EFFECTS OF TRYPSIN DIGESTION

ON FLAGELLAR STRUCTURES AND

THEIR RELATIONSHIP TO MOTILITY

KEITH E. SUMMERS and I. R. GIBBONS

From the Department of Biochemistry and Biophysics and the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822. Dr. Summers' present address is Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.

ABSTRACT

Flagellar axonemes isolated from sea urchin sperm were digested with trypsin for variou^{s} time periods. The course of digestion was monitored turbidimetrically and was found to take two different courses depending on the presence or absence of ATP in the digestion mixture. It was found that ATP induced active disintegration of the axonemes after slight digestion. Samples of the digested axonemes were examined with the electron microscope to determine the effects of trypsin digestion on the substructures of the axonemes. The rate at which trypsin sensitized the axonemes to ATP paralleled the rate at which it damaged the radial spokes and the nexin links, while the dynein arms were removed much more slowly. The results suggest that inactive dynein arms form cross bridges between the adjacent doublet tubules in digested axonemes, and that when activated by the addition of ATP, they induce an active shearing force between adjacent doublets. The radial spokes and the nexin links are not directly involved in the production of mechanical force, but they may participate in regulating the sliding between tubules to produce a propagated bending wave.

INTRODUCTION

The flagellum of sea urchin sperm consists of a membrane-enclosed axoneme which has the form of a cylinder of nine doublet tubules surrounding a central pair of single tubules (1). The nine doublet tubules are interconnected circumferentially by *nexin links* between adjacent doublets (2, 3), and they are connected radially to the central pair by *spokes* (1). Each doublet tubule bears two parallel rows of projections called *arms* which project asymmetrically from one component tubule of each doublet toward the adjacent doublet tubules (1), and these arms contain the flagellar ATPase protein *dynein* (2, 4). The doublet and single microtubules consist of the protein *tubulin* (5, 6).

It is possible to render permeable the membrane of sea urchin sperm by glycerination, or to remove it completely by extraction with Triton X-100, and, in both cases, the cell's motility can be reactivated by placing it in a suitable solution containing ATP (7, 8). These reactivated sperm can be used to study the specific requirements of the motile mechanism of the flagella. In favorable cases, it is also possible to separate the demembranated flagella from the sperm heads and reactivate the isolated anonemes (9).

We have recently reported that brief tryptic digestion of a suspension of isolated flagellar axonemes modifies the internal structure of the axonemes so that subsequent addition of low

concentrations of ATP to the suspension induces a rapid disintegration of the axonemes (10). Observation by dark-field light microscopy has shown that this ATP-induced disintegration results largely from active sliding movements between adjacent doublet tubules of the axoneme. The disintegration reaction is specific for ATP and requires the presence of a divalent cation. Either Mg^{+2} or Mn^{+2} can fill the cation requirement equally well. Ca^{+2} will also activate the disintegration, but it occurs more slowly and to a lesser extent than in the presence of Mg^{+2}. The specificity of the disintegration reaction is very similar to the requirements for reactivation of demembranated sperm flagella and, for activation of the flagellar ATPase, dynein (8, 11). It is probable that the ATP-induced disintegration of trypsin-modified flagella is due to forces produced within the flagella by the basic energy-transducing mechanism which is no longer coordinated or restrained as a result of the internal damage caused to the flagella by digestion.

This demonstration that ATP induces active sliding between adjacent doublets of the flagellar axoneme in digested preparations provides strong support for a sliding tubule model of flagellar motility. Theoretical studies have shown that the formation and propagation of flagellar bending waves can be explained on the basis of an active shearing force between the longitudinal elements which, when opposed by an elastic resistance to shear, gives rise to localized sliding and generates bending movement (12–14). In one such possible model, the magnitude of the local shearing force is controlled by the degree of local bending (15). A model of this type has the advantage of being able to explain the uniform propagation of the flagellar bend against the apparently nonuniform local viscous moments experienced by the beating flagellum (16, 17). Experimental work is now being done to explain these generalized theoretical results in terms of the flagellar substructure.

In this paper, we describe the effects of tryptic digestion on the axonemal structure, with special emphasis on the degree of the disruption of spokes, nexin links, and dynein arms as a function of the extent of digestion. The functional roles of these structures are discussed.

MATERIALS AND METHODS

Axonemes were prepared at 0°–4°C from sperm of the sea urchin *Tripneustes gratilla* by extraction and differential centrifugation in a solution containing 1% Triton X-100, 0.1 M KCl, 5 mM $MgSO_4$, 1 mM ATP, 1 mM dithiothreitol, 0.5 mM EDTA, and 10 mM Tris-phosphate buffer, pH 7.0, as described by Gibbons and Fronk (11). Dark-field microscopy was used to check the purity of the axonemal suspension. Care was taken to remove all sperm heads, because even small numbers of them contributed disproportionately to the turbidity of the suspension. Axonemes were suspended in Tris-Mg solution (2.5 mM $MgSO_4$, 0.2 mM EDTA, 0.1 mM dithiothreitol, 30 mM Tris-HCl buffer, pH 7.8), centrifuged, and resuspended in fresh Tris-Mg solution at a concentration of about 0.6 mg protein/ml.

Trypsin stock solutions were prepared in Tris-Mg solution, 250 μg/ml, using twice crystallized and lyophilized trypsin. Standardization of these solutions by digestion of benzoyl arginine ethyl ester (BAEE), showed an activity of 8,000–10,000 BAEE units/mg trypsin (18). A known volume of the stock trypsin solution was added to the axonemal suspension at room temperature (about 25°C) for digestion of the axonemes. The course of digestion was followed routinely by measuring the change in turbidity of the suspension with a Zeiss PMQII spectrophotometer (Carl Zeiss, Inc., New York) at 350 nm. When desired, the digestion was terminated by adding an excess of soybean trypsin inhibitor. Digested samples were either used immediately after the addition of inhibitor, or they were chilled to 0°C until used.

A quantitative measure of the rate of peptide bond hydrolysis during digestion was obtained by titration with 2 mM NaOH in a recording pH-stat. In this case, the axonemes were rinsed and suspended in 40 mM KCl and 2.5 mM $MgSO_4$, pH 7.8. The rate of peptide bond hydrolysis was calculated by assuming an average of 0.6 proton liberated per peptide bond hydrolyzed (19).

The effects of digestion on the various structural components of the axoneme were studied by electron microscopy of specimens that had been digested to differing extents. Preliminary observations showed that the axonemal components most rapidly damaged by digestion were the radial spokes which join each outer doublet tubule to the central sheath (1), and the nexin links which run circumferentially joining adjacent doublets (2, 3); the dynein arms and the central tubules appeared affected at a slower rate, while the outer doublet tubules were the most resistent to digestion. In more detailed experiments, we concentrated upon determining the relationship between the degree of structural disruption and the sensitivity of the digested axoneme to disintegration by ATP.

For this purpose, a large preparation of axonemes was digested with trypsin in the usual way. A series of samples were removed at various stages during the digestion and mixed with trypsin inhibitor.

Each sample was divided into three portions. One portion was fixed immediately by adding it to five volumes of fixative containing 2% glutaraldehyde, 2.5 mM $MgSO_4$, and 10 mM phosphate buffer, pH 7.8. The second portion was mixed with 0.1 mM ATP, and then, after 3 min, it was added to the fixative as above. (Previous work [10] has shown that the changes due to ATP are essentially complete within 1 min.) The third portion was dialyzed overnight against Tris-EDTA solution (5 mM Tris-HCl buffer, 0.1 mM EDTA, and 0.5 mM mercaptoethanol, pH 7.8), and then also fixed as above. After fixing in the glutaraldehyde solution for 1 h, each specimen was centrifuged at 35,000 g for 5 min. The resultant pellets were washed with fresh buffer, postfixed with 2% OsO_4, dehydrated in acetone, and embedded in Araldite epoxy resin. They were thin-sectioned with a glass knife. Sections with a reflection interference color of gold were found to give the best photographs. The sections were stained with uranyl acetate, followed by lead citrate. Electron micrographs were taken with a Philips EM 300 at 80 kV.

The samples fixed without further treatment were examined to determine the structural damage resulting directly from the digestion. From these micrographs, it was possible to determine the disruption of the radial spokes, the dynein arms, and the central and outer tubules. However, the nexin links could not be observed with sufficient clarity for quantitative estimation in these micrographs so they were determined from the samples that had been dialyzed after digestion. Dialysis against Tris-EDTA solution removes the dynein arms and part of the radial spokes, so that the nexin links are the only structures holding the doublet tubules together in groups of nine (2, 3). The size of the groups of tubules present after dialysis thus provides an assay for the nexin links. The approximate percentage of undamaged nexin links in each sample was calculated from the formula:

$$\% \text{ of undamaged links} \approx \frac{\sum\limits_{n=1}^{10} (n-1)m_n}{\sum\limits_{n=1}^{10} nm_n},$$

where n is the size of a group of tubules and m_n is the number of groups containing n tubules. The sensitivity of each of the digested samples to ATP was determined from the size of the tubule groups present in the portion exposed to ATP before fixation.

In order to obtain an unprejudiced estimate of the distribution of tubules from disintegrated axonemes into groups of various sizes as a function of the degree of digestion of the axonemes, the electron micrographs which had been taken of a series of embedded specimens were identified by code numbers, mixed together, and then selected in a random manner for counting. For simplicity, the central pair was counted as one unit since they usually remained together after the disintegration. The axoneme was, therefore, considered to be made of 10 tubules for the purpose of counting. A similar procedure was followed in counting disruption of spokes and of dynein arms. Where counts were made to determine the distribution of tubules into groups, the equivalent of 60–100 axonemes from each sample were counted. In counting the disruption of spokes, between 80 and 200 axonemal cross sections from each sample were examined. In counting the disruption of arms, 10 or more axonemal cross sections from each sample were examined.

Trypsin and soybean trypsin inhibitor were obtained from Worthington Biochemical Corp., Freehold, N.J.; sources of other chemicals were as given previously (8).

RESULTS

The effect of trypsin digestion on the turbidity of a suspension of axonemes follows two very different courses depending on the absence or presence of ATP. Fig. 1 shows the data from a typical experiment in which the trypsin:protein ratio was 1:2,000. In the absence of ATP, the turbidity decreased steadily with time, falling to about 90% of its initial value after 5 min and to 70% after 30 min. However, in the presence of ATP, there was an early, rapid decrease in turbidity down to about 40% of its initial value after 5 min, and then the rate of decrease became much slower, falling to about 30% of its initial value after 30 min. When ATP was added after
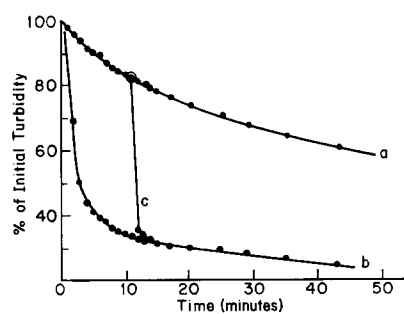

FIGURE 1 Change in turbidity of three samples of axonemes suspended in Tris-Mg solution because of digestion with trypsin. (a) No ATP present, (b) 0.3 mM ATP present initially, (c) 0.1 mM ATP added to suspension after 11 min of digestion without ATP. Trypsin-protein ratio in all three cases was 1:2,000.

the digestion had proceeded for a period of time (Fig. 1, curve c), the turbidity of the suspension dropped very rapidly to about the same value as that of the sample digested in the presence of ATP. Observations by light and electron microscopy have shown that the rapid ATP-induced decrease in turbidity results from a disintegration of the digested axonemes into individual doublet tubules and small groups (10). This disintegration reaction is very sensitive to ATP, concentrations of less than 50 μM being sufficient to give a complete reaction.

The rate of hydrolysis of peptide bonds, as measured by the pH-stat (Fig. 2), was found to follow a curve similar to that by which the turbidity of a suspension decreased when digested in the absence of ATP. This relationship indicates that the amount of turbidity decrease of a suspension being digested in the absence of ATP provides a direct indication of the extent of digestion, and that it can be used as a convenient assay of the digestion. By assuming that the amino acids of the axonemal proteins have a mean molecular weight of 115, and that 0.6 mol of H^+ is liberated per mol of peptide bond hydrolyzed (19), it can be calculated from the rate of NaOH added by the pH-stat (Fig. 2) that approximately 0.3% of the total peptide bonds in the axonemal proteins have been hydrolysed at a stage in digestion corresponding to a decrease in turbidity to 70% of its initial value.

When using the turbidity assay, it is important that the axonemal suspensions be completely free of sperm heads, because even small numbers of them contribute disproportionately to the turbidity. Any sperm heads present become lysed at an early stage of the digestion. The lysis causes a rapid decrease in turbidity at this stage of di-
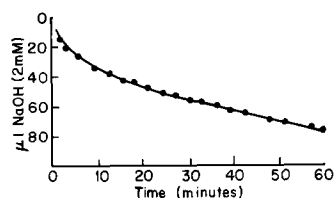

FIGURE 2   pH-stat assay of the digestion of axonemes. The assay vessel contained 4.7 mg of axonemal protein and 1.3 μg of trypsin. The abscissa indicates the volume of 2 mM NaOH added by the pH-stat. The speed of digestion corresponds to a decrease in the turbidity of the suspension to about 70% of its initial value after 10 min.

gestion, and it results in a sigmoid turbidity curve for contaminated suspensions being digested without ATP, rather than the uniform decrease obtained with pure suspensions of axonemes (Fig. 1).

In order to determine whether the form of the digestion curve was dependent upon the absolute rate of digestion, the turbidity curves of suspensions being digested with and without ATP were studied over a range of trypsin:axonemal protein ratios of from 1:660 to 1:13,200. At the highest ratio, the turbidity in the absence of ATP was reduced to 80% of its initial value in 2.75 min, while at the lowest ratio, it took 48 min to reach this turbidity. When the resultant digestion curves were plotted with the time-scale normalized with respect to the trypsin:protein ratio, they were essentially identical. This result indicates that the speed of digestion is proportional to the relative amount of trypsin present, and that the form of the digestion curve is not affected by the speed of digestion.

The sensitivity of the digested axonemes to disintegration by ATP presumably results from the destruction of certain trypsin-sensitive structural components which function to maintain the integrity of the normal axoneme. In order to determine which components are responsible for this effect, we have investigated the relationship between the degree of structural destruction caused by digestion and the sensitivity of the axoneme to disintegration by ATP. In a typical experiment, a large preparation of axonemes was digested with trypsin, with a series of samples being removed at various stages during the digestion and mixed with trypsin inhibitor. Each sample was then divided into three: one portion of which was fixed immediately, one after exposure to ATP, and the third fixed after dialysis against Tris-EDTA solution, as described in the Materials and Methods section.

It is convenient to describe first the changes which result directly from the digestion. The principal change in axonemal structure, visible in electron micrographs, due to digestion from 100% to about 90% of the initial turbidity was a separation of many of the radial spokes from their normal point of attachment to the sheath surrounding the central pair of tubules (Figs. 3 and 4). This separation of the spokes does not appear to occur randomly, for lightly digested preparations contain mostly axonemal sections in which

all of the spokes, or the half of them to one side of the central tubules, or none of them are detached, and it is uncommon to see a single spoke disconnected while its neighbors on either side remain attached. This pattern suggests that the disruption of the spokes may result from digestion of the central sheath to which they are attached, rather than from breakage of the spokes them-
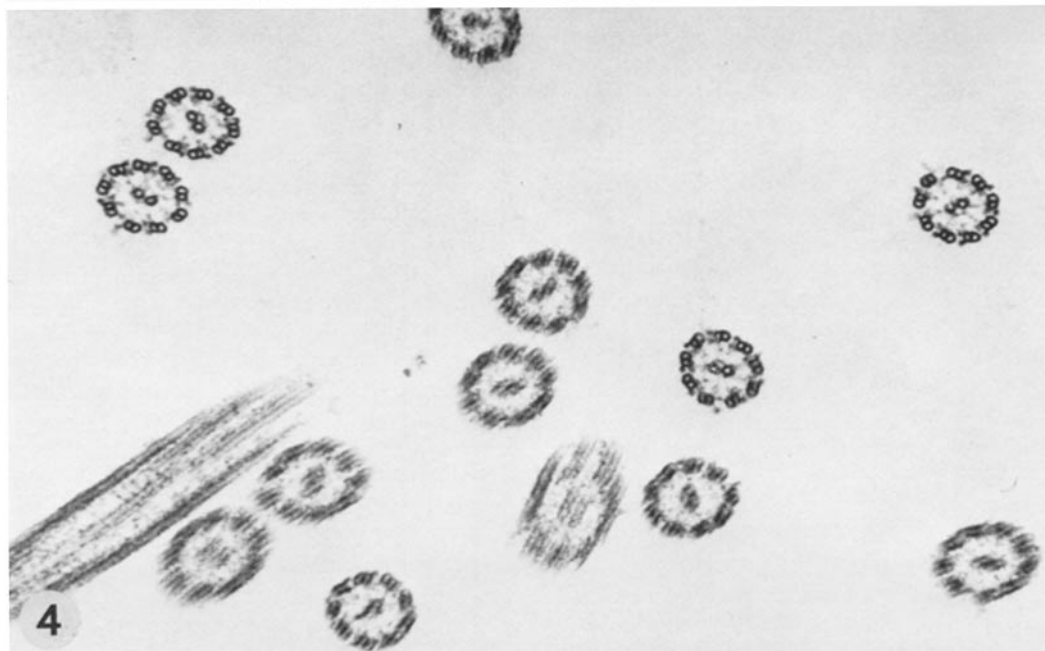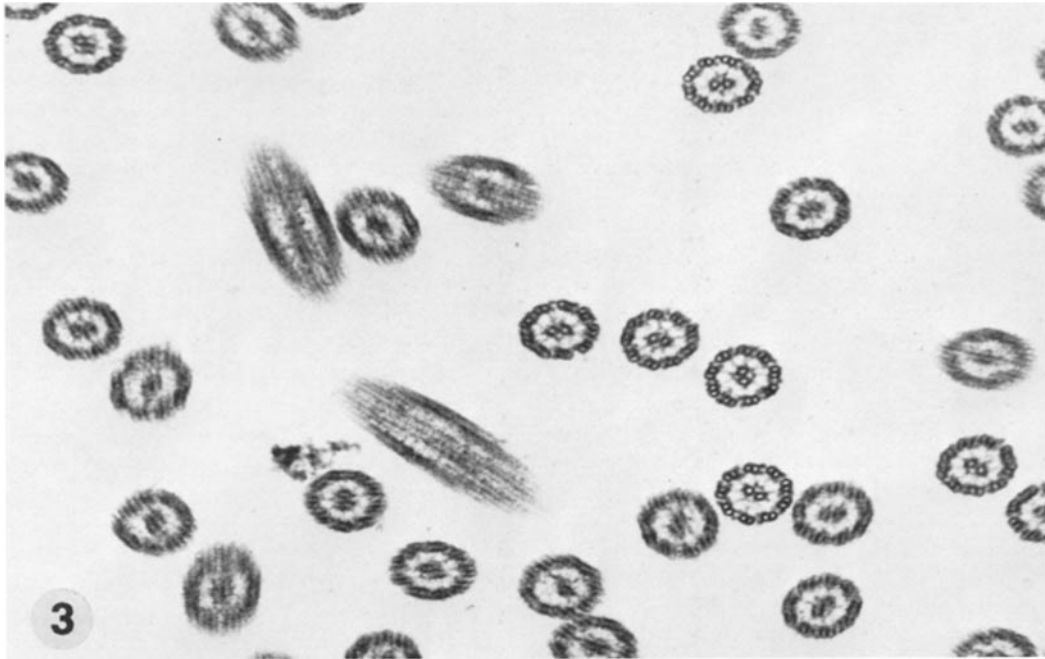

FIGURE 3   Electron micrograph of a preparation of undigested axonemes. × 30,000.

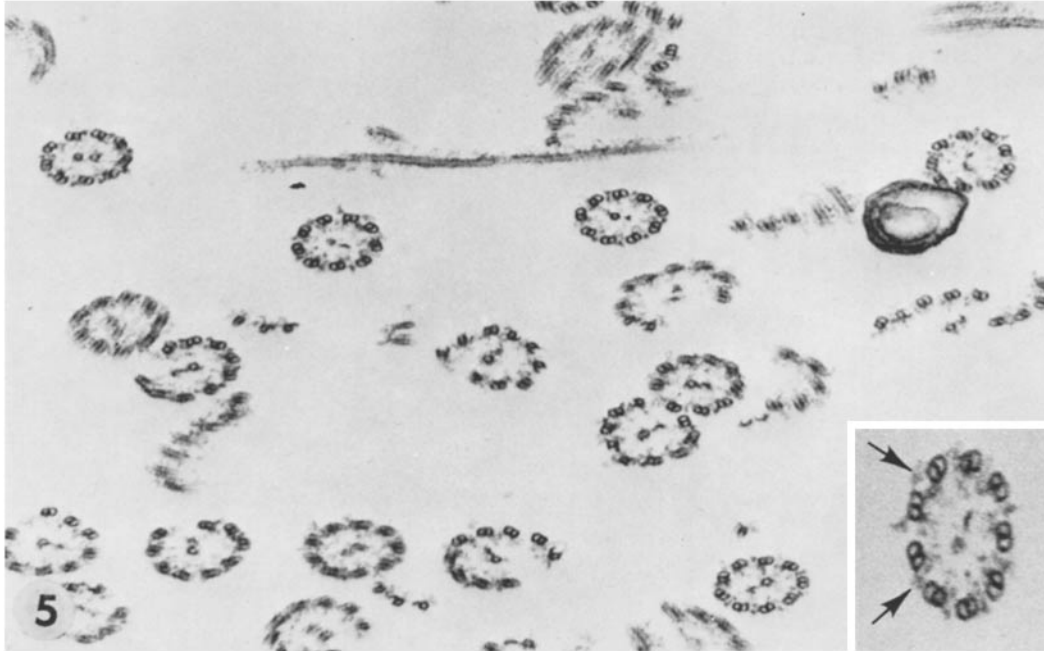FIGURE 4   Electron micrograph of same preparation of axonemes after digestion to 89% of the initial turbidity. × 30,000.

FIGURE 5   Same as Fig. 4, but after digestion to 80% of the initial turbidity. × 30,000. Inset shows bridging between adjacent doublet tubules by dynein arms. × 60,000.

selves. In more highly digested preparations in which digestion had reduced the turbidity to 80% of its initial value, the spokes were completely disconnected from the central sheath in all axonemal sections, and many of the central tubules and some outer doublet tubules were themselves visibly damaged (Fig. 5). About half of the axonemes had split open at one point in their periphery, although most still remained together as groups of 10 tubules (counting the remaining fragments of the central pair as one unit) (Fig. 6). After the turbidity had decreased further to 60%, the central tubules were almost completely absent and nearly half of the axonemes had broken down into smaller groups of tubules (Figs. 6 and 7). Counting the fraction of intact spokes at the different stages of digestion showed that there was an initial rapid drop during the early stage of digestion (to about 90% of initial turbidity), at which point only about a quarter of the cross sections showed completely intact spokes (Fig. 8). The disruption of the dynein arms occurred much more slowly, with about four-fifths of them remaining apparently intact after the turbidity had decreased to 90%, and about three-quarters of them intact at 80% turbidity (Fig. 8).

Determination of the nexin links by analysis of micrographs of the dialyzed portion of each sample showed that they were very rapidly attacked by the trypsin. Even as early as 90% turbidity, about two-thirds of the tubules were released by dialysis as individual tubules and the remainder were in small groups (Figs. 6, 9, 10). Calculation indicates that this distribution of tubules corresponds to the presence of, at most, one-fifth of the original nexin links (Fig. 8). Taken together, these results thus indicate that both the nexin links and the radial spokes are disrupted rapidly at an approximately equal rate in the early stages of the digestion. The nexin links and the radial spokes are almost completely disrupted before the dynein arms appear to be substantially damaged.

Analysis of the micrographs of the specimens that had been exposed to ATP before fixation indicated that the axonemes had almost all become sensitive to disintegration by ATP by the time that digestion had reduced the turbidity to about 90% of its initial value (Figs. 6, 11–13). The appearance of sensitivity to ATP thus appears to parallel approximately the disruption of the nexin links and the radial spokes.

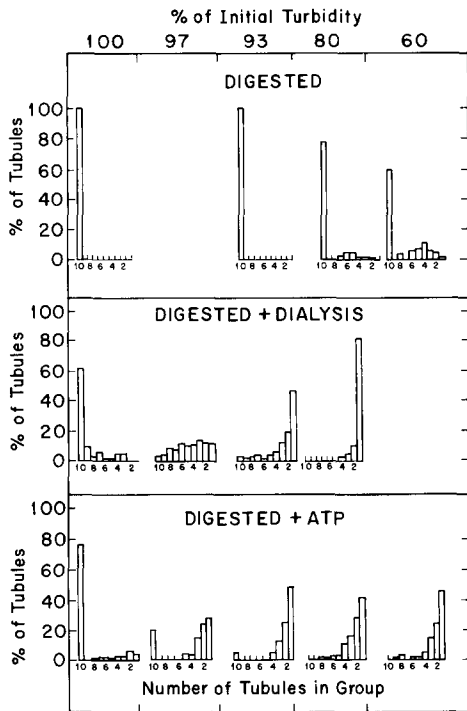The size-distribution of the tubule groups

% of Initial Turbidity

FIGURE 6 Size-distribution of tubule groups seen after different degrees of digestion. The upper row gives data from the portion of each sample fixed immediately after digestion. The middle row gives data from the portions of some samples dialyzed against Tris-EDTA solution before fixation. The bottom row gives data from portions of samples exposed to ATP before fixation. The dialyzed sample illustrated in the 80% turbidity position was actually only digested to 86% turbidity.

produced by ATP-induced disintegration shows certain characteristic differences as compared to that produced by dialysis of the digested axonemes. After ATP-induced disintegration, the preparation contained mostly a mixture of intact axonemes and small groups containing mostly one to three tubules, while there was a lack of larger groups containing five to nine tubules. The proportion of intact axonemes decreased rapidly with the degree of digestion, with only about one-quarter remaining intact after digestion to 97% turbidity, and almost none after digestion to about 90% turbidity. However, among the disintegrated groups at all stages of digestion, the relative numbers of groups containing one, two, or three tubules remained about the same (Fig. 6). If this unusual distribution of tubules is because of the nature of the

forces causing the disintegration, then these characteristics suggest that the ATP-induced disintegration is a cooperative phenomenon in which the shearing forces developed by interaction between groups of two and three tubules are mechanically effective in breaking up large groups of tubules but are less effective in separating the small groups into individual tubules. However, it is also possible that the small groups of tubules represent tubule groupings within the axoneme which are intrinsically less active in producing shearing forces. For example, the tubules which lie on opposite sides of the axoneme and through which the plane of bending passes might be such less active groups. Another possible explanation for the small groups of tubules is that they arise because of reassociation of individual tubules, although it is not clear why reassociation should not continue so as to produce larger groups of tubules as well.

In contrast, the digested axonemes subjected to dialysis appear to break down into smaller groups of tubules in a much more random manner, proceeding from numerous large groups of tubules early in digestion to smaller groups and eventually to almost all individual doublet tubules as the digestion progressed (Fig. 6). The distribution of size groups differs from that produced by ATP-induced disintegration by the substantial number of groups containing five to nine tubules at the early stages of digestion and by the great predominance of individual tubules over groups containing two or three at the later stages of digestion. Such a distribution might be expected to result from the random digestion of nexin links.

A small amount of disruption was induced by ATP even in the undigested axonemes (Fig. 8) in these preparations. This effect may have been due to weak spots caused within the axonemes during their preparation or to slight proteolysis by enzymes from the sperm head.

In preliminary experiments to test the effects of other proteolytic enzymes, digestion by either papain or chymotrypsin was found to sensitize the axonemes so that subsequent addition of ATP produced disintegration. This observation suggests that the sensitization resulting from digestion is an effect due to digestion of exposed peptide chains, rather than being due to the cleavage of a few bonds that are particularly sensitized to trypsin.
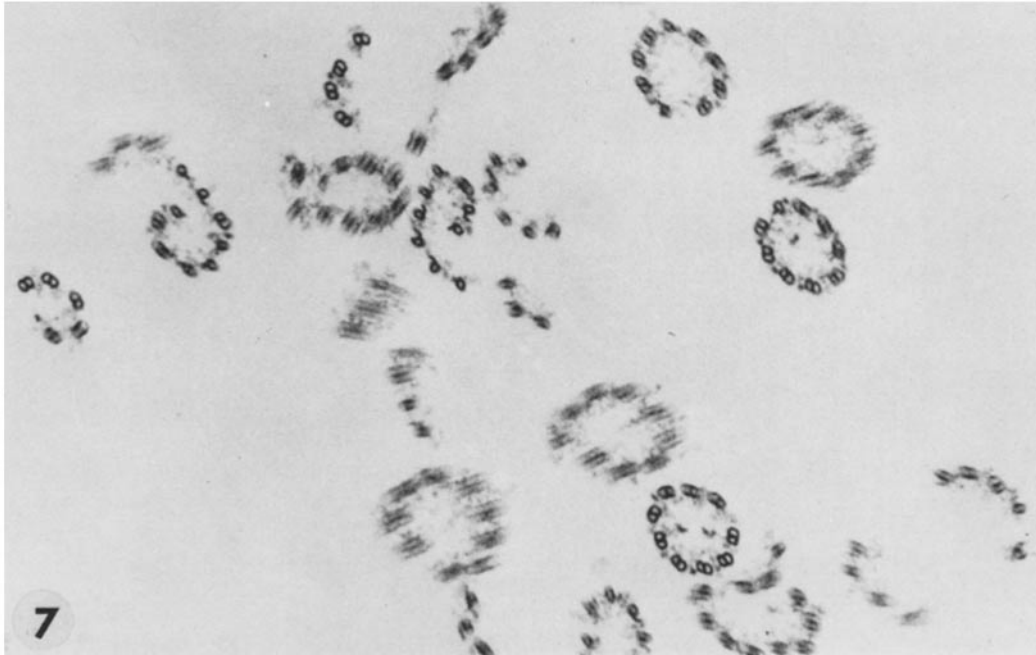
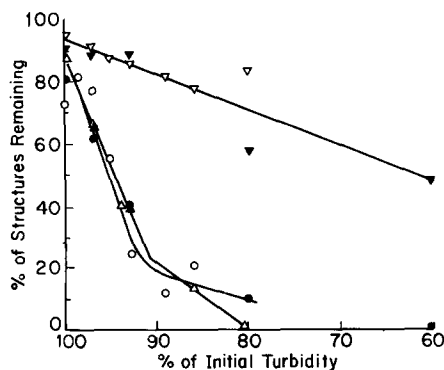FIGURE 7   Same as Fig. 4, but after digestion to 60% of the initial turbidity. × 30,000.

FIGURE 8   Damage of axonemal substructures as a function of tryptic digestion. (△) percent of axonemal cross sections with undisrupted spokes, (○) percent of undamaged nexin links as calculated from the size-distribution of tubule groups seen in digested preparations after dialysis against Tris-EDTA solution, (▽) percent of arms remaining in cross sections of axonemes. Data are from two preparations of axonemes as indicated by the outline and solid characters.

DISCUSSION

Previous work based upon direct observation by light microscopy has shown that the ATP-induced disintegration of digested axonemes is due to active shearing forces which produce relative sliding movements between the outer doublet tubules of the axoneme (10). The chemical conditions necessary for obtaining this active disintegration of digested axonemes closely resemble the conditions necessary for reactivating normal motility in demembranated flagella that have not been exposed to trypsin, suggesting that the same ATP-induced shearing force is involved in both cases. The propagated bending waves of normal flagella can reasonably be explained as the result of this ATP-induced shearing force between adjacent doublet tubules which, when opposed by the elastic resistance of the native structure, leads to localized sliding and generation of an active bending moment. The detailed studies reported in this paper provided more detailed support for our previous suggestion that the dynein arms participate directly in generating the active sliding between tubules, while the nexin links and the radial spokes may provide the elastic resistance to limit the amount of sliding that normally occurs and the coordination to produce propagated bending waves (10).

There is by now a wide variety of evidence indicating that the dynein arms play a major role in generating the shearing force that leads to sliding between tubules. The arms are appropriately located between adjacent doublet tubules

(1). The specificity properties of dynein ATPase closely resemble the conditions necessary for generating motility in demembranated flagella (8, 11) and for inducing active disintegration in digested axonemes (10). Even more direct evidence is provided by the recent report that partial removal of the dynein arms causes a proportionate reduction in beat frequency of reactivated
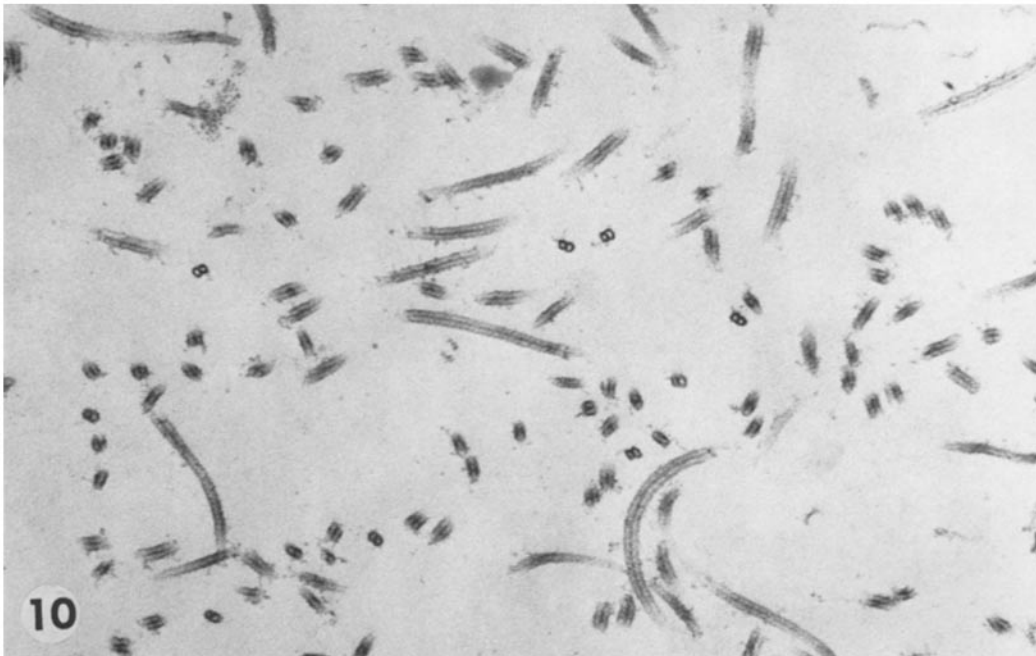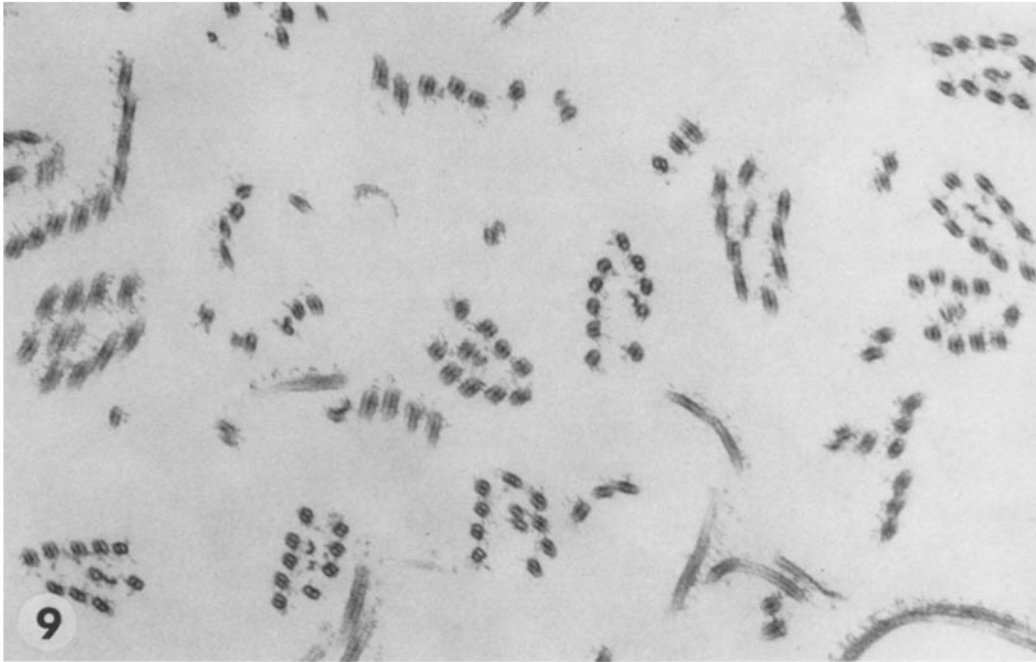

FIGURE 9 Undigested axonemes dialysed to remove the dynein arms and embedded for electron microscopy. X 30,000.

FIGURE 10 Axonemes digested in suspension to 93% of the initial turbidity, dialysed to remove the dynein arms, and embedded for electron microscopy. X 30,000.

flagella, indicating that the rate of sliding between tubules is proportional to the number of functional dynein arms present (20). In this paper, we have shown that the dynein arms appear to be relatively resistant to digestion, and that, apart from the doublet tubules themselves, they appear to be the only structural component remaining largely intact after sufficient digestion to sensitize the axonemes to disintegration by ATP.
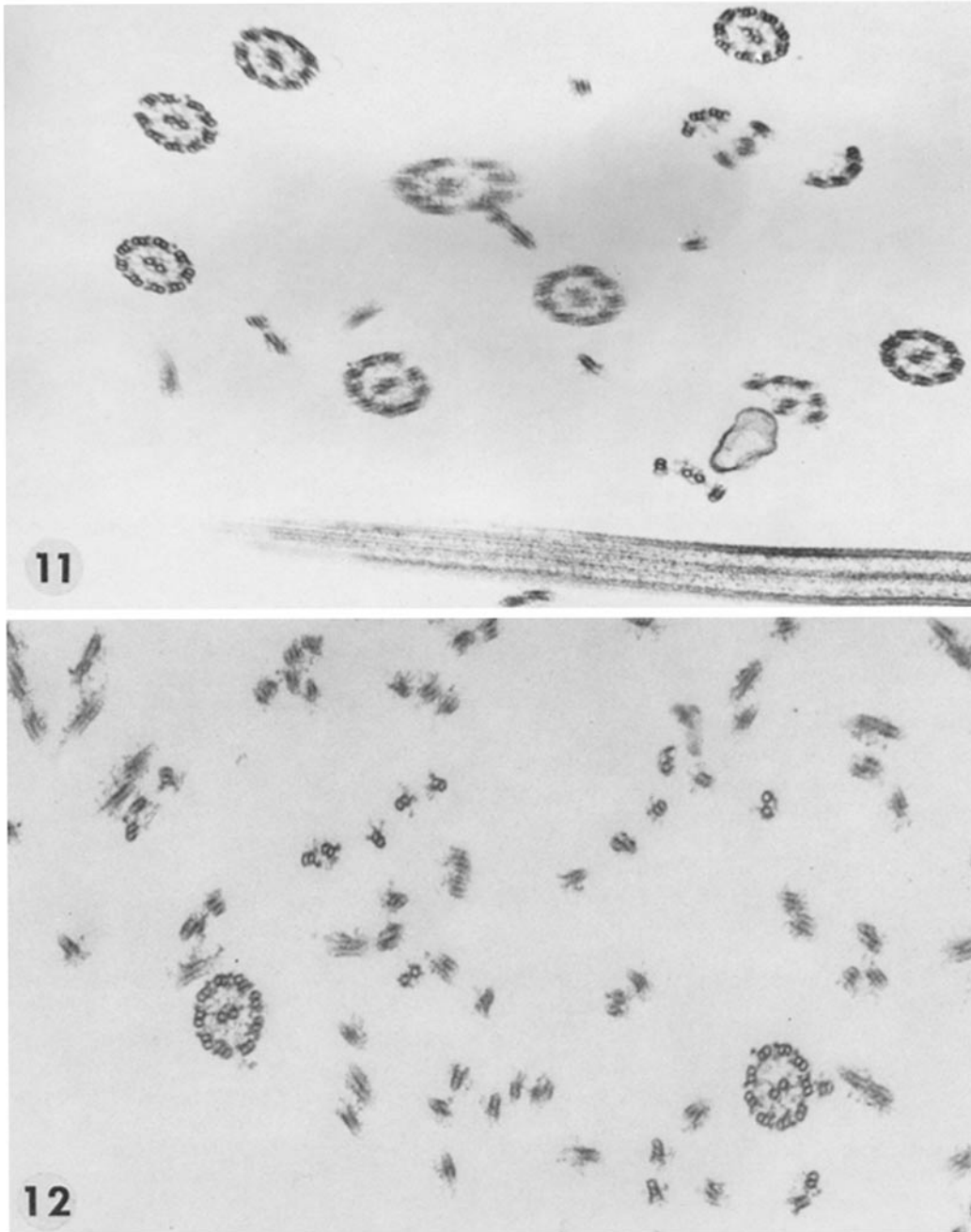

Figure 11   Undigested axonemes reacted with ATP and embedded for electron microscopy. × 30,000.
Figure 12   Axonemes digested in suspension to 95% of the initial turbidity, reacted with ATP, and embedded for electron microscopy. × 30,000.
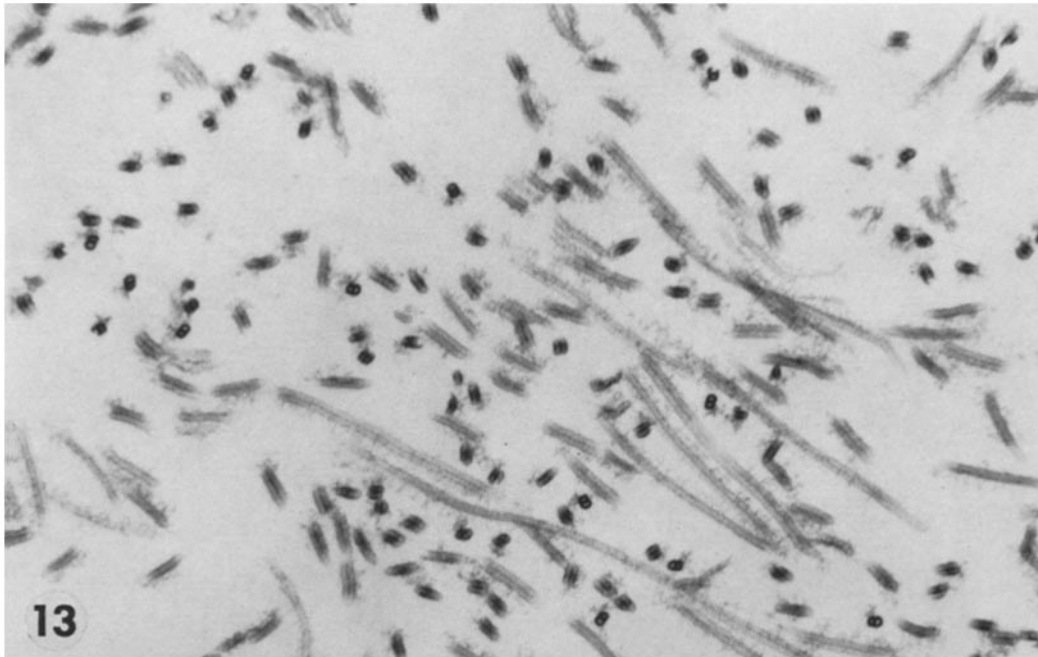
FIGURE 13 Axonemes digested in suspension to 80% of the initial turbidity, reacted with ATP, and embedded for electron microscopy. × 30,000.

Our results here have shown that the outer doublet tubules of the axoneme tend to remain together in a cylinder long after digestion appears to have destroyed the nexin links and to have broken the connection of the spokes to the central sheath and tubules. We have no direct evidence as to what structure is holding the tubules together at this stage, but, in the absence of other possibilities, it appears likely that the dynein arms are responsible and that they hold the tubules together by forming cross bridges linking adjacent doublet tubules. This hypothesis is supported by the fact that the tubules of digested axonemes immediately separate when dialyzed against Tris-EDTA solution, which removes the dynein arms from the tubules (2).

The occurrence of the dynein arms as cross bridges between adjacent doublet tubules is directly implied by the evidence mentioned above for their role in the mechanism producing active sliding. Such cross bridges are visible in many of our micrographs of digested axonemes (see inset, Fig. 5), and they have been observed recently in micrographs of undigested flagella prepared under particular conditions (20), although they have not been observed in most

earlier micrographs of cilia and flagella (21–23) either because only a small fraction of the arms form such bridges at any one time during normal movement or because the bridges are difficult to preserve by fixation for microscopy.

The digestion of the axonemes with trypsin disrupts the nexin links and the radial spokes at about the same rate as it sensitizes the axonemes to disintegration by ATP (Fig. 6). This result supports the hypothesis that the nexin links and the spokes are involved in providing the elastic resistance that normally limits the amount of sliding between tubules and in coordinating the sliding to produce propagated bending waves. However, since these two structures are digested by trypsin at approximately the same rate, our results do not provide evidence regarding their individual roles of providing resistance and coordination.

Assuming that the flagellar tubules are completely inextensible (24), a maximum bend of about 3 radians would require a displacement of approximately 400 nm between doublet tubules on opposite sides of the axoneme with at least 100 nm between adjacent tubules. To accommodate such sliding, the spokes and nexin links must either

be very elastic or they must shift their points of attachment as do the dynein arms. However, if all three structures were capable of shifting their points of attachment, the axoneme might be expected to fall apart under the influence of ATP without any digestion. The spokes in straight regions of a bent axoneme remain approximately perpendicular to the tubules (25) and do not appear to be subjected to a high degree of elastic stress that would be expected if their points of attachment had remained fixed. This suggests that it may be the nexin links which have fixed points of attachment and provide the elastic resistance to sliding, although the orientation of these structures in bent and straight flagellar segments has not yet been determined. The recent observations of McIntosh (26) on bent regions of axostyles in *Saccinobaculus* suggest that the microtubules in this organelle are not inextensible. If a similar amount of passive elongation were to occur in flagellar tubules, it would substantially reduce the amount of sliding for a given degree of bending and make it possible to accommodate the sliding without requiring an apparently unreasonable degree of elasticity in the nexin links or the radial spokes. However, the interpretation of such observations is difficult because it is not easy to determine how faithfully a mechanically stressed structure has been preserved by fixation for microscopy.

Although there is now abundant evidence implicating the dynein arms in the mechanism which generates the sliding movements between tubules, further data will be needed before it will be possible to specify the roles of the various structural components in coordinating the sliding to produce propagated bending waves.

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