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Isolation and Characterization of Tubules and Plasma Membranes from *Cytophaga columnaris*

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Tubular structures are released from cells of *Cytophaga columnaris* after lysis of the cells. To determine the nature of these tubules, they were purified and their composition was determined. Tubules were isolated after treating cell lysates with 1.0% sodium dodecyl sulfate at pH 8.1, which solubilizes all structural components except tubules. Plasma membranes from the same organism were isolated by discontinuous sucrose gradient centrifugation of lysed cells. Both tubules and membranes are composed of lipids and proteins. Lipids extracted from tubules and plasma membranes produced similar patterns when examined by thin-layer chromatography. Proteins solubilized from membranes were separated into 14 bands by polyacrylamide gel electrophoresis, whereas those solubilized from tubules separated into only 5 bands. The presence of lipids in tubules from *C. columnaris* supports the idea that they are derived from membranes of intact cells. In this respect they are similar to tubules produced by cells of *Clostridium botulinum* and different from other tubular structures ("rhapidosomes") found in cells of *Saprospira grandis*.

Tubular structures are released from cells upon lysis of certain types of bacteria. These bacteria include *Saprospira grandis* (21), *Archangium violaceum* (31), *Sporocytophaga myxococcoides* (16), *Sorangium* no. 495 (27), *Azotobacter vinelandii* (30), *Proteus mirabilis* (20), *Photobacterium harveyi* (39), *P. vulgaris* (39), *Spirillum itersonii* (7), *Myxococcus xanthus* (14), *Pseudomonas aeruginosa* (2), *Treponema* sp. (18), *Clostridium botulinum* (38), and *Cytophaga columnaris*, formerly *Chondrococcus columnaris* (15, 26, 27).

Lewin proposed the name "rhapidosome" for the type of tubules he discovered in lysates of *S. grandis* (21). Rhapidosomes are hollow tubules, some of which possess a wick or tail which protrudes from one end of the tubule. Structures similar to those found in lysates of *S. grandis* are also found in lysates of *A. violaceum*, *S. myxococcoides*, and *Sorangium* no. 495. Rhapidosomes isolated from *S. grandis* were reported to contain ribonucleic acid (RNA), protein, and carbohydrate (8). Recent work has demonstrated the absence of nucleic acids from rhapidosomes of *S. grandis* (10). There has been speculation that rhapidosomes are bacteriophage particles (4, 10, 32) or remnants of a contractile sheath involved in gliding motility (16).

Other tubules found after lysis of bacterial cells have no tails or wicks and resemble the tubules released during lysis of *C. columnaris* and described by Pate et al. (27). These tubules are 30 nm in diameter and break into sections 50 to 1,500 nm in length. They appear to be rigid and have a hollow core which widens and narrows with a regular periodicity of about 40 nm and which is penetrable by negative stains. Clark-Walker (7) has suggested that this type of tubule may be formed by self-assembly of excess bacteriophage parts. Pate et al. (27) have presented evidence indicating that tubules from *C. columnaris* are not present in growing cells but are produced from intracytoplasmic membranes (mesosomes) during lysis of cells. Ueda and Takagi (38) have demonstrated a similar origin for tubules from *C. botulinum*.

If tubules are derived from membranes upon lysis of cells of *C. columnaris*, their chemical composition should reflect that origin; they should be composed almost entirely of protein and lipid. In this paper we report a method for the isolation of tubules and the results of chemical characterization of tubules and membranes from *C. columnaris*.

MATERIALS AND METHODS

Chemicals. Tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, ethylenediaminetetraacetic acid (EDTA), pancreatic deoxyribonuclease, and

N-2-hydroxyethylpiperadine-*N'*-2-ethane sulfonic acid (HEPES) were purchased from Sigma Chemical Co., St. Louis, Mo. Mannogram precoated thin-layer chromatography (TLC) plates (250 μm thick, Silica Gel G with an inert polymer binder) were from Mann Research Laboratories, New York, N.Y.; Coomassie Brilliant Blue from Schwartz/Mann, Orangeburg, N.Y.; Parlodion from Mallinkrodt Chemical Works, St. Louis, Mo.; phosphotungstic acid from Baker and Adamson, Morristown, N.J.; and sodium dodecyl sulfate (SDS) from Fisher Scientific Co., Fair Lawn, N.J. All other standard reagents were of the highest quality available.

Bacteria and culture conditions. *C. columnaris* 1-R43, used throughout the study, was obtained from E. J. Ordal, University of Washington, Seattle. A mutant derived from strain 1-R43 (designated NT-1) which does not produce tubules upon lysis was also used for comparison of membrane proteins by disk-gel electrophoresis. Cells were grown at 25 C with rapid shaking in 2-liter Erlenmeyer flasks containing 500 ml of enriched cytophaga broth (28) and harvested after 12 h of growth (late exponential phase) by batch centrifugation at 4 C.

Isolation of tubules. Exponentially growing cells were harvested from 1 liter of cytophaga broth, and tubules were isolated as described in Fig. 1.

Isolation of plasma membrane. Cells were harvested as in the procedure for isolation of tubules and treated as in Fig. 2. Discontinuous sucrose gradients were prepared and centrifuged by the method of Schnaitman (35). Samples were removed drop-wise from the bottom of the gradients.

Chemical analyses. Each purified and washed sample was suspended in distilled water. The total yield was estimated gravimetrically after drying a portion of the suspension to constant weight. The remainder of the suspension was analyzed for protein by the Lowry method (22) after being stirred overnight with 1.0 M NaOH, RNA (19), deoxyribonucleic acid (5), carbohydrate (12), and phosphate (7). Hexosamines were determined by the method of Boas (3). Total extractable lipid was estimated by refluxing a dried, weighed sample for 24 h with a mixture of ethanol and acetone (1:1) acidified with 3 M HCl (5% [vol/vol] of the reflux mixture) in a tared soxhlet thimble. After the thimble and contents were dried to constant weight, the total lipid was calculated by difference. Loosely bound lipid was estimated by the method of Salton and Freer (33).

Extraction and TLC of lipids. Purified fractions (0.1 to 0.5 g [wet weight]) were suspended in about 10 ml of methanol-chloroform-water (10:5:4) in a 30-ml Corex test tube and allowed to stand for 1 h on crushed ice (1). The mixture was diluted with 1 vol of chloroform and 1 vol of water and centrifuged at 10,000 × g for 10 min. The chloroform phase was drawn from the bottom of the tube with a pipette, and traces of aqueous solvent were removed with a separatory funnel. The extract was dried in vacuo and

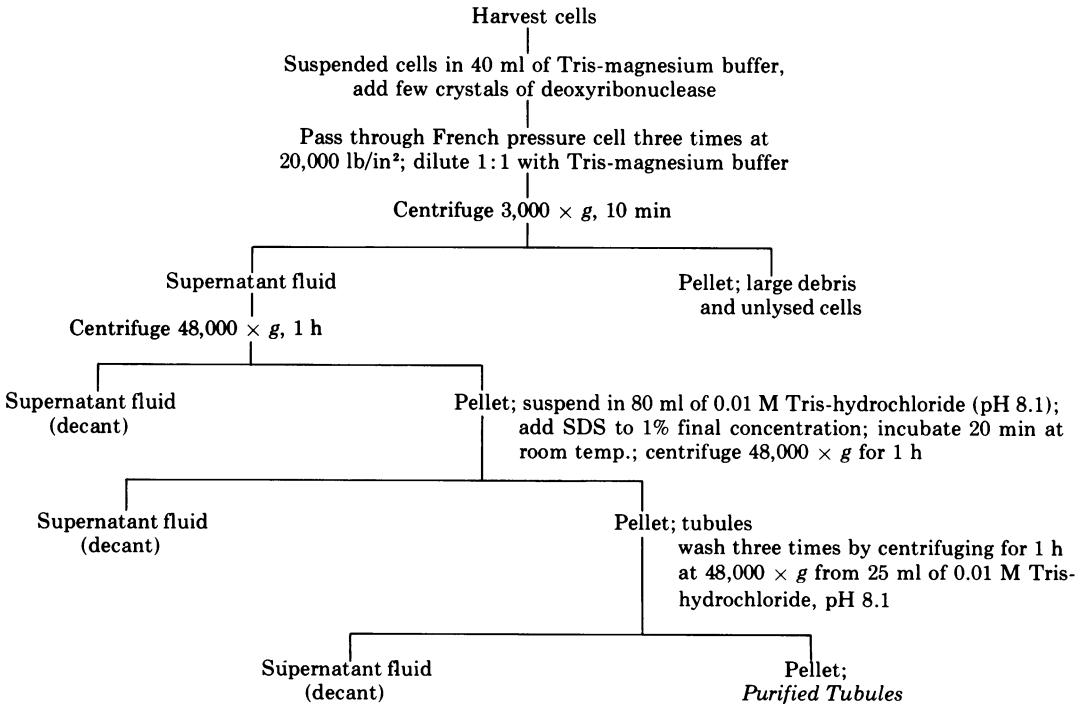

FIG. 1. *Flow diagram of the procedure for isolating tubules by treating crude lysates of C. columnaris with 1% SDS at pH 8.1. Tris-magnesium buffer: 0.05 M Tris-hydrochloride (pH 7.4) containing 0.02 M* $MgCl_2$.

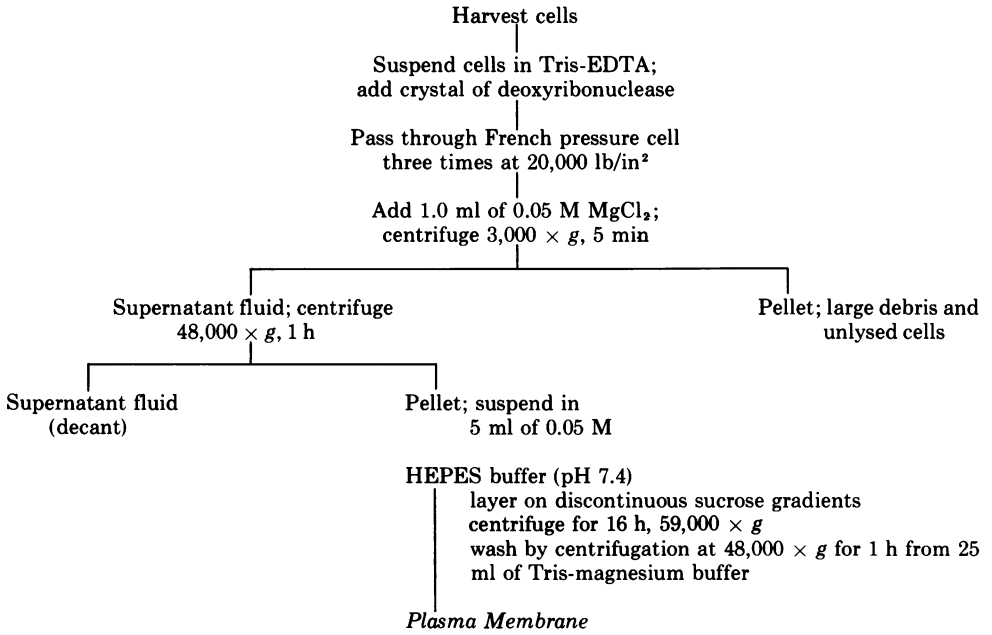

FIG. 2. *Flow diagram of the procedure for isolating plasma membranes from crude lysates of C. columnaris. Tris-EDTA: 0.05 M Tris-hydrochloride (pH 7.8) containing 1 mM EDTA. Tris-magnesium buffer: 0.05 M Tris-hydrochloride (pH 7.4) containing 0.02 M $MgCl_2$.*

dissolved in a minimal volume of chloroform. The phosphate content of the extract was estimated by the method of Chen et al. (6).

Samples were chromatographed on commercially prepared plates. Samples were run with chloroform-methanol-7 M ammonia (60:35:5, vol/vol). Lipid spots were detected by the methods of Ames (1). Iodine (1% in methanol), ninhydrin (0.2% in acetone), and Dittmer reagent (11), were sprayed on the plates sequentially to identify total lipid, amino lipids, and phospholipids.

Polyacrylamide disk-gel electrophoresis. Most samples were solubilized by the method of Schnaitman (unpublished data) devised for membranes not soluble by less rigorous methods such as treatment with 1% Triton X-100. Samples were suspended in distilled water (not more than 15 mg of protein per ml). One volume of the suspension was mixed with two volumes of a solution containing 3% SDS, 0.15% 2-mercaptoethanol, and 7.5 mM EDTA dissolved in 0.1 M sodium phosphate buffer (pH 7.2), sealed under nitrogen in a test tube, and incubated for 2 h at 37 C. The sample was then dialyzed overnight against 50 to 100 vol of a solution containing 480 g of urea, 0.19 g of sodium EDTA, 1 g of SDS, and 1 ml of 2-mercaptoethanol all dissolved in 1 liter of 0.1 M sodium phosphate buffer (pH 7.2). The dialysis was done in a nitrogen atmosphere. After dialysis, the sample was boiled for 3 min, concentrated by vacuum dialysis, and stored under nitrogen.

Proteins were separated on 7.5% polyacrylamide gels prepared by the method of Maizel (23). Sodium phosphate buffer (0.1 M, pH 7.2) was used with SDS added to a concentration of 1% in the upper chamber. Samples containing about 50 μg of protein in 50- to 100-μliter volumes were layered directly atop the gel. Migration was toward the anode. Electrophoresis was run for 5 h at 5 mA per gel. Gels were removed and stained with 0.25% Coomassie Brilliant Blue dissolved in 50% methanol containing 10% acetic acid. The gels were destained by soaking in a solution of 5% methanol containing 7% acetic acid.

Whole membrane preparations obtained by washing lysed spheroplasts produced by the method of Miura and Mizushima (25) were solubilized by treatment with Triton X-100. Membrane suspensions were adjusted to a protein concentration of about 2 mg/ml. Triton X-100 was added to a concentration of 1%, and the suspensions were incubated for 30 min at room temperature and clarified by centrifugation. About 150-μg amounts of protein from each sample were layered atop 7% polyacrylamide gels containing 1% Triton X-100 and run according to the method of Dulaney and Touster (13). Protein patterns were recorded by scanning photographic negatives of the gels with a Gilford model 2400 recording spectrophotometer equipped with a linear transport drive.

Electron microscopy. Copper grids (200 mesh) were coated with Parlodion. A thin layer of carbon was deposited on the Parlodion film by vacuum evaporation. Grids were cleaned before use by dipping them in chloroform.

A sample to be examined by the negative stain technique was diluted with buffer or distilled water until the liquid was faintly turbid. A drop of the dilution was placed on the grid, and the excess

moisture was removed with filter paper. A drop of phosphotungstic acid (PTA) adjusted to pH 6.8 with NaOH was added to the grid, and excess moisture was removed by blotting.

Material to be prepared by shadow casting was diluted and applied to grids in drops also. After excess moisture was blotted off, the grids were completely dried. Platinum wire was evaporated from a carbon-graphite electrode placed at a 15° angle to the surface of the grid.

Electron micrographs were made with a Zeiss EM-9A operated at 60 kV with a 50-μm objective aperature or a Hitachi HU-11E operated at 50 kV with a 30-μm objective aperature.

RESULTS

Envelopes from *C. columnaris* are dissolved or altered in appearance by various reagents at different concentrations and conditions; these conditions include treatment with 8 M urea (17), 67% dimethylsulfoxide (9), 1 M guanidine-hydrochloride, 0.03 M 2-mercaptoethanol in the presence of 0.3 M adenosine triphosphate (24), 1% Triton X-100 in the presence of 0.05 M EDTA (36), 1% deoxycholate (29), 1% SDS (37), 90% acidic dimethylformamide (34), and 1 N NaOH. Tubules can be dissolved by treatment with dimethyl formamide (34), boiling SDS (as described), or 1.0 M NaOH and are stable under all other conditions. Anionic detergents are the most effective for dissolving envelopes.

Treating lysates of *C. columnaris* with 1% SDS for 20 min at 25 C in the presence of 0.01 M Tris-hydrochloride (pH 7.3) dissolved nearly all structural material in the lysates except tubules. If the same treatment was carried out at pH 8.1, only tubules remained (Fig. 3). About 3 mg of purified tubules per liter of culture could be obtained after concentrating the tubules by centrifugation and removing SDS with thorough washing.

Two fractions of material were separated from a crude lysate of *C. columnaris* with sucrose gradient centrifugation. The top band was yellow, and its position corresponded to that of the band containing plasma membranes in an experiment with *E. coli* (35). The bottom band was gray and appeared about 1 cm below the top band. This position corresponds to the position of the band containing cell walls in the preparation from *E. coli*. The top band contained fragments of membranes varying in diameter from 80 to 650 nm (Fig. 4). The bottom band contained tubules and large fragments of material which appeared to be portions of the cell envelope (outer membrane and peptidogylcan). These fragments often were large enough to retain the shape of a portion of a cell (Fig. 5). Only the bottom band contained hexosamines, and for that reason the fragments were assumed to be derived from cell envelopes.

The results of quantitative analyses of plasma membranes and tubules from *C. columnaris* are listed in Table 1. Tubules contain protein and lipid in a ratio of approximately 1.3:1.0. Purified plasma membranes contain a small amount of carbohydrate and have a protein-to-lipid ratio of only 0.4. This value is unusually low for bacterial membranes. If this value is erroneous, it could be due to loss of protein from the plasma membrane during preparation. Alternatively, the value for total lipid in plasma membranes may be high because some protein could be removed with the procedures used to extrac' total lipid from the sample. No nucleic acids are present in purified preparations of plasma membranes or tubules.

Extracts were made from fractions enriched for plasma membranes or for tubules. A similar pattern of lipids was seen in each extract when examined with TLC (Fig. 6). The third and fourth spots were quite faint and often merged. Extracts from plasma membranes contained yellow pigments. The pigments traveled in the spot nearest the solvent front. The extract from tubules contained neutral lipids which traveled in a spot near the solvent front even though no pigments were present in this extract. This spot moved a bit faster than the fastest spot in the plasma membrane pattern.

In the course of the work, a mutant of *C. columnaris*, NT-1, arose spontaneously from the wild-type 1-R43. NT-1 does not produce tubules upon lysis. Proteins solubilized from membranes of this mutant were compared to those of the wild type which produces tubules upon lysis. A total membrane preparation from each strain was prepared by extensively washing lysed spheroplasts. Proteins were extracted by treatment with 1% Triton X-100. After electrophoresis, proteins from *C. columnaris* 1-R43 formed a pattern of 15 bands. The pattern of proteins from NT-1 cells was similar except that one band was missing (Fig. 7).

Proteins from tubules and fractions of purified plasma membranes isolated from 1-R43 and NT-1 were extracted with hot SDS and examined by disk-gel electrophoresis. Proteins from purified 1-R43 plasma membranes can be separated into 14 bands (Fig. 8). Proteins from tubules can be separated into five bands, three of which are common to bands in the preparation from 1-R43 plasma membranes (Fig. 8). The pattern of proteins from NT-1 cells was very similar to the pattern from 1-R43 except

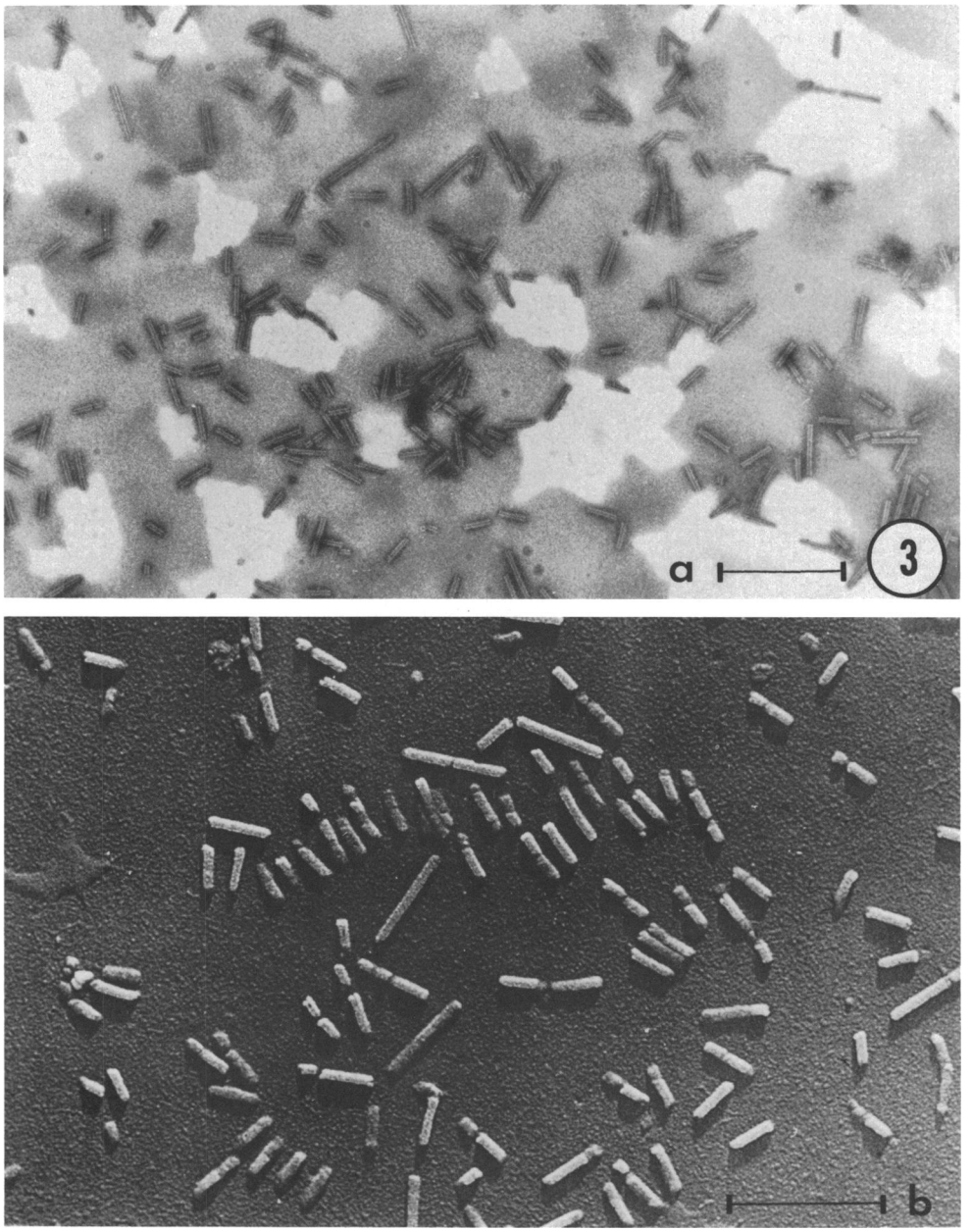

FIG. 3. *Tubules from lysates of C. columnaris purified by treatment with 1% SDS at pH 8.1, PTA negative stain (a), platinum-carbon shadowed (b). Markers in this and subsequent figures represent 0.5 μm.*

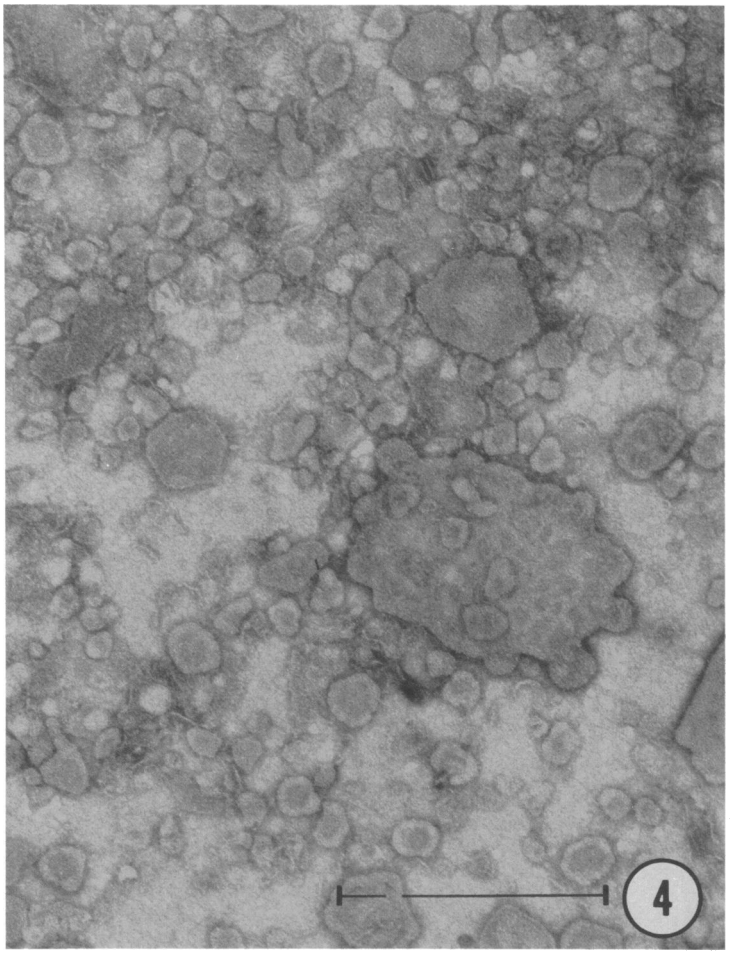

FIG. 4. *PTA preparation of C. columnaris membrane pieces from upper band of discontinuous sucrose density gradient.*

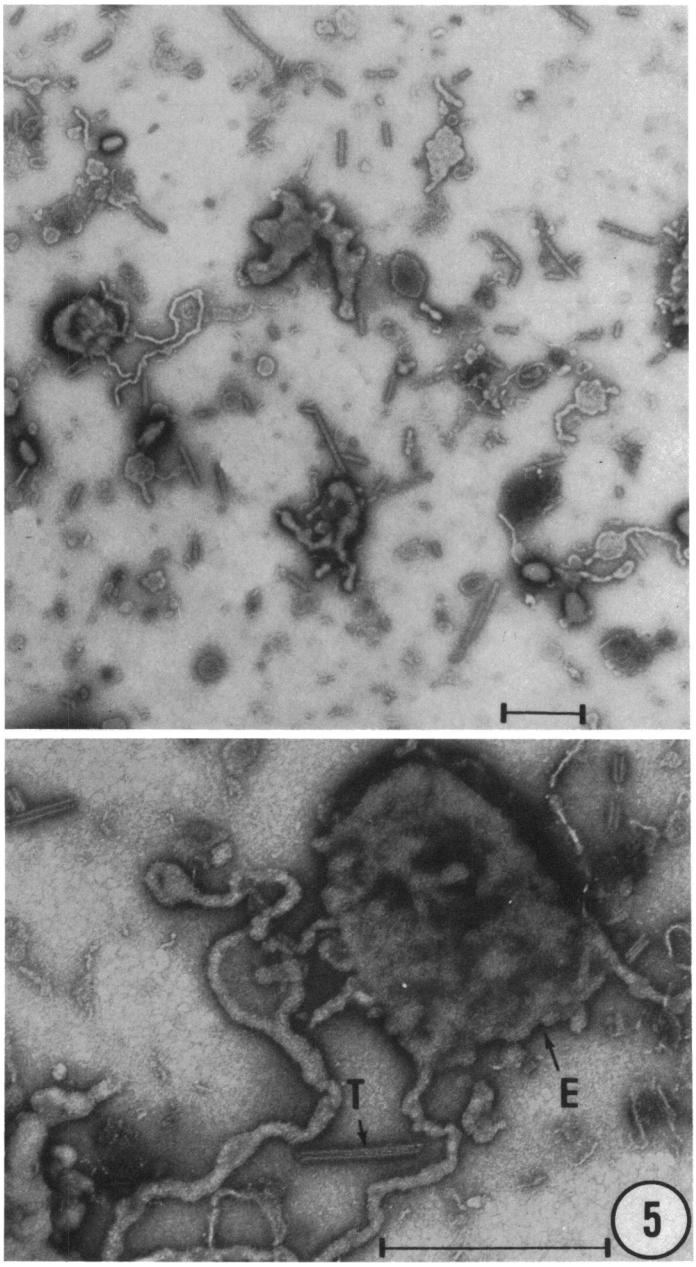

FIG. 5. *PTA preparation of tubules (T) and cell envelope (E) from lower band of discontinuous sucrose gradient centrifugation. Note large piece of envelope (E) that retains the outline of the cell.*

TABLE 1. *Chemical composition of tubules and plasma membranes*[a]

Fraction	Protein	Lipid			Carbohydrate	Phosphate	RNA	DNA[b]
		Tightly bound	Loosely bound	Total				
Plasma membrane	29.0	50.5[c]	24.0	74.5[d]	6.7	1.9	0.0	0.0
Tubules	58.2			41.8[e]	0.0	0.9	0.0	0.0

[a] Values expressed as percent total dry weight.
[b] Deoxyribonucleic acid.
[c] Value determined by subtracting loosely bound lipid from total lipid.
[d] Value determined by weight loss after refluxing tared sample.
[e] Value determined by subtracting protein from total dry weight.

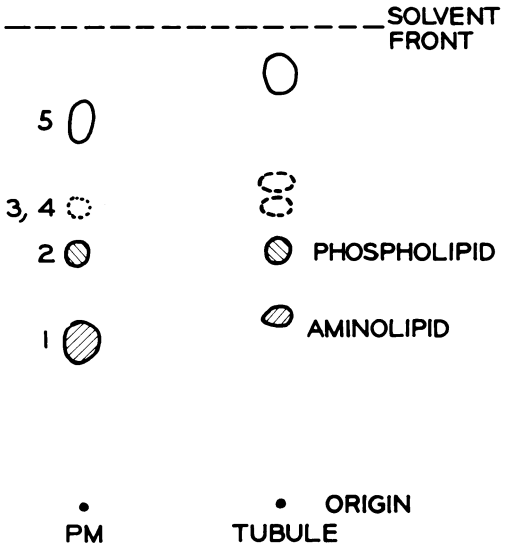

FIG. 6. *Thin layer chromatograph of lipids extracted from plasma membranes and tubules from C. columnaris. Chloroform-methanol-7 M ammonia (60: 35:5, vol/vol). Each sample contained 4.7 μg of lipid phosphate.*

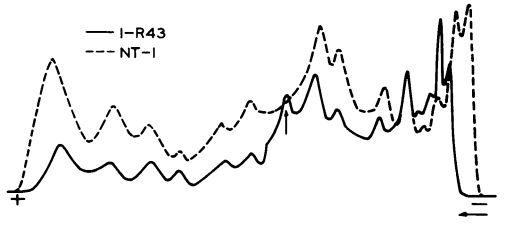

FIG. 7. *Comparison of electrophoretic patterns of proteins extracted from plasma membranes of C. columnaris 1-R43 (wild type) and NT-1 (does not produce tubules upon lysis). Proteins extracted with 1% Triton X-100. Note extra band in 1-R43 pattern (arrow).*

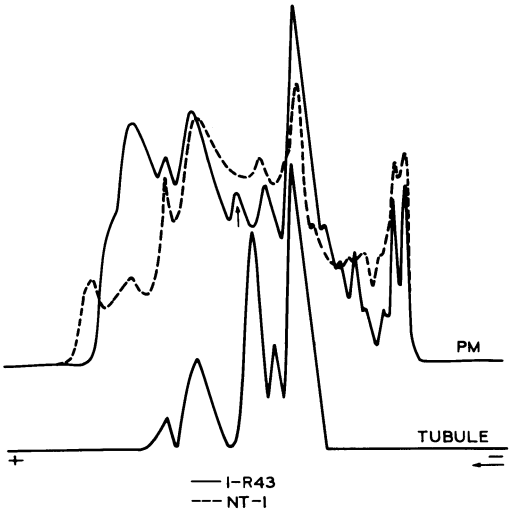

FIG. 8. *Comparison of electrophoretic patterns of proteins extracted from plasma membranes of C. columnaris 1-R43 (wild type) and NT-1 (does not produce tubules upon lysis). Protein pattern from tubules of 1-R43 included for comparison. Note that extra band (arrow) in 1-R43 plasma membrane pattern migrates to the same area as the major bands of the tubule pattern. Proteins extracted with hot SDS.*

that one protein was missing. This protein migrated to a position in the gel near the major bands of protein solubilized from tubules (Fig. 8).

DISCUSSION

Tubules from *C. columnaris* contain only protein and lipid. The composition of tubules from *C. columnaris* is different from that of rhapidosomes from *S. grandis*. Delk and Dekker have reported that no lipids or nucleic acids are present in rhapidosomes from *S. grandis* and that they consist entirely of proteins (10).

Mesosomes present in cells of *C. columnaris* may fragment to form tubules upon lysis of the

cell. Pate et al. (27) presented evidence that such a conversion could take place. (i) Tubules are not seen in intact, actively-growing cells that have been fixed, embedded, sectioned, and examined by electron microscopy. (ii) Tubules are always seen when cells have undergone lysis and no longer possess mesosomes of the type seen in growing cells. (iii) Sections of cells undergoing autolysis do not contain the type of mesosomes seen in actively growing cells. Those mesosomes present appear to be a transition form between the folded membrane and tubule.

A mesosome-to-tubule transition has been reported to occur in lysing cells of *C. botulinum* (38). Tubules from *C. botulinum* contain protein, lipid, and a trace of RNA (38).

The presence of lipids in tubules from *C. columnaris* suggests that the tubules are derived from membranes. Tubules do not contain pigments. Otherwise, only slight differences are noted in chromatographic patterns of polar lipids extracted from tubules and plasma membranes. The procedure for isolation of tubules involved treatment with SDS at 25 C and pH 8.1. These conditions could account for the slight differences in patterns.

Patterns of proteins solubilized from plasma membranes by hot SDS treatment contain 14 bands, whereas only 5 bands can be separated from protein preparations from tubules by disk-gel electrophoresis. The same proteins present in tubules may also be present in plasma membranes. Some of these might migrate to different positions in the gels due to the formation of different-sized aggregates during solubilization of the two fractions. On the other hand, it is possible that none of the proteins present in tubules is identical to proteins from plasma membranes. It is not possible to distinguish between these two alternatives at the present time.

One protein is missing from the membranes of a mutant strain (NT-1) of *C. columnaris*. This mutant strain does not produce tubules when lysed. It is possible that the absence of this protein alters the membranes in such a way that they do not reorganize to form tubules upon lysis of the cell.

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LITERATURE CITED

1. Ames, G. F. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli:* structure and metabolism. J. Bacteriol. **95**:833-843.
2. Baechler, C. A., A. R. Taylor, and R. S. Berk. 1972. Electron microscopic examination and quantitation of rhapidosomes, viruses and bacteria concentrated at an aqueous-air interface. Prep. Biochem. **2**:287-296.
3. Boas, N. F. 1953. Determination of glucosamine in hydrolyzates. J. Biol. Chem. **204**:553-563.
4. Bradley, D. E. 1965. The isolation and morphology of some new bacteriophages specific for *Bacillus* and *Acetobacter* species. J. Gen. Microbiol. **41**:233-241.
5. Burton, K. 1956. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315-323.
6. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756-1758.
7. Clark-Walker, G. D. 1969. Association of microcyst formation in *Spirillum itersonii* with the spontaneous induction of a defective bacteriophage. J. Bacteriol. **97**:885-892.
8. Correll, D. L., and R. A. Lewin. 1964. Rod-shaped ribonucleoprotein particles from *Saprospira*. Can. J. Microbiol. **10**:1-10.
9. Cummings, D. J., W. A. Chapman, and S. S. DeLong. 1968. Disruption of T-even bacteriophages by dimethyl sulfoxide. J. Virol. **2**:610-620.
10. Delk, A. S., and C. A. Dekker. 1972. Characterization of rhapidosomes of *Saprospira grandis*. J. Mol. Biol. **64**:287-298.
11. Dittmer, J. C., and R. L. Lester. 1964. A simple specific spray for the detection of phospholipids on thin layer chromatograms. J. Lipid Res. **5**:126-128.
12. Dubois, M., K. A. Gilles, J. K. Hamilton, R. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350-356.
13. Dulaney, J. T., and O. Touster. 1970. The solubilization and gel electrophoresis of membrane enzymes by use of detergents. Biochim. Biophys. Acta **196**:29-34.
14. Dworkin, M. 1966. Biology of the myxobacteria. Annu. Rev. Microbiol. **20**:75-106.
15. Garnjobst, L. 1944. *Cytophaga columnaris* (Davis) in pure culture: a myxobacterium pathogenic to fish. J. Bacteriol. **49**:113-128.
16. Gräf, W. 1965. Bewegungsorganellen bei Myxobacterien. Arch. Hyg. Bakteriol. **149**:518-526.
17. Horzinek, M., and M. Mussgay. 1971. Studies on the substructure of togaviruses. I. Effect of urea, deoxycolate, and Saponin on the sindbis virion. Arch. Gesamte Virusforsch. **33**:296-305.
18. Hougen, K. H., and A. Birch-Andersen. 1971. Electron microscopy of endoflagella and microtubules in *Treponema reiter*. Acta Pathol. Microbiol. Scand. **79**:37-50.
19. Kerr, S. E., and K. Seraidarium. 1945. The separation of purine nucleosides from free purines and the determination of the purines and ribose in these fractions. J. Biol. Chem. **159**:211-225.
20. Iterson, W. van, J. F. M. Hoeniger, and E. N. van Zanten. 1967. Microtubules in a bacterium. J. Cell Biol. **32**:1-10.
21. Lewin, R. A. 1963. Rod-shaped particles in *Saprospira*. Nature (London) **198**:103-104.
22. 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.
23. Maizel, J. V. 1966. Acrylamide-gel electrophoresis by mechanical fractionation: radioactive adenovirus proteins. Science **151**:988-990.
24. Marchesi, V. T., and E. Steers Jr. 1968. Selective solubilization of a protein component of the red cell

membrane. Science **159**:203-204.
25. Miura, T., and S. Mizushima. 1968. Separation by density gradient centrifugation of two types of membranes from spheroplast membranes of *Escherichia coli* K12. Biochim. Biophys. Acta **150**:159-161.
26. Ordal, E. J., and R. R. Rucker. 1944. Pathogenic myxobacteria. Proc. Soc. Exp. Biol. Med. **56**:15-18.
27. Pate, J. L., J. L. Johnson, and E. J. Ordal. 1967. The fine structure of *Chondrococcus columnaris*. II. Structure and formation of rhapidosomes. J. Cell Biol. **35**:15-35.
28. Pate, J. L., and E. J. Ordal. 1967. The fine structure of *Chondrococcus columnaris*. I. Structure and formation of mesosomes. J. Cell Biol. **35**:1-14.
29. Philipport, J. 1971. Studies of human red blood cell membrane using sodium deoxycholate. I. Mechanism of the solubilization. Biochim. Biophys. Acta **225**:201-213.
30. Pope, L., and P. Jurtshuk. 1967. Microtubules in *Azobacter vinelandii* strain O. J. Bacteriol. **94**:2062-2064.
31. Reichenbach, H. 1965. Rhapidosomen bei Myxobakterien. Arch. Mikrobiol. **50**:246-255.
32. Reichenbach, H. 1967. Die wahre Natur der Myxobakterien Rhapidosomen. Arch. Mikrobiol. **56**:371-383.
33. Salton, M. R. J., and J. H. Freer. 1965. Composition of the membrane isolated from several gram-positive bacteria. Biochim. Biophys. Acta **107**:531-538.
34. Schnaitman, C. A. 1970. Examination of the protein composition of the cell envelope of *Escherichia coli* by polyacrylamide gel electrophoresis. J. Bacteriol. **104**: 882-889.
35. Schnaitman, C. A. 1970. Protein compositions of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890-901.
36. Schnaitman, C. A. 1971. Solubilization of cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. **108**:545-552.
37. Shafa, F., and M. R. J. Salton. 1960. Disaggregation of bacterial cell walls by anionic detergents. J. Gen. Microbiol. **22**:137-141.
38. Ueda, M., and A. Takagi. 1972. Rhapidosomes in *Clostridium botulinum*. J. Gen. Appl. Microbiol. **18**:81-98.
39. Yamamoto, T. 1967. Presence of rhapidosomes in various species of bacteria and their morphological characteristics. J. Bacteriol. **94**:1746-1756.