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Purification and Characterization of Axial Filaments from *Treponema phagedenis* Biotype *reiterii* (the Reiter Treponeme)

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Axial filaments have been purified from *Treponema phagedenis* biotype *reiterii* (the Reiter treponeme) and partially characterized chemically. The preparations consist largely of protein but also contain small amounts of hexose (3%). Filaments dissociate to subunits in acid, alkali, urea, guanidine, and various detergents. Amino acid analyses show an overall resemblance to other spirochetal axial filaments and to bacterial flagella. Dissociated filaments migrate as a single band upon acrylamide gel electrophoresis at pH 4.3 (in 4 M urea and 10^{-3} M ethylenediaminetetraacetate) and at pH 12, but in sodium dodecyl sulfate gels, three bands are obtained under a wide variety of conditions. Two of these bands migrate very close together, with molecular weights of 33,000 ± 500. The other band has a molecular weight of 36,500 ± 500. Analysis of axial filaments by the dansyl chloride method yields both methionine and glutamic acid as amino terminal end groups. Sedimentation equilibrium measurements on dissociated axial filaments in 7 M guanidine hydrochloride yield plots of log C against χ^2 which vary with the speed and initial protein concentration used. Molecular weight values calculated from these plots are consistent with a model in which axial filament subunits are heterogeneous with respect to molecular weight in the approximate range of 32,000 to 36,000.

Axial filaments in spirochetes are commonly believed to be organelles of locomotion. The basis for this belief is largely their morphological resemblance to bacterial flagella, both throughout the filament portion and in the basal structures (4, 16, 22). (In this paper, as in a previous publication [5], we will follow common practice in using the term "axial filament" to refer to the filament portion of the total organelle, unless otherwise specified.) There is at present no direct evidence that axial filaments are involved in motility, although an attempt to obtain such evidence by immunological techniques has been reported (6). We have purified axial filaments from *Spirochaeta* (*Treponema*) *zuelzerae*, a free-living organism (10, 37), and demonstrated that, like flagellar filaments from many eubacteria, they are composed of identical monomer protein subunits with a molecular weight of approximately 37,000, held together by noncovalent bonds (5). Axial filaments from a related free-living spirochete, *Spirochaeta stenostrepta*, have been purified by Joseph and Canale-Parola (21) and also show a general chemical similarity to bacterial flagella. In contrast to this, there is evidence that axial filaments in *Leptospira* may be more complex structures, both morphologically and chemically (30, 32), although recent studies by Birch-Andersen et al. (7) suggest that leptospiral axial filaments are more similar morphologically to those of *Spirochaeta* and *Treponema* than was previously thought. In host-associated treponemes, both pathogenic and nonpathogenic, axial filaments resemble those of free living *Spirochaeta* species morphologically (16, 17, 19, 20, 34), but have not previously been characterized chemically. Cell structure in general is ill-defined chemically in this group of spirochetes, in spite of the medical importance of some members of the group. Perhaps the principal reason for this is the difficulty and expense of growing cells with yields sufficient for chemical analysis. It has not yet been possible to cultivate the pathogenic treponemes *Treponema pallidum*, *Treponema pertenue*, and *Treponema carateum* outside of an animal host.

One nonpathogenic host-associated treponeme, *Treponema phagedenis* biotype *reiterii* (the Reiter treponeme; also known as the Reiter strain of *Treponema pallidum* and *Treponema reiteri*) (9), was originally isolated from a human syphilitic lesion and thought to be a nonpatho-

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genic variant of *Treponema pallidum* (33). Later work revealed that it is antigenically and morphologically distinct, but that, like other similar host-associated nonpathogenic treponemes, it does display considerable immunological cross-reactivity with virulent *Treponema pallidum* (16, 20, 38). This property of cross-reactivity has several clinical applications, including the use of a protein extract from the Reiter treponeme in a standard diagnostic test for syphilis, the Reiter Protein Complement Fixation Test (11). There is evidence from immunofluorescence studies that axial filaments constitute a major component of this protein extract (8). Since this organism may be grown in complex laboratory medium with cell yields adequate for chemical analysis, we set out to isolate and characterize its axial filaments. The results of this study are reported here.

MATERIALS AND METHODS

Organism. The culture of *Treponema phagedenis* biotype *reiterii* used in these studies was obtained from E. G. Hampp, National Institute of Dental Research, Bethesda, Md. Cultures were grown at 32 C in spirolate broth (BBL) containing 10% (vol/vol) sterile bovine serum (North American Biologicals). An inoculum of 10% was required to get maximal cell yields, which were approximately 2 to 4 g (wet weight)/liter. For large quantities of cells, cultures were grown in 2-liter, round-bottomed, long-necked Pyrex flasks for 4 to 5 days. After 3 to 4 days incubation, these cultures were agitated with a magnetic stirring bar to break up clumps of cells settling at the bottom of the flask, which increased cell yields. Cells harvested for preparative purposes were washed twice with distilled water and stored frozen at −20 C. Electron microscope observations showed that this procedure disrupted the outer envelope but did not affect axial filament morphology.

Purification of axial filaments. Methods previously evolved to purify axial filaments from *Spirochaeta zuelzerae* (5) were not satisfactory for the Reiter treponeme. Various alternative methods were tried and the method of choice, described here, combines our previous methods with procedures previously used for purifying bacterial flagella (12, 14). This procedure was monitored by electron microscopy.

Approximately 10 to 15 g (wet weight) of cells was stirred for 1 h in 150 ml of distilled water saturated with 1-butanol to dissolve and disrupt components of the outer envelope. Cells were sedimented by centrifuging at 10,000 × *g* for 20 min, and the supernatant fluid (in which filaments were not found) was discarded. Cells were then frozen in acetone-dry ice, thawed, and suspended in 100 ml of distilled water. From this stage, preparations were kept at 4 C or below. The cell suspension was blended at maximal speed in a Sorvall Omnimixer for 1 min. Triton X-100 was added to the suspension to 0.5% (vol/vol) final concentration and it was allowed to stand overnight. Cell debris was removed by centrifuging at 6,000 × *g* for 10 min, and the crude axial filament preparation was sedimented in the ultracentrifuge at 100,000 × *g* for 2 h. The sediments obtained were resuspended in 10 ml of water and most of the remaining Triton X-100 was removed by overnight dialysis against water. Sodium chloride was added to the suspension to 2 M and it was stirred for 2 h. The filaments were sedimented by centrifuging at 100,000 × *g* for 2 h and resuspended in 10 ml of 0.01 M sodium phosphate buffer, pH 8.0, containing 0.01 M ethylenediaminetetraacetate (EDTA). Any remaining sodium chloride was removed by dialysis overnight against the same phosphate-EDTA buffer. These treatments with detergent and salt removed substantial amounts of contaminating material of membranous appearance from our crude filament preparations. The preparation was frozen in acetone-dry ice and thawed two times, then blended at maximal speed in a Sorvall Omnimixer for 1 min. Freezing and thawing had the effect of breaking up the filaments to fragments which were more readily separable from membranous-appearing material in the crude preparations. More cell debris was removed by centrifuging at 5,000 × *g* for 10 min.

The crude filament preparation was then placed on a column of diethylaminoethyl-cellulose (DE 52, Whatman; equilibrated with buffer, bed volume 50 ml), washed with 1 liter of buffer, and eluted by passing a linear gradient of 0 to 2 M NaCl in 300 ml of buffer. As with other axial filaments and flagella (5, 26), these filaments eluted at approximately 0.15 M NaCl, separating them from much membranous-appearing and ribose-rich material. Filament fractions were pooled and treated for 24 h with more Triton X-100 (0.2% vol/vol). Filaments were sedimented at 100,000 × *g* for 2 h, suspended in 2 ml of buffer, and dialyzed overnight against buffer to remove remaining detergent.

Filaments in phosphate-EDTA buffer were then banded by density gradient centrifugation in cesium chloride (optical grade) as previously described (5). As with filaments from *S. zuelzerae*, these filaments banded as a gelatinous layer at a density of approximately 1.29 g/cm^3, a value characteristic of proteins. Equilibrium was not reached at 3 h, as appeared to be the case with filaments from *S. zuelzerae*, but, in this case, bands increased in sharpness and separation up to 12 h, after which they remained constant. Filaments were removed with a Pasteur pipette, usually at 18 to 24 h, dialyzed exhaustively against distilled water for 24 h, lyophilized, and stored at −20 C.

Yields of axial filaments ranged from 0.5 to 1 mg of filaments (dry weight) per 10 to 15 g (wet weight) of cells.

All experiments to be described were performed with the purified axial filaments thus obtained.

Electron microscopy. Axial filaments were examined by negative contrast, using glutaraldehyde as fixative, uranyl acetate as stain, and copper specimen-support grids coated with Parlodion and carbon, all as previously described (5). Specimens were examined in a Hitachi HU 11E electron microscope (50 kV, 30-μm objective aperture).

Protein and carbohydrate estimations. Protein was estimated by the method of Lowry et al. (23). Hexose and pentose were estimated by the anthrone (35) and orcinol (28) methods, respectively. Bovine serum albumin, glucose, and ribose, respectively, were used as standards.

Dissociation of axial filaments. Dissociation of filaments to subunits by various treatments was monitored by electron microscopy and by visual observation of loss of opalescence.

Amino acid analysis. Amino acid analyses were done in a Beckman 120B amino acid analyzer by the methods of Spackman et al. (36). Hydrolysis was carried out at 110 C in 6 N HCl in sealed evacuated tubes for 24 h. Cysteine was determined as cysteic acid following performic acid oxidation.

Acrylamide gel electrophoresis. Axial filaments were dissociated and run on acrylamide gels at pH 4.3 and pH 12 as described for flagella by Martinez et al. (27). At pH 4.3, EDTA (disodium salt) was additionally incorporated into the buffer and dissociated sample at 10^{-3} M. Runs in sodium dodecyl sulfate (SDS) were done in 10% gels according to the methods of Weber and Osborn (39), except as noted in the text. Bovine albumin (68,000 daltons), immunoglobulin G heavy chain (50,000 daltons), immunoglobulin G light chain (23,500 daltons), and egg white lysozyme (14,300 daltons) were used as standards in estimating molecular weight. Protein loads were in the range 10 to 30 μg per gel. In all runs, blank controls were included in which dissociating buffer alone, without sample, was loaded onto a gel. This served to control for contamination of gels with material other than that in the sample, and for cross-contamination via the upper buffer chamber.

Amino terminal end group analysis. Amino terminal end group analysis was done by the dansyl chloride method (15). Filaments (0.5 mg of protein) were suspended in 0.5 M $NaHCO_3$, pH 8.6, and 8 M urea (1 ml). To this was added 0.5 ml of dansyl chloride in acetone (1 mg/ml). The mixture was stirred overnight at room temperature wherein the dansylated axial filament protein precipitated. The precipitate was washed four to five times with 10% formic acid (wt/vol), lyophilized to dryness, and hydrolyzed at 110 C for 12 h in 6 N HCl, and the HCl was removed by placing in a vacuum desiccator for 16 h over pellets of NaOH. Dansyl derivatives were separated and identified by thin-layer chromatography on silica gel plates (Eastman 6061, 20 by 20 cm) according to the methods of Morse and Horecker (29).

Sedimentation equilibrium. Sedimentation equilibrium studies were performed in 7 M guanidine hydrochloride plus 0.5% β-mercaptoethanol (vol/vol) on dissociated axial filaments, by the method of Yphantis (40).

RESULTS

Electron microscopy. Axial filaments purified from *Treponema phagedenis* biotype *reiterii* resemble those observed on the whole cell (16) in diameter (20 nm), fine structure, and the occasional presence of a hook (Fig. 1). In this respect, the filaments also resemble bacterial flagella of the "beaded" type (24) and axial filaments from *S. zuelzerae* (5). The filaments showed considerable fragmentation to pieces of shorter length caused by blending and repeated freezing and thawing in the purification procedure. There were occasional thin filaments in these preparations, similar to those observed in preparations of axial filaments from *S. zuelzerae* (5).

Gross analysis. Gross analyses of axial filaments from the Reiter treponeme are shown in Table 1. Like other axial filaments and bacterial flagella (1, 5), these filaments consisted largely of protein, with small amounts of anthrone-positive material present. Although pentose was detected in cruder preparations at various stages in the preparative procedure (possibly reflecting the presence of ribosomes), no detectable pentose was present in purified filament preparations.

Dissociation. Axial filaments dissociated to subunits in acid (pH 2), alkali (pH 12), urea (4 M and above), guanidine (6 M), and some cationic and anionic detergents, such as SDS (above 0.5% wt/vol) and dodecyl trimethyl ammonium bromide (0.2% wt/vol), but not in nonionic detergents such as Nonidet P-40 and Triton X-100. This indicates that, like flagella and axial filaments from other species (5, 27), subunits in these filaments are not held together by covalent bonds. The hook portion of the total axial filament organelle was more resistant to dissociation than the filament portion. For example, filaments dissociated in concentrations of SDS above 0.5%, whereas hooks remained intact at concentrations as high as 2.0%.

We were not able to produce reaggregated filaments from dissociated subunits by methods previously used for reaggregating the subunits of bacterial flagella (1, 3).

Amino acid analysis. The amino acid composition of axial filaments from the Reiter treponeme showed an overall resemblance to that of bacterial flagella and, especially, to that of axial filaments of *S. zuelzerae* (5; Table 2). Small amounts of half-cystine were present, of the order of 0.1 mol% or 1 mol per 3 mol of protein of molecular weight 35,000. Direct analysis for tryptophan was not done, but spectrophotometric examination of axial filaments suggested that little, if any was present.

Acrylamide gel electrophoresis. Dissociated axial filaments, run either in 0.037 M glycine-acetate buffer at pH 4.3 (in the presence of 4 M urea and 10^{-3} M EDTA) or at pH 12, migrated as one major band, with small amounts (judged

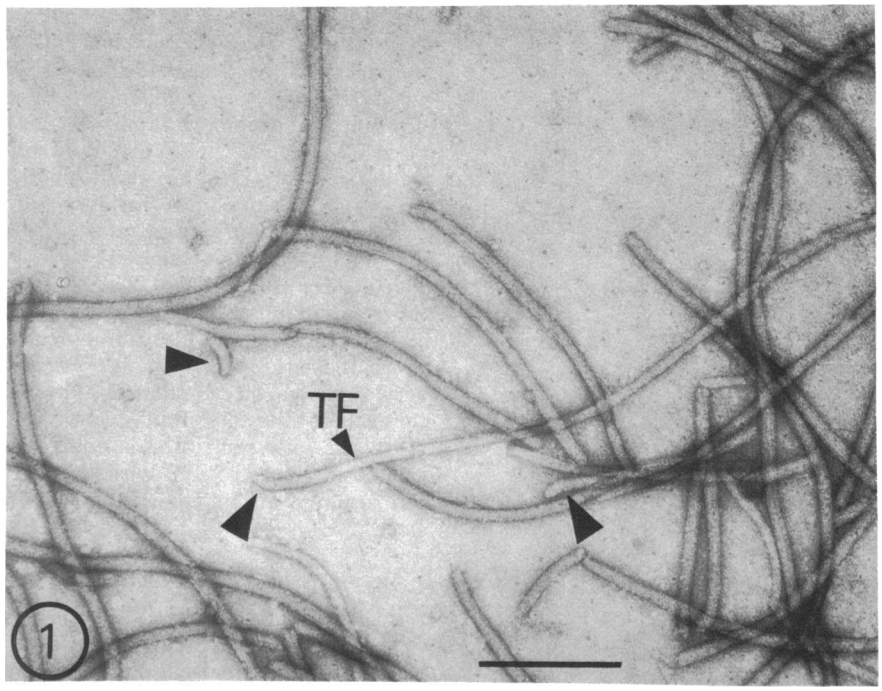

FIG. 1. *Electron micrograph of purified axial filaments from the Reiter treponeme. Fixed with glutaraldehyde and stained with uranyl acetate. Several hooks (arrows) are visible in this field as well as one of the thin filaments (TF) which are occasionally observed. Bar equals 0.2 μm.*

TABLE 1. *Gross analysis of axial filaments of the Reiter treponeme*

Determinants	Concentration in axial filament suspension (mg/ml)
Protein[a]	0.310
Hexose[b]	0.008
Pentose[c]	<0.005

[a] Method of Lowry et al. (23).
[b] Anthrone method (35).
[c] Orcinol method (28).

TABLE 2. *Amino acid analysis of axial filaments from the Reiter treponeme*

Determinants	Molar ratios	Calculated average no. of residues for molecular weight of 35,000
Lysine	3.52	12.6
Histidine	1.30	4.6
Arginine	4.41	15.7
Aspartic acid	10.88	38.8
Threonine	4.80	17.1
Serine	8.30	29.6
Glutamic acid	13.87	49.5
Proline	2.62	9.4
Glycine	9.82	35.1
Alanine	12.80	45.7
Half-cystine	0.10	0.35
Valine	4.47	16.0
Methionine	2.42	8.6
Isoleucine	3.37	12.0
Leucine	5.74	20.5
Tyrosine	2.40	8.6
Phenylalanine	2.22	7.9
Tryptophan	Not done	

as less than 5%) of contaminating material (Fig. 2).

In SDS gels, results were somewhat different. The axial filament subunits migrated as three major bands of approximately equal intensity (Fig. 3A). The two faster migrating bands were very close together and, in many gels, particularly if overloaded, these appeared as a single band (Fig. 3B). Fainter staining bands were also present in these gels. The two faster migrating major bands had molecular weights of 33,000 ± 500 (Fig. 4). The other band had a molecular weight of 36,500 ± 500. These results were constant from batch to batch of filaments. They were also constant whether the concentration of SDS used in pretreatment of filaments was 1 or 2%, whether pretreatment was at 37 C (2 h) or in a boiling-water bath (15 or 30 min), and whether or not β-mercaptoethanol was included in the mixture used for pretreatment. The relative intensities of the three bands did not vary from run to run.

Amino terminal end group analysis. Hy-

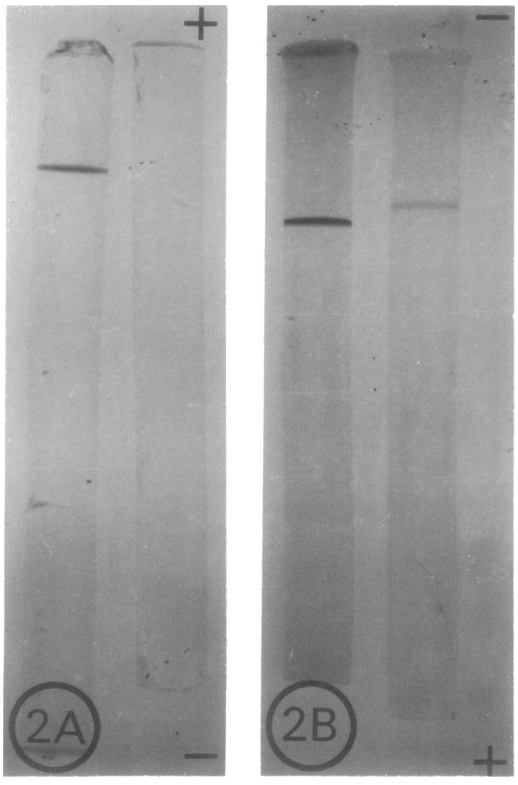

FIG. 2. *Acrylamide gel electrophoresis of dissociated Reiter treponeme axial filaments (ca. 15 μg of protein per gel). In each picture, blank control is shown on right. (A) In 4 M urea and 10^{-3} M EDTA at pH 4.3. (B) In 0.01 N NaOH and 0.01 N KCl at pH 12. Electrophoresis was carried out for 2 h at pH 4.3 and 1 h at pH 12, both at 6 mA per gel.*

drolysates of dansylated preparations of dissociated axial filaments from the Reiter treponeme contained two dansyl-amino acyl derivatives in addition to *O*-dansyl-tyrosine and monosubstituted epsilon-*N*-dansyl-lysine. In thin-layer chromatography on silica gel, using solvent systems B, C, and D of Morse and Horecker (29), these dansyl derivatives migrated with the dansyl derivatives of methionine and glutamic acid.

Sedimentation equilibrium. Sedimentation equilibrium studies on dissociated axial filaments in 7 M guanidine hydrochloride and 0.5% β-mercaptoethanol yielded plots of log C against χ^2 which varied according to initial protein concentration and speed. At the highest protein concentrations used (4 mg/ml), plots showed a gradient which increased slightly with increasing values of χ^2. At protein concentrations in the range of 0.5 to 2.0 mg/ml, plots of log C against χ^2 were almost linear. For each initial protein concentration and speed, the slope was measured at an arbitrarily chosen value of log C, such that all slope measurements were made at the same value of log C. Molecular weights (Table 3) were calculated for each slope using partial specific volume, $\bar{v} = 0.732$, calculated from amino acid analysis. At each speed, the estimated molecular weight decreased with increasing initial concentration of protein. For each initial protein concentration, the estimated molecular weight decreased with increasing speed. Molecular weight values obtained by this method fell within an approximate range of 32,000 to 36,000, close to the molecular weight range of the three major bands obtained in SDS acrylamide gel electrophoresis. These data tend to exclude the possibility that the subunits associate with each other under these conditions, but are consistent with a model in which the subunits are heterogeneous with respect to size.

DISCUSSION

In general, the filament portion of the total eubacterial flagellar organelle has been found to be composed of identical monomer protein subunits with molecular weights in the range 30,000 to 40,000 (flagellin molecules), characterized by the absence of cysteine (27). One exception to this generality is observed in the flagella of *Bacillus pumilus*, in which two closely related flagellins are copolymerized in a ratio of 7:3 (G. Somkuti, J. C. Bui, R. W. Smith, and H. Koffler, Abstr. Annu. Meet. Amer. Soc. Microbiol. G233, p. 69, 1972). Several bacterial species have sheathed flagella (25), but there are at present no published reports on the chemical composition of these flagella.

In all genera and species of spirochetes examined, the axial filaments show an overall morphological resemblance to bacterial flagella (4, 7, 22, 34). In spite of this general similarity, there are some differences from flagella reported in the literature, the most obvious being the distinctive location of axial filaments between two cell envelopes approximately along the axis of the cell (4, 22). Previous chemical analysis of axial filaments from *S. zuelzerae* by a variety of methods provides strong evidence that these filaments, like those of most eubacterial flagella, contain only a single species of protein subunit (5). In contrast to this, there are other reports that acrylamide gel electrophoresis of dissociated axial filaments from *Spirochaeta* and *Leptospira* species yields multiple protein bands, six in the case of *Leptospira* (21, 30). It was therefore of interest to find out whether axial filaments from other species of spirochetes resembled those of *S. zuelzerae* chemically, or

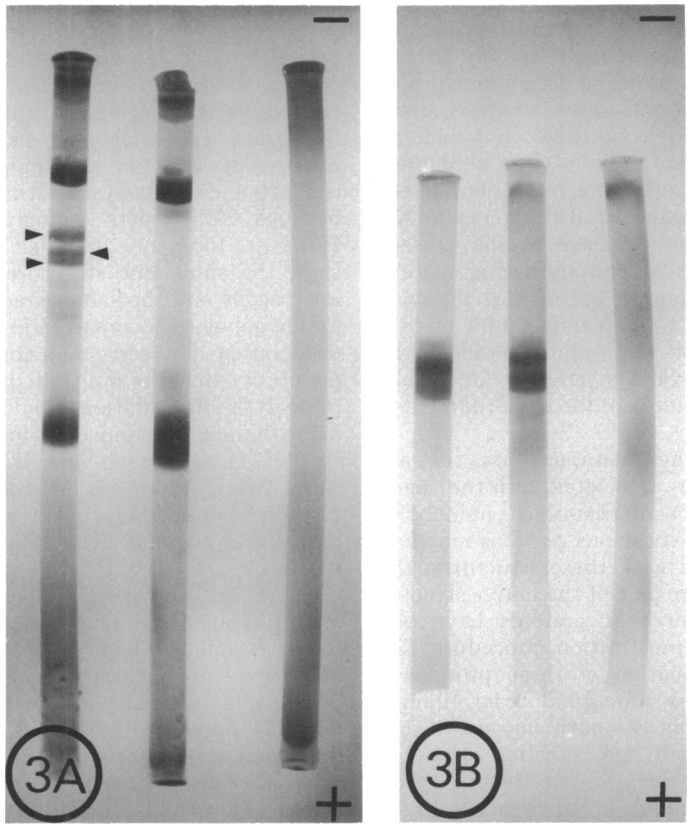

FIG. 3. *Acrylamide gel electrophoresis of dissociated Reiter treponeme axial filaments in SDS, according to method of Weber and Osborn (39). In each picture, blank control is shown on right. (A) Gel on left shows filaments (arrows) plus bovine albumin (above) and egg white lysozyme (below). Middle gel shows same without filaments. (B) Two different batches of filaments. Protein load is 15 to 30 μg per gel.*

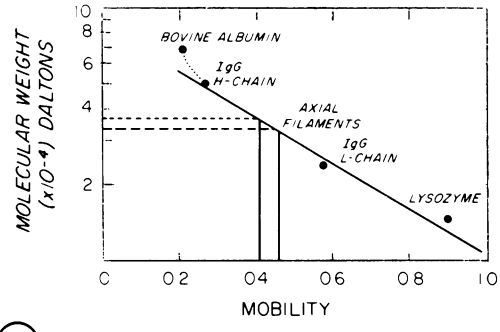

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FIG. 4. *Graph showing calibration of Reiter treponeme axial filament subunit proteins on SDS acrylamide gels for molecular weight. Mobilities were calculated according to the method of Weber and Osborn (39). Deviation from linearity in molecular weight range of bovine albumin is consistent with previous findings of those authors.*

whether they contained more than one protein.

In the case of the Reiter treponeme, purified

TABLE 3. *Sedimentation equilibrium of Reiter treponeme axial filament subunits in 7 M guanidine hydrochloride at pH 8.3*[a]

Speed (rpm)	Initial protein concentration (mg/ml)	Estimated molecular weight
37,500	0.8	35,700
	1.4	33,900
	1.7	33,500
42,040	0.8	32,800
	1.4	32,300

[a] Method of Yphantis (40). Molecular weights were estimated from plots of log C against χ^2, measuring slopes for all plots at the same protein concentration within the cell.

axial filament preparations resemble those obtained from *S. zuelzerae* in the electron microscope. However, chemical analyses by three independent methods (SDS acrylamide gel electrophoresis, amino terminal end group analysis, and sedimentation equilibrium in 7 M

guanidine hydrochloride) indicate that our preparations contain more than one protein, probably three, in major and constant proportions. In other respects (gross analysis, amino acid analysis, and conditions in which the filaments dissociate to subunits), axial filaments from the Reiter treponeme resemble bacterial flagellar filaments and axial filaments from *S. zuelzerae* (5), with evidence of the presence of minor amounts of contaminating material (judged from acrylamide gels as less than 5%). Furthermore, the three bands found on SDS acrylamide gels all fall approximately within the size range previously reported for flagellin molecules and subunits of the axial filaments of *S. zuelzerae* (5, 27).

In interpreting these data, one faces the same kinds of difficulties associated with the chemical characterization of ribosomes, namely the possibilities that extraneous proteins may have become associated with these structures, that proteins which were part of the native structure have been lost, or that proteins have been modified in the purification procedure (31). Definitive identification of these proteins as components of the functional axial filament organelle must await the purification and characterization of each one, labeling studies to demonstrate their location in the intact cell and, possibly, studies on their roles in the function of the organelle. Nevertheless, there are several considerations which indicate that these three proteins are indeed separate components of the axial filaments of the Reiter treponeme. One is that the filament preparations appeared free of obvious contamination in the electron microscope. It is unlikely that a soluble protein contaminant would be present because there are several steps in the purification procedure in which filaments are sedimented from suspension. A second consideration is that the filament subunits migrated as a single band on acrylamide gel at pH 4.3 and pH 12. This implies a close association of the subunits in vivo. A precedent for this is the observation that peptide fragments obtained by cyanogen bromide digestion of flagellin from *Bacillus subtilis* 168 flagella migrate as a single band under similar conditions (J. Y. Chang, personal communication). A third consideration is that we have been able to isolate axial filaments from the Reiter treponeme by an alternate method and obtain a similar banding pattern in SDS acrylamide gel electrophoresis to that reported here. In this method, cells were lysed by suspending in 0.2% SDS in buffer, liberating the axial filaments, which were then purified by washing and differential centrifugation, followed by density gradient centrifugation in cesium chloride. This method was not considered suitable for preparing purified filaments for extensive chemical analysis because electron microscopy and gel electrophoresis indicated the presence of contaminating material, and because SDS reacts strongly with proteins. However, filaments prepared by this method show the same fine structure in the electron microscope and the same three bands on acrylamide gel as those reported in this paper. This observation may serve as a control against the possibility that the multiple proteins represent proteolytic degradation products of a single axial filament protein (see, for example, J. C. Bui, H.-D. Tauschel, and H. Koffler, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1974, G9, p. 21), since, in our judgement, it is unlikely that proteolytic enzymes would be active in 0.2% SDS.

There are two further possibilities which must be considered. One is that one of the three bands is axial filament hook protein. There is adequate evidence that flagellar hooks are composed of a different protein from eubacterial flagellar filaments (2, 13). The presence of hook protein in major proportions is unlikely because relatively few hooks were visible in our preparations. The second possibility is that there are several different species of axial filaments present in these preparations, each with a distinct subunit protein. This possibility must be considered because (i) these filaments resemble bacterial flagella morphologically, (ii) the overall composition of these filaments resembles that of flagellin, (iii) the subunit molecular weights all fall in the range reported for different flagellin molecules, and (iv) because, in general, any particular kind of bacterial flagellum contains only a single species of protein subunit. These subunits differ more in size than do the two flagellins of *Bacillus pumilus* and there is no precedent among bacterial flagellar filaments for the presence of subunits which differ by 3,000 in molecular weight copolymerized in the same flagellum. If there are, in fact, different kinds of axial filaments present in our preparations, it would be of interest to know whether these are present in the same cell or whether a heterogeneity of cell type exists within our population of Reiter treponemes. This organism cannot be cloned because it requires large inocula to grow at all. Thus, the possibility of it being a mixed culture cannot, at this stage, be rigorously excluded. There is, however, no morphological evidence of hetero-

geneity and the constancy of our results from batch to batch would also tend to argue against this. There are precedents for the concept of a single organism possessing more than one kind of flagellum, notably phase variation in *Salmonella* (18). Further clarification of these points must await the availability of greater yields of these axial filaments and the isolation and characterization of each protein component, employing techniques such as chromatography and peptide fractionation (5). Meanwhile, our studies suggest that the chemical nature of treponemal axial filaments, at least in the Reiter treponeme, is somewhat more complex than our earlier studies on axial filaments from *S. zuelzerae* had led us to anticipate.

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