Journal of Supramolecular Structure
and Cellular Biochemistry 17:183-196 (1981)

Factors Implicated in Determining the Structure of Zinc Tubulin-Sheets: Lateral Tubulin-Tubulin Interaction Is Promoted by the Presence of Zinc

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Addition of increasing amounts of zinc to a cold microtubule protein solution results in the disappearance of 30 S oligomer found in the absence of that cation and in the appearance of new tubulin oligomers, 90 S and 23 S. When a microtubule protein solution is warmed in the presence of zinc, tubulin-sheets are assembled. We have tested the influence of microtubule associated proteins and the zinc:tubulin ratio on the polymerization process. Depletion of microtubule associated proteins results in wider and longer tubulin-sheets than those polymerized in the presence of microtubule associated proteins. However by increasing zinc concentration wider but shorter tubulin-sheets were found. These results suggest that microtubule associated proteins and zinc could promote nucleation of tubulin-sheets, but zinc also promotes lateral tubulin-tubulin interaction. This interpretation was confirmed when microtubule protein was assembled at a low zinc:tubulin ratio. In such conditions composite structures of microtubules and zinc tubulin-sheets are formed. These composite structures are consequence of a lateral attachment of a zinc tubulin-sheet on a microtubule protofilament.

Key words: microtubule protein, tubulin-sheets, zinc, MAPs, tubulin lateral interaction

Microtubules can be assembled in vitro by warming a tubulin solution in the conditions first described by Weisenberg [1] and Shelanski et al [2]. These polymers have a variable length and an essentially constant number of protofilaments. In vitro assembly of these polymers is facilitated by the presence of several microtubule associated proteins (MAPs) [3-5]. MAPs favor the initiation of microtubule polymerization, and once the polymer has been initiated the final length of the structure appears to be determined by the number of nucleation centers and the available tubulin, that is, it depends on the monomer-polymer equilibrium [6]. Thus, when MAPs are removed, longer microtubules were found [7-9]. Little is known about the factors that determine the protofilament number of a microtubule, but Pierson

Received April 25, 1981; accepted July 31, 1981.

0275-3723/81/1702-0183$04.00

et al [10] after finding a population of microtubules reassembled in vitro, by several polymerization-depolymerization cycles, with an abnormal protofilament number, have suggested that MAPs might be responsible for such differences. Also Amos [11] has suggested that MAPs could somehow determine the protofilament number in the microtubule. MAPs are also present in a ring shaped tubulin oligomers with a sedimentation coefficient of 30 S, found in depolymerized microtubule solution [12, 13]. Although these oligomers are usually found during the microtubule polymerization-depolymerization process, they are not, probably, obligatory intermediates in such process [8,14,15].

Zinc addition to microtubule protein results in the formation of sheet shaped structures [16], named sheets I [17], which are structurally different from open microtubules or sheets found in the absence of zinc [18,19]. Gaskin and Krees have shown that zinc tubulin-sheets have a variable width and length [17]. These structures are composed of tubulin protofilaments, just as microtubules are; however, the lateral interactions among these protofilaments and their polarity differ from those in microtubules [18,19].

In this report we have studied (a) whether zinc addition to a microtubule protein solution results in the appearance of different tubulin oligomers; (b) whether the factors implicated in determining the length of zinc tubulin-sheets and those that determine the length of microtubules are similar (ie, number of nucleation centers and tubulin available); and (c) the role of zinc and MAPs in determining the protofilament number (width) in zinc tubulin-sheets.

METHODS

Microtubule Protein Assembly

Microtubule protein was obtained from porcine brain by three cycles of temperature dependent assembly and disassembly in buffer A (0.1 M piperazine-N, N′-bis(2-ethane sulfonic acid) (Pipes), 0.5 mM $MgCl_2$, and 1 mM GTP), adjusted to pH 6.7 at 23°C with NaOH, essentially as indicated by Shelanski et al [2]. The first polymerization step was carried out in the presence of 4 M glycerol and the two further polymerization steps were performed without glycerol. Microtubule protein was frozen in liquid nitrogen and stored at −70°C until it was used.

Microtubule protein, in buffer A, was assembled in the presence or absence of zinc, by incubation at room temperature. Controlled pore glass chromatography CPG-10 (1195 Å) (Electro-Nucleonics Inc) was performed on a column (0.45 × 26.5 cm) in buffer A containing 0.3 mM zinc chloride, at 6 mg/ml. Sepharose 4B chromatography was performed on a column (1.4 × 30 cm) under similar conditions. Poly dAdT (Miles) was added up to a mass ratio, to microtubule protein, of 1:40 to obtain longer sheets following a protocol similar to that described for microtubules [20].

To study the association of ^{3}H-GTP and $^{65}ZnCl_2$ to polymerizing structures, we have added ^{3}H-GTP (10 μCi/ml), 0.1 mM final concentration, to a microtubule solution containing acetate kinase and acetyl phosphate as a GTP-regenerating system [21] or $^{65}ZnCl_2$ (10 μCi/ml) at the indicated concentrations. After polymerization, the samples were centrifuged in buffer A containing 50% sucrose as previously indicated [22]. The pellets were washed and resuspended in 1% sodium dodecyl sulfate (SDS), 10 mM Tris pH 6.8, and the radioactivity and protein content were measured.

Analytical Ultracentrifugation

Sedimentation velocity experiments were carried out with a Beckman model E analytical ultracentrifuge equipped with Schlieren optics. Samples were run in a An-D rotor with 12-mm double sector cells. Sedimentation profiles were recorded on Kodak metallographic plates. Distances on the photographic record were determined with a Nikon microcomparator. Sedimentation coefficients were corrected to water at 20°C, using a value of $\bar{v} = 0.736$ cm^3 g^{-1} for the partial specific volume of tubulin [23]. The relative concentrations of molecular species observed in the sedimentation profiles were estimated from the areas under the peaks by planimetry.

Electron Microscopy

Negative staining of microtubule samples were performed by brief adsorption of the sample to grids coated with collodion and carbon. The sample was displaced with several drops of 1% (w/v) uranyl acetate, excess stain was removed with filter paper and the sample was air dried. Grids were examined with a Jeol electron microscope (JEM 100B). Magnifications were determined with the use of a carbon replica of 2160 line/mm (Emscope Laboratory Ltd). Length and width were determined from the electromicrographs taking into account the magnification in each case.

Optical Diffraction

Micrographs were examined in an optical diffractometer [24]. Sheets and open microtubule areas most suitable for analysis were selected on basis to the number and quality of the spots in the diffraction plane. Image filtration of composite sheets was achieved as indicated by Aebi et al [24]. To determine the localization of sheet and microtubule region in the composite structures, a mask, containing holes in the position corresponding to both diffraction patterns (zinc tubulin-sheets and open microtubles), was placed in the diffraction plane. The filtered images were averaged [25] following the protofilament direction to avoid distortion of a possible interphase.

RESULTS

Oligomers Found in the Presence of Zinc

When 0.3 mM zinc is added to a microtubule protein solution (4 mg/ml) several tubulin oligomers appear (Fig. 1). These oligomers have a common feature, namely, the lack of stability. Usually two types of oligomers are found, a broad component with about 90 S found only at early centrifugation times (Fig. 1A, a and b), and another with a sedimentation coefficient of 23 S (Fig. 1A, b); 23 S proportion decreases relative to total protein and 90 S usually disappears, at longer centrifugation times (Fig. 1A, b). This behavior indicates a main difference between these oligomers and the 30 S complex found in the absence of zinc. This difference could be related to the distinct structures assembled in the presence or absence of zinc. Moreover, when the dependence of the sedimentation coefficient upon protein concentration was studied in the 23 S oligomer, a behavior that differs from that reported for 30 S was found. For 30 S oligomers a sharp decrease was found at increasing protein concentrations [13], whereas for 23 S the sedimentation coefficient was almost independent of protein concentration (Fig. 1B). Figure 2 indicates the fraction of protein mass in tubulin oligomers at different zinc concentrations. The

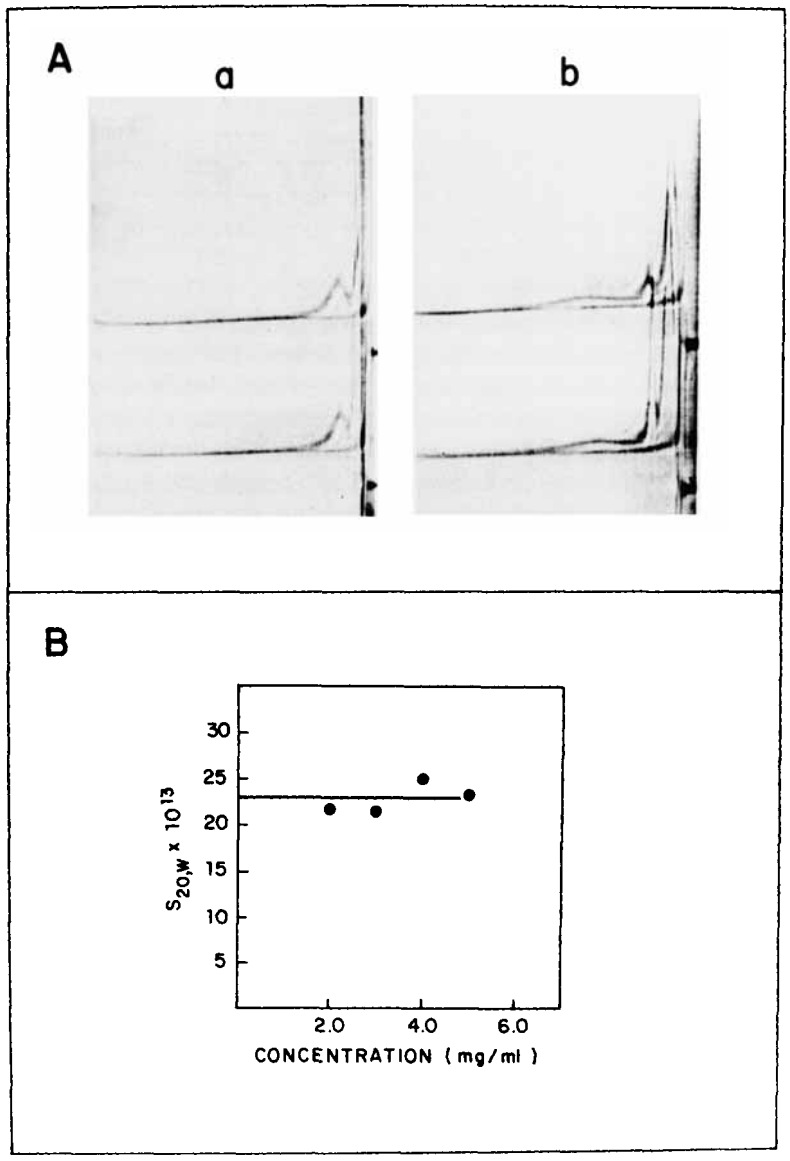
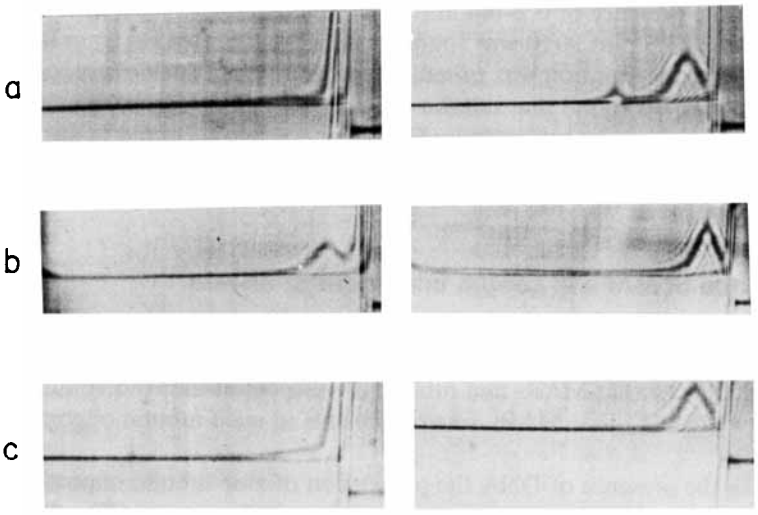


Fig. 1. Protein oligomers found in the presence of zinc. (A) Schlieren photographs showing the sedimentation pattern of microtubule protein in the presence of zinc. A solution of microtubule protein at 5 mg/ml (upper traces) and 4 mg/ml (lower traces) in buffer A plus 0.3 mM $ZnCl_2$ was sedimented at 36,000 rpm at 5°C and photographed at a phase angle of 55° at 4(a) and 12 min(b) after reaching final speed. (B) Concentration dependence of the sedimentation coefficient of the 23 S microtubule protein oligomer. A solution of microtubule protein was prepared in buffer A plus 0.3 mM $ZnCl_2$ and high protein concentration. Dilutions from the concentrated stock were made just prior to analytical ultracentrifugation at 36,000 rpm at 5°C in double sector cells. Sedimentation coefficients were measured and corrected as described under Methods. Each point in the graph is the average of two experiments.

A



B

Sample	$ZnCl_2$(mM)	% Protein in oligomers	
		30S	other oligomers
1	—	40	—
2	0.1	18	—
3	0.2	13	14
4	0.3	—	40
5	0.4	—	35
6	0.6	—	—

Fig. 2. Tubulin oligomers found at different zinc concentrations. (A) Schlieren phtographs showing the sedimentation pattern of microtubule protein at different zinc concentrations. A solution of 4 mg/ml in buffer A plus 0.1 mM (a), 0.4 mM (b) and 0.6 mM $ZnCl_2$ (c) was sedimented at 36,000 rpm at 5°C and photographed at 4 (left) and 30 min (right) after reaching final speed. (B) Tubulin oligomers proportion in the presence of increasing zinc chloride concentration. Sedimentation profiles of microtubule protein (4 mg/ml at 5°C) in the presence of variable $ZnCl_2$ concentration were recorded in metallographic plates. These plates were examined on a microcomparator, and the areas under the peaks were estimated by planimetry. Due to the variation in the proportion of the peaks during the run, the values of the figure were obtained as follows. Photographs were taken every 2 min and the one with the highest oligomer proportion was used to calculate the values indicated in the figure. The protein solution volume placed in the ultracentrifuge cells was 0.45 ml in each case. Under "other oligomers" we have indicated the proportion of aggregates with a sedimentation coefficient higher than 6 S and different from 30 S; they are essentially oligomers with sedimentation coefficients of 23 S and 90 S. These oligomers were found at a concentration between 0.2 and 0.5 mM. 23 S was not always present and it disappears at a zinc concentration above 0.4 mM. In sample 3 only 90 S proportion was considered, because we could not distinguish between 30 S and 23 S. For concentrations equal and higher than 0.3 mM zinc, a protein concentration dependence experiment, as indicated in Figure 1B, was done to characterize 23 S oligomers.

results show a decrease of 30 S complex and an increase of the 23 S and 90 S oligomers in the presence of 0.2–0.4 mM zinc. The highest increase of zinc-dependent oligomers (23 S plus 90 S) was found at 0.3 mM zinc (see Fig 1) or 0.4 mM (Fig. 2); when zinc concentration was raised, the proportion of such oligomers decreases. At 0.6 mM zinc we found that tubulin polymerizes into irregular aggregates but not into regular sheets as indicated by Gaskin and Krees [17], and at such concentration no 23 S or 90 S oligomers were present in solution. On the other hand, 23 S oligomers were not always found at 0.3 mM, and they are usually missing at 0.4 mM $ZnCl_2$.

Influence of MAPs in Length and Width of Sheets

We have determined whether MAPs were present in the 90 S and 23 S zinc-oligomers by gel filtration on a controlled-pore glass or Sepharose 4B columns. Our results indicate that MAPs and tubulin are present in the void volume of both columns, suggesting that MAPs are components of such tubulin oligomers (data not shown).

In the presence of DNA the proportion of zinc-tubulin oligomers decreases (Fig. 3) and a possible conversion of 90 S into 23 S was seen (Fig. 3). At a ratio of 1:40 (w/w) of DNA to microtubule protein, the proportion of tubulin oligomers decreases from 40% (found in the absence of DNA) to 20% in the presence of nucleic acid (Fig. 3).

Since DNA binds to MAPs, but not to tubulin [26], we suggest that the presence of MAPs facilitates the zinc-tubulin oligomers formation. Depletion of MAPs results in longer and wider tubulin-sheets. As indicated above, MAPs facilitate tubulin aggregation and oligomer formation. This aggregation could result in the formation of nucleation centers needed for polymerization of sheets. Therefore, a removal of MAPs could produce longer structures as it happens in microtubule polymerization [9]. We have found that this is the case when we have blocked MAPs from microtubule protein solution by adding DNA. Figure 4, left, shows the length distribution of zinc tubulin-sheets polymerized in the presence or absence of DNA. The values in the figure indicate and average length of 0.3 μm for sheets polymerized in the absence of DNA, whereas the average length for sheets assembled in the presence of nucleic acid was 0.8 μm. This increase in length was smaller than the indicated for microtubules [9] suggesting a different role for MAPs in aggregation or stabilization of zinc tubulin-structures compared with their role in microtubule polymerization. Addition of DNA also results in wider sheets, as is indicated in Figure 4, right, that shows a slight but significant increase in the width of tubulin-sheets assembled after MAPs depletion.

Influence of Zinc:Tubulin Ratio on Length and Width of Tubulin-Sheets

The length and width of tubulin-sheets is determined by the zinc:tubulin ratio. The histograms in Figure 5 indicate that at low zinc:tubulin ratio, tubulin polymerization results in longer and narrower sheets than those polymerized at higher zinc:tubulin ratio.

To minimize the influence of MAPs in the polymerization process, we fixed a constant concentration of zinc in solution, and to increase the zinc:tubulin ratio, we did successive dilutions of microtubule protein. The histograms on the right of Figure 5 show that at high zinc:tubulin ratio, wider sheets are found. The average

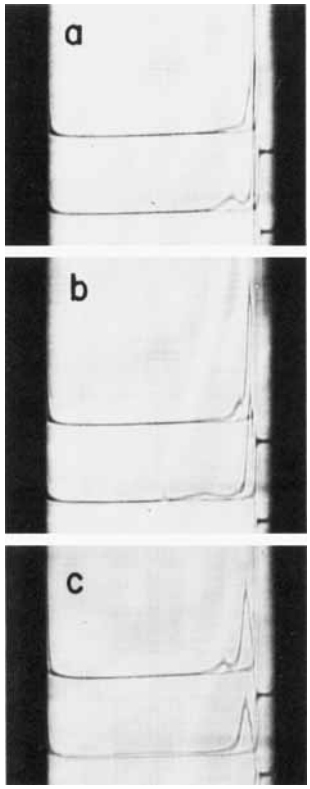


Fig. 3. Influence of DNA addition on the tubulin oligomer proportion found in the presence of zinc. Schlieren photographs shows the sedimentation pattern of microtubule protein (4 mg/ml at 5°C) in buffer A plus 0.3 mM $ZnCl_2$, in the absence (lower traces), or in the presence of poly dAdt (1:40 w/w to protein((upper traces). Because of the difference in the sedimentation coefficient of 90 S and 23 S oligomers and their lack of stability, we show the sedimentation pattern at different times. At early times only 90 S was found; however, at later times 23 S was observed. The photographs were taken at 4(a), 8(b) and 16 min(c) after reaching final speed.

width of sheet polymerized at 4 mg/ml in the presence of 0.3 mM zinc was 0.12 μm, whereas the average width for sheets assembled at 2 mg/ml and the same zinc concentration was 0.21 μm. This result suggests that zinc can promote lateral tubulin interaction. Similar results were described by Gaskin and Krees [17] but different findings were reported by Baker and Amos [19].

On the other hand, in this experiment we have also found that sheets polymerized at 2 mg/ml are shorter than the sheets assembled at 4 mg/ml. The average length was 0.24 and 0.52 μm, respectively.

The above results suggest that zinc could promote not only nucleation but possibly lateral tubulin-tubulin interaction. This possibility was confirmed when microtubule protein was assembled at a lower zinc:tubulin ratio. Figure 6 shows the structures polymerized in the absence of zinc (microtubules), low zinc (simultaneous appearance of sheets, microtubules, and structures composed of both microtubule and sheet), and high zinc (sheets).

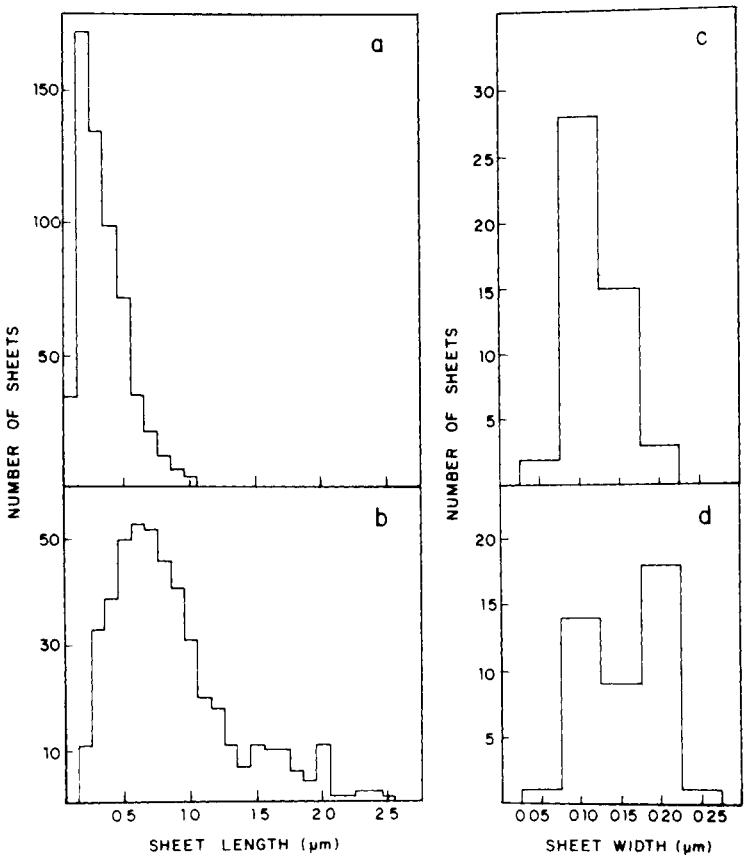


Fig. 4. Influence of DNA addition on the width and length of zinc tubulin-sheets. Left histograms show the length of sheets (4 mg/ml, 0.3 mM zinc) in the absence (a) and presence (b) of poly dAdT (100 μg/ml). Right histograms indicate the width of sheets (4 mg/ml of microtubule protein, 0.3 mM zinc chloride) in the absence (c) and presence (d) of poly dAdT (100 μg/ml). Polymerization was performed at room temperature and length determination was carried out as indicated under Methods.

Composite Tubulin Sheet-Microtubule Structures Obtained in the Presence of Zinc

These structures (about 5% of total polymerized material) are composed by a tubulin-sheet laterally bound to a microtubule protofilament. To test whether tubulin-sheets are linked to microtubule in these composite structures, we have performed optical diffraction studies on the composite structure. Figure 7 indicates the diffraction pattern of (a) open microtubules, (b) composite structures, and (c) zinc tubulin-sheets. As indicated in Figure 7, composite structures present diffraction spots characteristic from both microtubule and sheet structures. We have determined the localization of sheet and microtubule regions in the composite structure (Fig. 7b), by placing a mask in the zone of the picture which appears like a longitudinal prolongation of the microtubule structure (right side) and in the zone of the

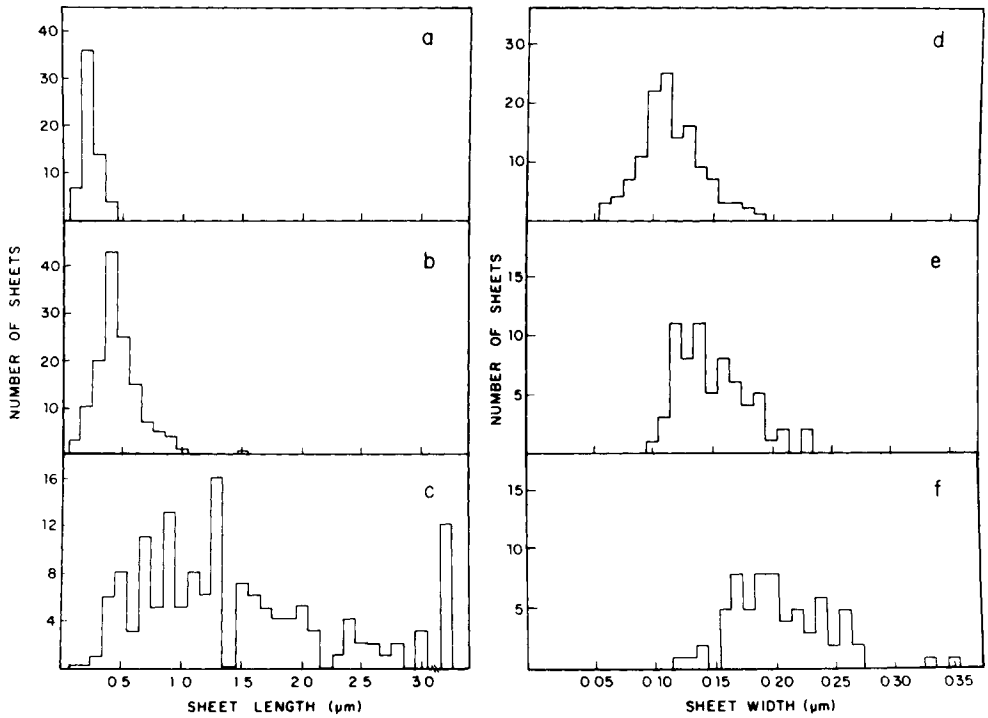


Fig. 5. Influence of zinc:tubulin ratio on tubulin-sheet width and length. Left histograms indicate the length of sheets, assembled at 4 mg/ml in buffer A, at 0.5 mM zinc (a), 0.25 mM zinc (b), and 0.1 mM zinc (c). Right histograms show the width of sheets assembled in buffer A plus 0.3 mM $ZnCl_2$ at 4 (d), 3 (e), and 2 mg of tubulin/ml (f). The width and length of polymerized sheets was measured as indicated under Methods.

picture which appears like a lateral addition to the polymer (left side). The results obtained indicate a diffraction pattern for the left side identical to the pattern on Figure 7c, whereas the right side shows a pattern identical to Figure 7a.

The barrier between microtubule and zinc-sheet structures is a protofilament. Image filtration of the area with both types of structures, using a composite mask containing holes for the spots of both types of diffraction patterns, results in the image shown in Figure 8. In our analysis we cannot distinguish the differences between both tubulin subunits because the low porportion of composite structures makes difficult the analysis of the sample using a low dose technique in the electron microscope [18]. However, Figure 8 indicates that the transition between both structures is a protofilament, at least in the 10% of the length of the structure in which the analysis was done. This result indicates that zinc promotes lateral interaction between dimers, even if they already belong to a previously assembled structure.

Nucleotide Binding in the Presence of Zinc

The lateral tubulin-tubulin interaction promoted by zinc is different from that of microtubules [18,19]. However, the longitudinal tubulin-tubulin interaction,

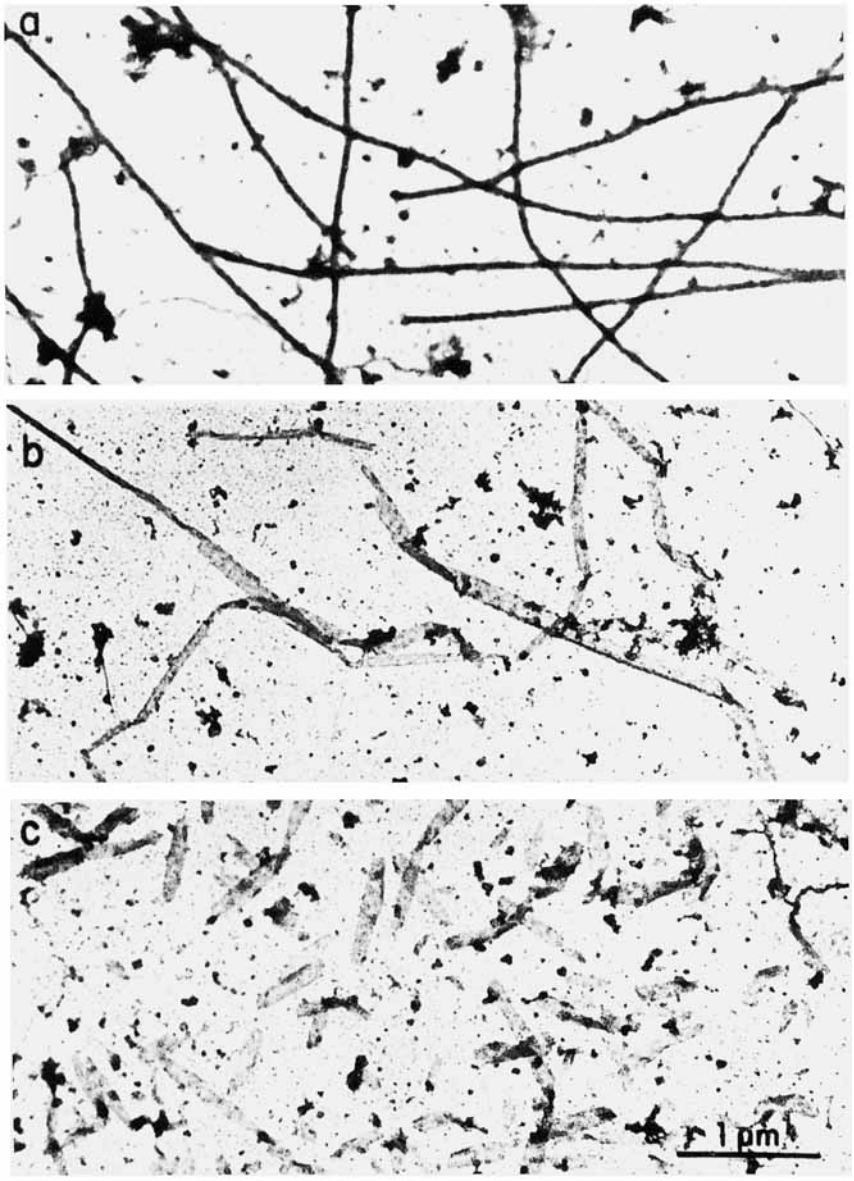


Fig. 6 Structures polymerized in the presence of variable zinc concentrations The figure indicates the structure polymerized in buffer A at 4 mg/ml and room temperature, in the absence of zinc (a), microtubules; at 0.1 mM zinc chloride (b), composite structures together with microtubules and long sheets; and at 0 5 mM zinc chloride (c), short sheets

which results in a protofilament, appears similar for zinc tubulin-sheets and microtubules [18,19]. Tubulin shows two binding sites for GTP, one exchangeable and the other nonexchangeable. The exchangeable one becomes nonexchangeable upon polymerization into microtubules [27]. We have tested whether it also happens when tubulin polymerizes into zinc tubulin-sheets.

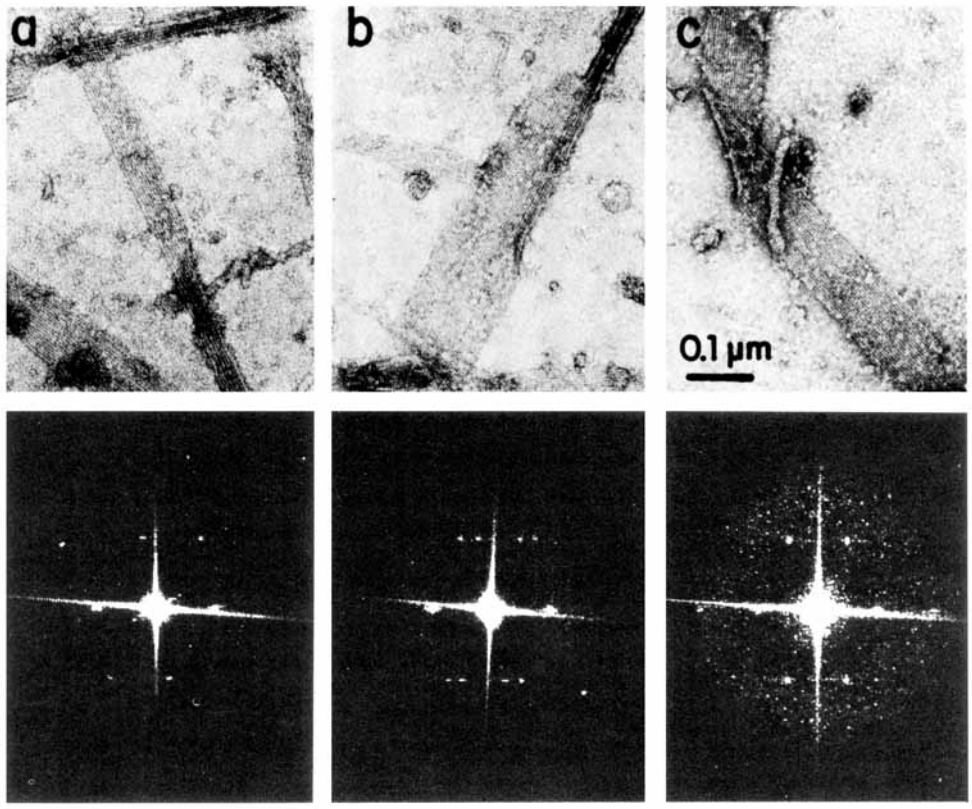


Fig 7 Optical diffraction patterns of the structure polymerized in the presence of variable zinc concentrations The structures obtained, (a) open microtubules, (b) composite microtubule-sheets, and (c) zinc tubulin-sheets (Fig 6), were photographed and micrograph plates were examined in an optical diffractometer as indicated under Methods

After adding increasing concentrations of $ZnCl_2$ to a microtubule protein solution, in the presence of $^{65}ZnCl_2$ and ^{3}H-GTP, polymerization was promoted by raising the temperature of the solution. The assembled protein was pelleted by centrifugation, and the binding of the labeled compounds was measured in the polymer. We have found an incorporation of GTP, in the absence of $ZnCl_2$, of 0.6 mol GTP/mol tubulin. Such incorporation remains at 0.15 mM $ZnCl_2$ (0.5 mol GTP/mol tubulin) under the conditions in which microtubules, zinc tubulin-sheets, and composite structures are present. However, at 0.3 mM $ZnCl_2$, a concentration in which only zinc tubulin-sheets are found, the GTP bound to the polymerized structures was only 0.1 mol GTP/mol tubulin. Simultaneously with the decrease of GTP binding, an increase in the amount of zinc bound to the polymer was found (0.4 mol zinc/mol tubulin at 0.15 mM $ZnCl_2$ and 1,4 mol zinc/mol tubulin at 0.3 mM $ZnCl_2$). From these results, we suggest that either (a) the exchangeable site of tubulin becomes nonexchangeable in microtubules due to the lateral interaction of tubulin (different in sheets and microtubules) but not due to the longitudinal interaction of tubulin (similar in both structures) or (b) the addition of zinc results in a decrease of

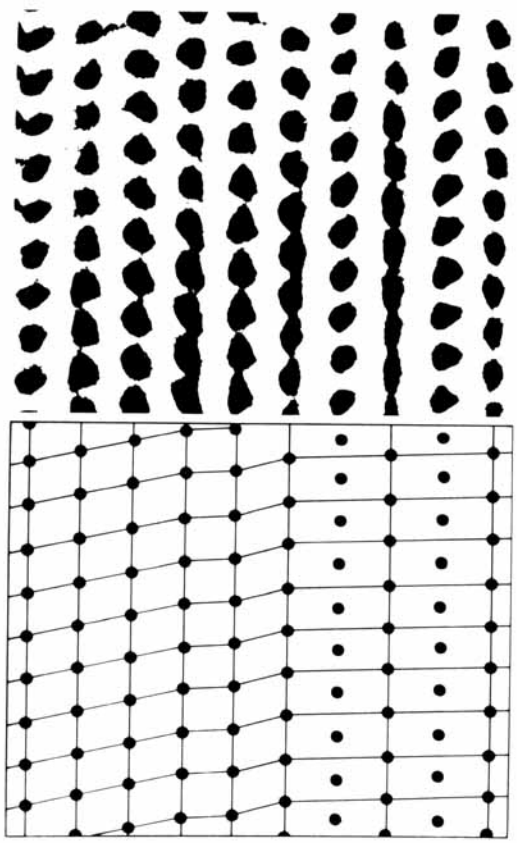


Fig. 8. Image filtration of the microtubule-sheet composite structure. From the diffraction patterns of Figure 7 image filtration was achieved as indicated under Methods. In the filtered image the tubulin subunits are represented as black spots (upper panel). Lower panel shows a diagrammatic representation of the image, in this case tubulin subunits are indicated as black dots. Microtubule structure is shown on the left of the figure and zinc tubulin-sheets on the right.

GTP requirement to tubulin polymerization. In fact, Gaskin and Krees [17] have indicated that zinc tubulin-sheets could be found, occasionally, in the absence of GTP together with other wider sheets that they named sheets II.

DISCUSSION

In this report we have studied the factors that can determine the length and width of a rectangular structure like a tubulin-sheet. Along the length of a tubulin structure, tubulin dimers interact longitudinally resulting in a protofilament. These protofilaments are present in microtubules and zinc tubulin-sheets but their lateral arrangement, perpendicular to longitudinal interaction, differs in both structures and this lateral interaction is the main difference between microtubule and zinc-sheet structures, as previously indicated [18,19]. Then, if an oligomer facilitates the polymerization in either case and these aggregates determine, somehow, the final shape of the structures, such oligomers must be different for each case. The stabil-

ity, variability, and proportion of such oligomers can be correlated with the constancy of the structure shape and the final length of the polymer. Our results indicate that oligomers found in the presence of zinc are less stable and different from the oligomers present when that cation is missing. When zinc is not present a 30 S complex was found, as reported by several authors [13,28]. Whether this oligomer is an obligatory precursor in microtubule initiation has been discussed [8,15], and it appears that is not an obligatory intermediate in the assembly process [8,14,15,20, 29], and probably it could be only an abortive association complex [14]. However, such oligomers are tubulin aggregates and such tubulin aggregation could facilitate the initial step for microtubule polymerization. Whenever the 30 S complex was found, microtubules were polymerized. Only when zinc concentration was increased, the 30 S oligomers were missing, new oligomers (90 S and 23 S) were present, and the assembled structures were exclusively zinc tubulin-sheets. In the presence of zinc, depletion of MAPs results in a decrease in the proportion of oligomers in solution. A possible consequence of this change is an increase in the length of the zinc tubulin-sheets following a mechanism similar to the indicated for microtubules [7–9].

When microtubule protein concentration was decreased under conditions in which the influence of MAPs and tubulin in protein aggregation decreases and, therefore, the relative influence of zinc in such aggregation increases, wider structures were found. It indicates that zinc promotes lateral tubulin binding. This result was confirmed in experiments in which we found that tubulin polymers can grow laterally on the edge of microtubules, the transition between both structures being a protofilament. Such structures composed by an open microtubule and a zinc tubulin-sheet (composite sheets), could be related to those found by Mandelkow and Mandelkow [30] in the absence of that cation and at a lower protein concentration.

Our results suggest that MAPs can promote tubulin aggregation into tubulin-sheets and somehow are involved in the final protofilament number of the polymer. The interaction between adjacent protofilaments appears determined by factors present in the assembly mixtures such as ions and nucleotides. For microtubule polymerization, Carlier and Pantaloni [31] have indicated the role of magnesium in lateral tubulin-tubulin interaction. Zinc promotes a lateral subunit interaction different to that of magnesium [18,19]. In our study we have found that in zinc tubulin-sheets, zinc is involved in a lateral tubulin interaction. However, longitudinal tubulin binding is similar in microtubules and zinc induced sheets, both composed of protofilaments. Protofilaments in zinc tubulin-sheets are formed in the presence or absence of GTP, as indicated by Gaskin and Krees [17], or in tubulin polymers in the presence (sheets) or absence (microtubules) of zinc. Therefore, the final shape of a tubulin structure will mainly depend on its lateral interaction in which different ions could be implicated and that could be followed by the retention or not of GTP. In this way we like to point out that the exchangeable site of tubulin for GTP becomes not exchangeable in 30 S oligomers or microtubules [27], suggesting a similar tubulin interaction in both structures, whereas our preliminary results suggest that GTP is not incorporated or it remains exchangeable into the oligomers found in the presence of zinc, in a similar way to that of zinc tubulin-sheets. This result agrees with the report of Gaskin [32] indicating that zinc induced structures are not due to a zinc-GTP complex.

In conclusion our results indicate that: (a) the oligomers found in a cold microtubule solution in the absence of zinc are different from those present when zinc was added; (b) increasing concentrations of zinc result in wider and shorter sheets; (c) the presence of MAPs produces the opposite effect of zinc addition, with respect to the width of tubulin-sheets, suggesting a role for these proteins in lateral tubulin-tubulin interaction and probably an implication in determining the final shape of tubulin structures, as previously suggested by Amos for microtubules [11]; (d) zinc promotes a form of lateral tubulin interaction that can give rise to the growth of a tubulin-sheet on a microtubule protofilament; and (e) the exchangeable site for GTP becomes nonexchangeable in microtubles but not in zinc tubulin-sheet, probably due to the lateral interaction of tubulin that is different in both structures.

ACKNOWLEDGMENTS

We thank Professor Viñuela for advice, Dr. Carrascosa for helpful suggestions, and Dr. Ramírez for critical reading of the manuscript.

We are indebted to J.A. Muñoz for analytical ultracentrifuge experiments. This work was supported by grants from the Fondo Nacional de Investigaciones Sanitarias and Comisión Asesora para el Desarrollo de la Investigación Científica y Técnica.

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