminating material not derived from the rodlets have become associated with them during preparation even though, as judged by electron microscopy, particulate impurities were essentially absent. When the same preparation procedure was applied to conidia of the *eas* mutant, which lacks rodlets, a small pellet, weighing about 3% of that found with wild type, was obtained. This pellet was orange in color, and it wetted and dispersed readily in water, suggesting it contained little lipid. It showed no fibrillar structure when negatively stained. It was composed largely of protein with a composition different from that of rodlet protein (estimated semi-quantitatively) and contained about 12% carbohydrate (anthrone test). Even if this non-rodlet material had been isolated from wild type along with rodlets, it would form too small a component to have significantly altered the main analytical results. However, the orange color of the pellet from the mutant indicates that the association of carotenoid pigment with rodlet preparations may well be an artifact of preparation.

DISCUSSION

The initial material used as a source of rodlets contained both conidia and aerial mycelium (about 80 to 20%, respectively, dry weight). Preliminary data show that some aerial hyphae do bear rodlets, although vegetative mycelium from liquid culture does not. It is thus probable that although most of the rodlets of the rodlet preparation came from conidia, a fraction came from aerial hyphae.

The only other fungal spore rodlet layer that has been well characterized chemically is that of *T. mentagrophytes* microconidia (14, 26), which, like that of *N. crassa*, is composed largely of protein (80 to 85%). Although there is a general resemblance in the molar ratios of the amino acids of these two rodlet preparations, the sulfur amino acid content was relatively much higher in *N. crassa* rodlets (half-cystine, 8.09 and 1.90%; methionine, 1.64 and 0.14%, for *N. crassa* and *T. mentagrophytes*, respectively). The rodlet layer from *Penicillium expansum* conidia has been

partially characterized and shown to be composed partly of protein which resists solubilization (12). Amino acid analysis of an alkaline partial extract showed that, in comparison with *N. crassa* rodlets, *P. expansum* rodlets appear to contain low levels of half-cystine but high levels of methionine. The rodlets of all three species resemble each other in their resistance to solubilizing by protein-denaturating buffers and in their incomplete solubility in warmed alkali.

The high half-cystine content of *N. crassa* rodlets (628 μmol/g of rodlets) deserves comment, since it has been shown (S. Brody, Abstr. Second Int. Mycol. Congress, 1977, p. 69) that the total proteins extractable from conidia have a higher half-cystine content (95 μmol/g of protein) than do those from mycelium (64 μmol/g of protein). In addition, conidia have a high proportion of residues in the disulfide form, of which at least 20% are present in a relatively low-molecular-weight buffer-soluble protein. In view of the insolubility of rodlets it is probable that they were not included as such in these analyses, but in view of their high half-cystine content it is tempting to suggest that the small protein might be a rodlet precursor. Also, dry conidia have relatively high oxidized glutathione and protein glutathione disulfide levels which drop rapidly when dry conidia contact water (10). These workers comment that "conidia must possess a marked capacity for excreting or reducing this disulfide." An alternative explanation is that rather than being intracellularly located these disulfides are associated with the rodlet layer and are lost along with the layer. In *Bacillus cereus* the formation of the "cross-patched" layer, which resembles the rodlet layer of *N. crassa* in appearance, is associated with incorporation of half-cystine residues across disulfide bonds of the protein at a relatively late stage in its development (1).

T. mentagrophytes rodlets contain 7 to 10% carbohydrate, compared with 2 to 3% for *N. crassa* rodlets, but, unlike *N. crassa* rodlets, apparently contain no lipid or phosphorus. We believe that the lipid is an integral part of *N. crassa* rodlets and plays an important role in conferring the hydrophobic properties to the conidium. Although surface lipid plays a role in making some fungal spores nonwettable, surface topography is also often important (11). We have elsewhere demonstrated that rodlets in *N. crassa* confer water repellency to the spore (2). It is plausible to suggest that this results from a combination of the rough surface topography and the presence of lipid on this surface. Thin sections of rodlets both on the conidium (9) and in rodlet preparations (Fig. 2) show osmiophilic material, reasonably attributed to lipid, concentrated on both sides of a lightly staining core, presumably of protein. Lipid-extracted spores retained some hydrophobic properties (9) which can be in part attributed to the surface topography, but as they retained the property of staining with osmium tetroxide, some lipid may have remained. In contrast to the situation in *N. crassa*, the rodlet layer of *T. mentagrophytes* is covered by another layer and is unlikely to play a direct role in water repellency.

The existence of fibrous structures similar in appearance to those of fungal spore rodlets has been reported for basidiomycete hyphae (20, 24) and the yeast phase of *Paracoccidioides brasiliensis* cells (8), but, because these structures have been shown to be composed of α-(1→3)-glucan (8, 24), the resemblance is superficial. On the other hand, the hyphal wall of *Epidermophyton floccosum* has an outer "exolayer" composed largely of protein (63%), but this layer does not have a rodlet appearance under the electron microscope (18). The existence of protein arranged in a regular hexagonal or tetragonal pattern on the surface of gram-negative bacteria is well known (23). Layers more closely resembling fungal rodlets in appearance have been reported from various *Bacillus* species and shown to be largely composed of resistant protein (1, 13, 16). Species of *Streptomyces* possess layers which have also been termed rodlets and which resemble those of fungi quite closely (25), although in this instance it has been suggested that they are largely composed of chitin (21).

In many studies of rodlets and rodlet-like structures, relatively destructive methods have been used in the purification steps. Thus, there is a problem in establishing that the material being analyzed indeed corresponds to the rodlets seen under the electron microscope. The simplicity and relative gentleness of the procedure used here suggest that the rodlets have been little altered during purification. A major focus of future work will be in solubilizing the protein component in a relatively nondestructive way so that studies can be made of the number and molecular weights of component subunits.

ACKNOWLEDGMENTS

Joy Leonard gave excellent technical assistance. The fatty acid analyses were ably carried out by Ross Holland (Plant Physiology Division, Department of Scientific and Industrial Research); the amino acid analyses were carried out by Alf Lambden (Meat Industry Research Institute of New Zealand), B. A. Tapper, and W. Bell (Applied Biochemistry Division, Department of Scientific and Industrial Research).

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