Journal of Supramolecular Structure 10:419–431 (1979)

Tubulin Rings: Curved Filaments With Limited Flexibility and Two Modes of Association

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Tubulin rings have been previously identified as composed of linear polymers of tubulin subunits, equivalent to a protofilament in the microtubule wall but in a curved rather than a straight conformation. We have examined and measured a number of different ring structures obtained under different conditions. The preferred curvature is indicated by a single ring of 380 Å outside diameter. Radially double rings consist of two coplanar rings of 460 Å and 350 Å outside diameter, held together by a pattern of eight identical contacts between the 40 Å subunits in the inner and outer rings. In some circumstances a larger ring, 570 Å diameter, can be added to the outside, or a smaller ring, 240 Å diameter, may be added to the inside of the radially double ring, in both cases repeating the pattern of eight radial contacts. The distortion of the filament from its relaxed 380 Å diameter curvature apparently can be made without disrupting the longitudinal bond between subunits in the filament, but must be stabilized by the energy of the radial contacts. All of these rings (single and radially double and triple) are observed to associate axially to form pairs or in some cases larger stacks. The radially double rings or an axially associated pair of these (quadruple ring) may also associate to form crystals. These are thin plates, up to 100 μm in extent and several μm thick which have been of limited use so far in diffraction studies because of irregularities in the packing of adjacent rings.

Key words: microtubules, assembly, protein–protein interactions, electron microscopy

Microtubules can be assembled from the protein subunits, tubulin, in a variety of solution conditions. In some solutions microtubule associated proteins (MAPs) or polycationic factors are incorporated into the microtubules and are required for assembly [1–5]. In other solution conditions microtubules can be assembled from purified subunits [6–8].

The tubulin subunits can also assemble to form a variety of small polymers, most of which have been identified as circular or ring-shaped by electron microscopy [9–14].

Received January 12, 1979; accepted April 2, 1979.

0091-7419/79/1004-0419$02.60

These tubulin rings are normally obtained under solution conditions in which microtubules are disassembled. When conditions are changed to favor microtubule assembly, the rings disappear as microtubules form [13, 15]. At least under certain conditions the ring-shaped polymers have been observed by electron microscopy to straighten out and be incorporated into the growing microtubule as a protofilament [10, 12]. The rings have thus been identified as linear polymers of tubulin subunits, equivalent to a protofilament of the microtubule wall, but uniformly curved when released from bonding to neighboring protofilaments. The proposal that these rings or a shorter segment of a protofilament are intermediates in microtubule assembly has been reviewed recently [16].

A number of significantly different ring-like structures have been described by different laboratories. These include single closed circles, double rings (a small ring inside a larger one), planar spirals, stacks of rings, and helices or coils. Different dimensions have been reported by different laboratories for apparently similar structures. We report here a structural analysis of rings formed by purified tubulin in the presence of magnesium. These illustrate the architectural features upon which all of the various ring forms may be based: curved filaments of limited flexibility, producing rings of different diameter, which can associate by either radial or axial contact between subunits. These rings are compared with those obtained under different conditions of assembly.

MATERIALS AND METHODS

The standard buffer used for isolation and purification of microtubule protein was 50 mM MES [2(N-morpholino) ethane sulfonic acid], 1 mM EGTA [ethyleneglycol-bis-(β-aminoethyl ether)-N,N′-tetra-acetic acid], 0.5 mM $MgSO_4$. The procedure for the first cycle of assembly of microtubules from pig brain homogenate has been described [5] and is a slight modification of the method of Shelanski et al [17]. For observation of rings from glycerol-free tubulin, protein was purified from pig brains in the complete absence of glycerol as described by Borisy et al [18]. The microtubules from the second cycle of assembly were chilled to 0°C for 30 min to produce rings. Negatively stained specimens were made in the cold after centrifugation at 0°C to pellet any large aggregates of protein.

Purification of tubulin by chromatography on phosphocellulose was essentially as described previously [4, 5], except that the 0.05 M MES buffer described above was used for application and elution of the tubulin. In most experiments the tubulin was chromatographed on phosphocellulose after one cycle of assembly from the brain homogenate. The purified tubulin was used immediately or was stored frozen after addition of glycerol to 3.4 M. For some purposes the tubulin was concentrated using an Amicon PM-30 membrane in a stirred pressure cell.

The quadruple rings identified and discussed below were prepared by adding magnesium chloride or magnesium sulfate to a concentration between 5 and 21 mM to phosphocellulose purified tubulin at a concentration of 1–5 mg/ml or more. The MES buffer contained 0.1–1.0 mM GTP but no glycerol. The solution was warmed to 37°C, and negatively stained specimens were made after different time intervals. The best specimens of rings were obtained after 5–60 min. Crystals of tubulin rings were obtained in similar preparations after 30 min–4 h at 37°C. Solution conditions favoring crystallization are described under Results.

Triple rings were formed after pelleting crystals of tubulin rings by centrifugation and resuspending them in MES buffer containing low magnesium. The solution was then put in the cold for several hours. After a clarification centrifugation, magnesium was

added to 15 mM. The solution was then warmed to 37°C, and negatively stained specimens were made after 15–30 min.

A Philips EM-300 was used for electron microscopy. Magnification was calibrated accurately by recording catalase crystal images as the first and last of each set of plates [19]. Two percent uranyl acetate was used for negatively stained specimens.

RESULTS

Rings Induced by Magnesium

Figure 1 shows several examples of the rings formed from purified tubulin in the presence of 5–25 mM magnesium. These are observed in two orientations: either in axial projection (face view) or in edge view (looking in a direction perpendicular to the axis). The most commonly found images are the axial projections seen in Figure 1 a–d, which show two concentric rings. Edge views were found primarily in areas where the negative stain was relatively thick. Most of the edge views showed two parallel lines, as in Figure 1 e–g. Occasionally edge views were found showing only a single element, as in Figure 1 h and i.

Our identification of these as perpendicular views of the same structure is supported by the fact that the outside diameter of the face view is almost identical to the total length of the edge view. Furthermore, the bright patches at the ends of the edge view correspond to the total radial thickness of the double ring in the face view. This evidence strongly implies that these structures are quadruple rings, ie, two-layer stacks, each layer consisting of two concentric, coplanar rings.

As shown in Figure 1d, rings often are seen in lightly stained areas to have a beaded or subunit structure. Normally, this subunit structure could be seen only over a short length of the inner or outer ring, but this was often sufficient to measure the angle subtended, at the ring center, by adjacent subunits. These measurements were made where strings of 4–8 contiguous subunits could be resolved. The mean angle subtended per subunit was $14.1^\circ \pm 0.7^\circ$ for the inner ring and $10.4^\circ \pm 1.4^\circ$ for the outer ring. Dividing these figures into 360° yields 25.5 and 34.6 subunits for the inner and outer rings, respectively. Similar values, 15.1° and 11.3°, were found by measuring the magnesium-induced rings in the image published by Frigon et al [20].

Rings that appear in face view to be composed of three concentric rings (Fig. 2) have been formed from crystallized tubulin, as described in Methods. As was the case for the quadruple rings described above, these oligomers appear to be two layers thick (actually a sextuple ring) (Fig. 2 c and d). The lengths of the edge views are in good agreement with the diameters measured in axial projections. The outer diameter of the middle ring of the radially triple ring structures is identical to that of the outer ring of the radially double ring (Table I). The radial thicknesses per ring, as well as the axial thickness or height, was essentially identical for the radially double and radially triple rings. The triple ring thus appears to be formed by addition of a third ring (or axial pair of rings) to the radially double ring.

Rings Induced by DEAE Dextran

The polycation diethylaminoethyl (DEAE) dextran, at concentrations of around 0.05 mg/ml, can induce purified tubulin to form a variety of ring structures [21]. One of the more common appears in axial projection to be a single ring with a thin wall. No

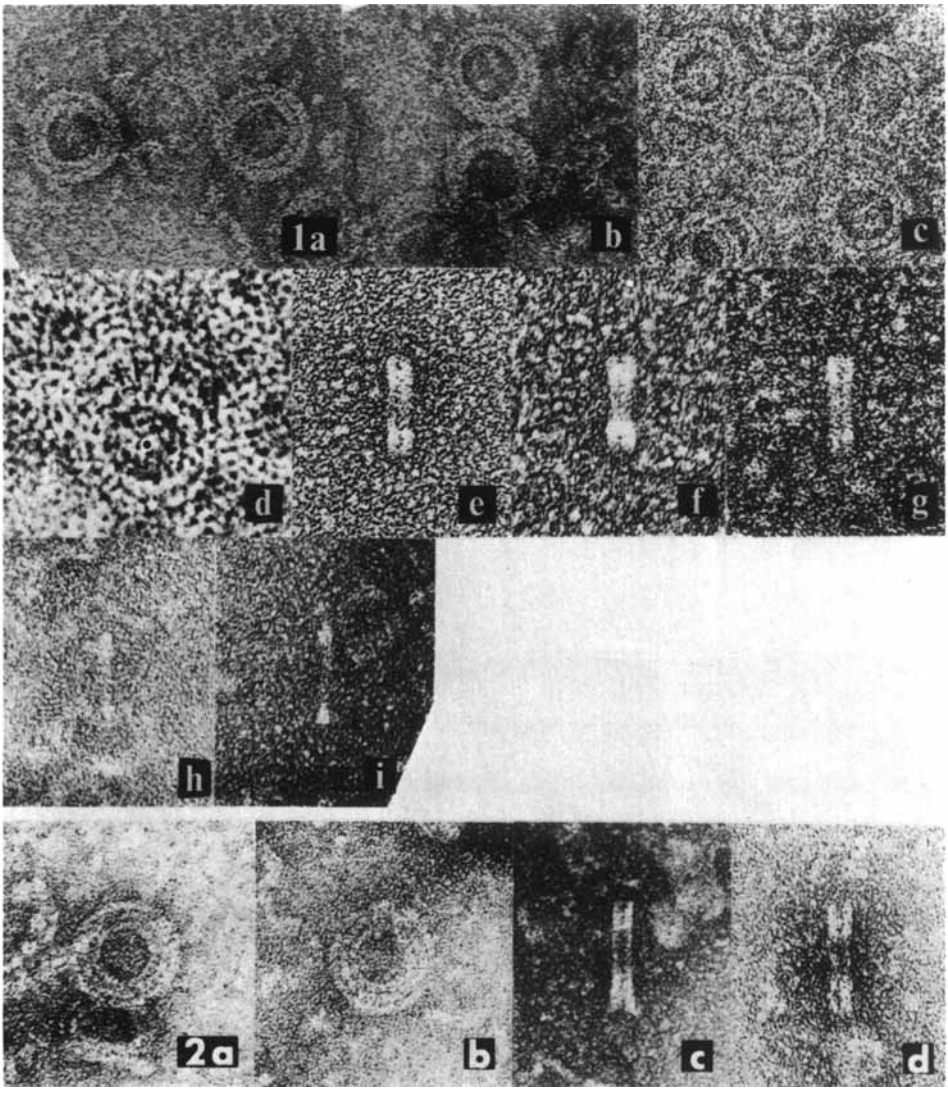

Fig. 1. a, b) Rings assembled from phosphocellulose-purified tubulin (8 mg/ml) in the presence of 13.5 mM $MgCl_2$ (magnification, 275,000 ×). c) Axial views of typical radially double rings are shown, as well as examples of a single-stranded version which appear to be about the same size as the outer ring of the more common double variety ($MgCl_2$ was 21 mM and specimen was made after 10 min at 37°C; protein concentration was 4 mg/ml; magnification, 275,000 ×). d) A radially double ring found in an area of light stain showing the subunit structure in the inner ring (magnification, 410,000 ×). e, f, g) Edge views of rings in a region of thick stain. These appear to be two-layer stacks (18 or 21 mM $MgCl_2$, 37° for 6 min; protein concentration was 4 mg/ml; magnification, 275,000 ×). h, i) Edge views of single-layered rings (conditions as in a, b; magnification, 250,000 ×).

Fig. 2. a, b) Radially triple rings, assembled from redissolved tubulin crystals in 15 mM magnesium (protein concentration was 1.5 mg/ml). c, d) Edge views of radially triple rings, from the same specimens as a and b. These appear to be two-layer stacks, like most of the radially double rings (magnification, 250,000 ×).

TABLE I. Dimensions of the Various Types of Tubulin Rings*

Ring structure					
Radial	Axial	Protein and solution		Outside diameter (Å)	Radial thickness (Å)
Double	Double	Tubulin and magnesium	Outer	459 ± 11	55
			Inner	349	55
Triple	Double	Tubulin and magnesium	Outer	569 ± 15	54.5
			Middle	460 ± 11	54.5
			Inner	351 ± 8	50
Single	Stack or helix	Tubulin and DEAE dextran		384 ± 17	61 ± 8
Thick wall	?	Tubulin and MAPs		441 ± 31	102 ± 16
Thin wall	?			429 ± 24	73 ± 8
Thick wall	?	Tubulin (glycerol free [18])		461 ± 31	102 ± 21
Thin wall	?	and MAPs		402 ± 20	60 ± 6

*Either 10 or 12 samples of each type of ring were measured, and standard deviations are given. Radial thicknesses were calculated from the measured outside and inside diameters.

double rings are found in face views. The diameter of 384 ± 17 Å is intermediate between that of the inner and outer rings of the radially double ring described above. Examples of the DEAE dextran-induced rings are shown in Figure 3.

A prominent feature of the accompanying micrographs is the variability of the contrast of axial views of the rings. This is probably due to variations in the axial length or height, which implies that the more contrasty rings are actually helices or stacks of rings. Edge views of such structures may be found as shown in Figure 3b. These coils or helices are similar to those reported by Erickson [12] and by Borisy et al [18] in preparations of tubulin plus MAPs. The average axial filament thickness is 57 Å, based on measurements of two such structures.

Rings Induced by MAPs

Rings formed in solutions containing MAPs and tubulin are basically similar to those described above, but they show some definite differences. Examples of the various rings are illustrated in Figure 4. They were more irregular in appearance than those described above and often appeared somewhat decorated with irregular adhering material. Most of these rings could be classified as one of two types: thick- and thin-walled varieties. The thin-walled rings were quite similar to those induced by DEAE dextran, but they measured about 40 Å larger in outside diameter and had a slightly thicker wall (Table I).

The thick-walled rings have about the same outside diameter as the radially double rings and have a wall thickness about twice that of the single filament seen in other ring forms. Occasionally a cleft is seen dividing the thick wall into an inner and outer filament, but more commonly the structure is irregular and no clear division is seen. Nevertheless, the simplest interpretation is that these are, in fact, radially double rings, but that the division between the inner and outer rings is obscured, perhaps by the MAPs.

Changing the pH of the MES buffer from 6.5 to 7.2 had little effect on the appearance or dimensions of the MAP-tubulin rings. Nearly all the rings from both pH values could be classified as either thick- or thin-walled, and in each category (thick- and thin-walled) the differences in size between pH 6.5 and 7.2 were considerably smaller than the

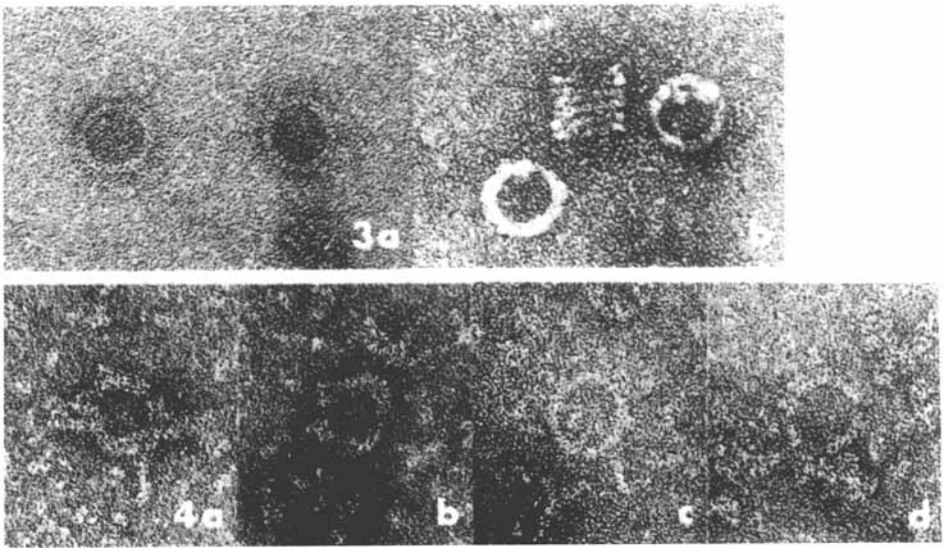

Fig. 3. Rings assembled from purified tubulin after addition of 0.05 mg/ml DEAE dextran (protein concentration was 0.25 mg/ml; magnification, 250,000 ×). a) Axial view. b) A stack of rings or possibly a helical coil lying horizontally on the carbon film.

Fig. 4. Microtubules were purified by two cycles of assembly in buffer at pH 6.5 containing glycerol. After a third cycle of assembly in the absence of glycerol, rings were induced by cooling the solution to 0°C for 35 min (magnification, 250,000 ×). a, b) Examples of the thick-walled rings seen both at pH 6.5 + pH 7.2. c, d) Examples of the thin-walled rings, also seen at both pHs.

standard deviation values. In each case the majority of rings were thick-walled. Rings made from tubulin purified by the method of Borisy et al [18] in the complete absence of glycerol were also indistinguishable from those made with tubulin prepared with glycerol (Table I).

The measurements of our non-glycerol tubulin rings may be compared to those presented by Vallee and Borisy [23] in their study of microtubule protein oligomers. They found an outside diameter of 405 ± 31 Å, virtually identical to that of our thin-walled rings (402 ± 20 Å), but the wall thickness we measured (60 ± 6 Å) was somewhat greater than theirs (48 ± 13 Å). One notable difference in our results is that nearly all of their rings were thin-walled, whereas we found a considerable number (one-third to two-thirds of the total) of the thick-walled species.

Crystals of Tubulin Rings

Many of the same preparations that gave the magnesium-induced quadruple rings produced crystals of these rings after 1–4 h of incubation at 37°C. Crystallization was favored by higher concentrations of magnesium (15–20 mM) and tubulin (> 2 mg/ml). Crystallization was enhanced by but did not require 0.1 mM colchicine, and 0.5–2.0 mM GTP or GDP. In many cases crystals could be obtained at 20°C. These were usually more regular but were smaller and the total yield was less.

Some of the cleanest preparations in terms of regularity of crystal shape and absence of amorphous precipitates were obtained by slowly dialyzing magnesium into a solution of tubulin at 4°C. For this a solution of tubulin (5–10 mg/ml) in 0.05 M MES,

0.5 mM GTP, was placed in a dialysis bag and left overnight without agitation in the same buffer containing 15 mM magnesium. The heavy white precipitate that formed displayed a pronounced "silky" turbidity when agitated, and light microscopy showed thin plates about 1 μm thick and 10–20 μm wide. This procedure for producing crystals is very similar to that reported by Weisenberg and Timasheff [24] to produce a precipitate that displayed a similar "silky turbidity." The "fibers" that these authors identified in this preparation were probably edge views of the crystals.

That we do not fully understand the conditions necessary for crystal formation is emphasized by the fact that our recent attempts to reproduce the crystals in the above solutions have failed. Crystals were finally obtained in 25 mM MES, 30–45 mM $MgSO_4$, pH 6.75, after several hours at 37°C.

The crystals could be partially solubilized by resuspending the pellet from a low-speed centrifugation in cold buffer with low magnesium. After several hours on ice about half of the protein remained as a precipitate, but the supernatant obtained after centrifugation contained active tubulin. Microtubules could be reassembled from this soluble tubulin at 37°C after addition of either DEAE dextran or 15 mM magnesium plus 3.4 M glycerol. Curiously, this tubulin showed a much stronger tendency to form rings than did the tubulin before exposure to magnesium and crystallization. The radially triple rings in Figure 2 were obtained from a supernatant of dissolved crystals.

Figure 5 shows a collection of crystals as seen in the light microscope. The crystals are thin plates, and in different preparations they varied from 1–20 μm in thickness and

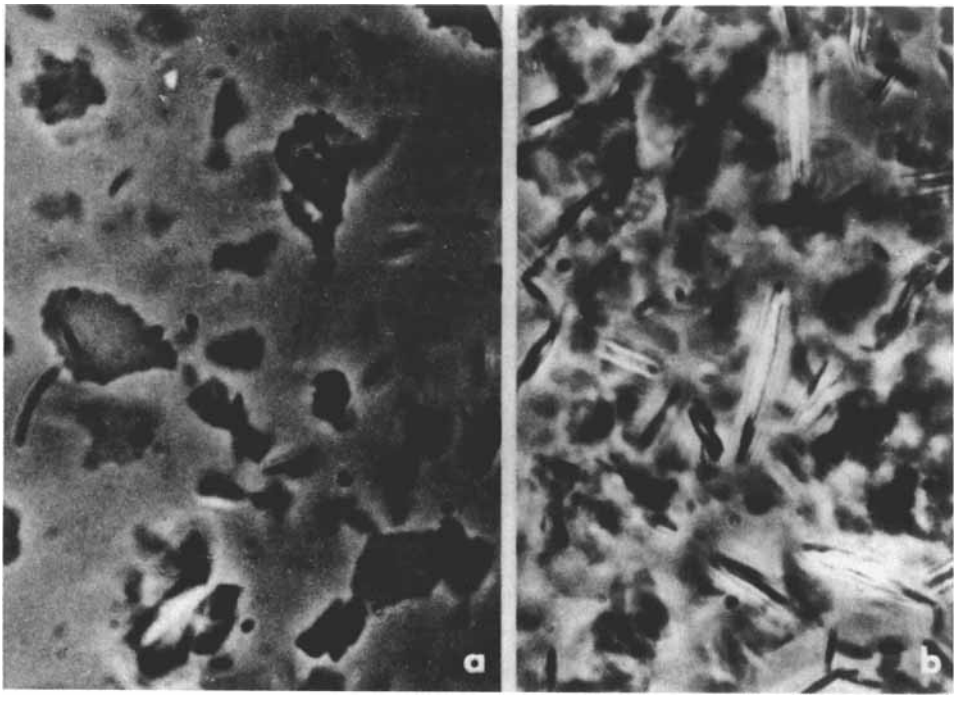

Fig. 5. Light microscope views of crystals of tubulin rings using phase contrast optics. The crystals appear in many different shapes depending on temperature and buffer conditions (magnification, 1,500 ×). a) A field of crystals oriented with the flat sides parallel to the surface of the slide. b) Several edge views of the thin crystals showing the highly refractile appearance.

20–100 μm in width. The plates generally had an irregular outline when viewed face on (Fig. 5a). When viewed edge on the crystals were highly refractile in phase contrast and showed birefringence in polarized light.

Small crystals can be visualized in negatively stained specimens prepared at early times in the crystallization. Before the crystals appear, large numbers of radially double rings may be found in well-stained areas (Fig. 6). At somewhat later times small crystals are seen (Fig. 7). Radially double rings may be seen both free and associated with these crystals, which appear to be composed of interlocking cylindrical stacks of rings. At later times much larger plates may be found (Fig. 8), and free rings are rare.

The lattice in the broad plane of the crystal appears to be hexagonal, with each ring surrounded by six nearest neighbors, but a closer examination shows that the contacts or association with these neighbors are of two types, giving a tetragonal lattice. The contact with four of the neighbors is characterized by a bright bar (black arrow, Fig. 7), which we interpret as an overlap of the outer rings. The center-to-center spacing of rings

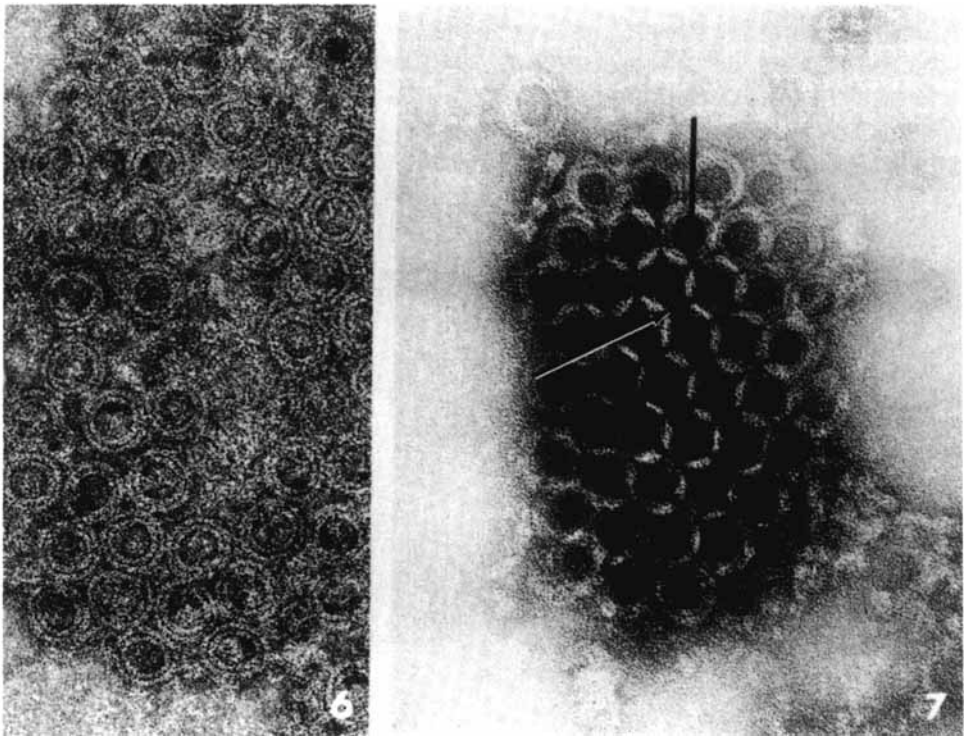

Fig. 6. Rings induced by the experimental conditions for crystal growth. Specimen was made after 10 min at 37°C. The $MgCl_2$ concentration was 21 mM and the protein concentration was 4 mg/ml (magnification, 175,000 ×).

Fig. 7. A small ring crystal showing the characteristic overlap zones as lightly stained regions on the circumference of some of the rings in the center of the structure. The black arrow indicates an overlap between two rings, and the other arrow indicates the non-overlapping contact or near contact between two rings. Two free rings showing the typical radial doubling can be seen above the crystal. Just below the left-hand one are one or two rings in edge view. The specimen was made after 16 min at 37°C ($MgCl_2$ concentration, 21 mM; protein concentration, 4 mg/ml; (magnification, 175,000 ×).

connected by the overlap is about 410–420 Å. The other two neighboring rings are farther apart, about 460 Å center to center, and there is no apparent overlap. The overlap contacts usually occur in pairs directly opposite each other. Thus, in the crystal shown in Figure 8 virtually all of the contacts between rings in the vertical direction are of the overlap type and the rings are aligned in a straight row. The second pair of overlap contacts forms a line at an angle of 67.8° to this vertical row. (Actual measurements gave values

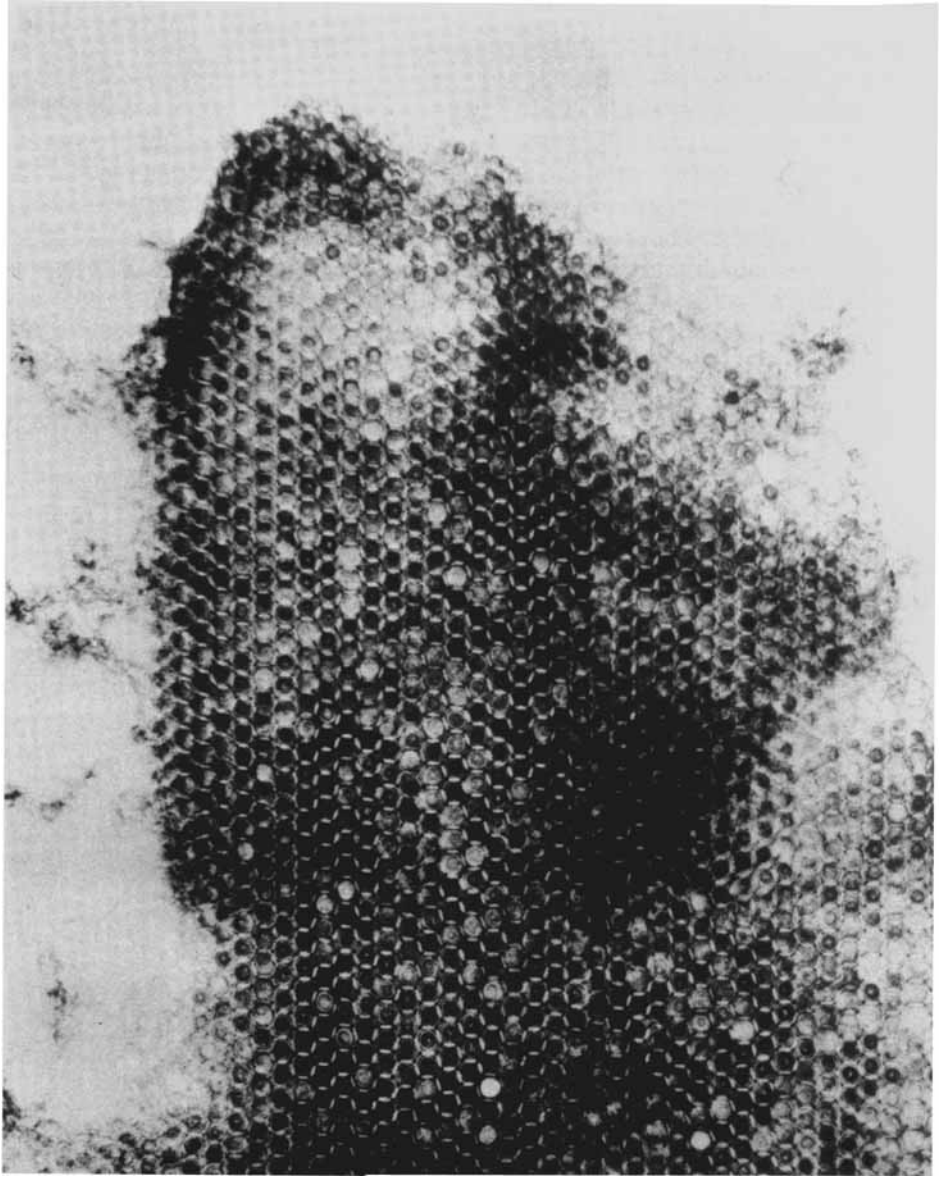

Fig. 8. An example of a very large, thin crystal grown at 0°C. The protein concentration was 8–10 mg/ml. In the vertical direction adjacent rings are all connected by overlaps and form straight rows. Alignment in the other directions is irregular as the second overlap occurs capriciously on one side or the other (magnification, ~75,000 ×).

from 65° to 70°; the value of 67.8° is consistent with the model (Fig. 9), in which the outer ring has 32 subunits, the second pair of overlap contacts occurring six subunits around the ring from the first.) An irregularity is introduced in the lattice, however, since this second pair of overlaps can apparently be formed in the clockwise or anticlockwise direction from the first pair. The rows of rings in these two directions are consequently crooked, as the overlap alternates from one side to the other.

DISCUSSION

The Identity of the Rings

The basic structural element of all of these ring forms is a 55 Å thick curved filament, which has been identified in previous studies as equivalent to the protofilament in the microtubule wall. The continuity of the straight protofilament in the wall with the curved filament of a ring is clearly seen in images of microtubules in the process of disassembly [25] and in reassembly from a fraction enriched for rings [12]. Another example, which has largely escaped attention since it was published before the tubulin rings were established as important in the in vitro assembly experiments, is in the work of Warner and Satir [26], showing outer doublet microtubules disintegrating under the action of phosphotungstate. Here, as in the later images, it appears that the filament is under some strain when it is straightened out in the microtubule wall. When it is released from the bonds attaching it to neighboring filaments it relaxes into the curved conformation, which appears to be energetically favorable for the isolated filament [16].

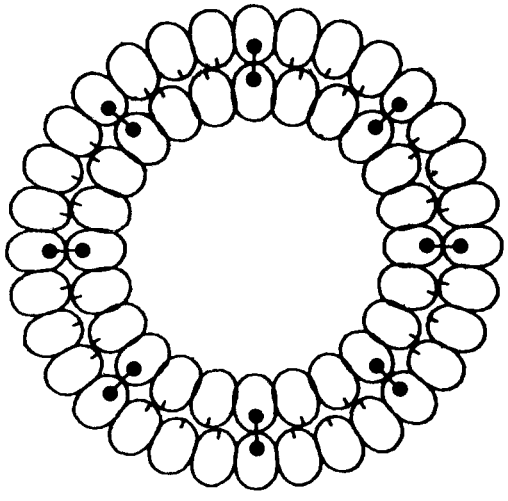

Fig. 9. A model for the radially double ring, showing the radial contacts between subunits in the inner and outer rings. The outer ring (460 Å outside diameter) contains 32 and the inner ring (350 Å outside diameter) 24 of the 40 × 55 Å subunits. A favorable contact (black circles) will be repeated eight times, between every fourth subunit in the outer and every third subunit in the inner rings. If alternate subunits around each ring are identified as alpha and beta tubulin, four of the radial bonds must be between homologous subunits (say, alpha–alpha), and the other four between heterologous subunits (alpha–beta). A larger ring of 40 subunits could be added on the outside, or a smaller ring of 16 subunits on the inside, in each case repeating the pattern of eight radial bonds.

Scheele and Borisy [22] have suggested that the ring might be identified with a string of subunits that forms an eight start helix in the intact microtubule. The rationale for this hypothesis was the need to account for the stoichiometry of MAPs to tubulin, which is about twice as high in rings as it is in microtubules. The proposal is indeed consistent with the lattice of attachment sites for MAPs proposed by Amos [27], but it is not the only way to account for the enrichment of MAPs attached to rings. For example, if the binding site for the MAPs in the microtubule were in the groove between two protofilaments, the isolated filament in the form of a single ring might bind MAPs on both sides. These would be shared with the adjacent protofilaments in the microtubule, so that the stoichoimetry of MAPs to tubulin in the rings would be twice that in microtubules. Arguments along this line are necessarily speculative, however, since so little is actually known about the structure and arrangement of the MAPs. The electron microscopy showing the continuity of the rings with protofilaments is, we believe, the most substantial and reliable evidence for the identity of the rings.

The sedimentation coefficient may be calculated for any of the ring models using the theory of Kirkwood [28], and calculations of this type have already been presented by several laboratories [14, 29, 30]. It is found that the sedimentation coefficient of a single ring, containing about 30 of the 55,000 mol wt subunits, should be about 20S, and that of a double ring about 40S. There is little difference between the sedimentation of the axially and radially double rings. A quadruple ring should have a sedimentation coefficient of 70–80S. Experimentally, values of about 20S [30, 31] and 36–42S [14, 23, 29] have been observed in cases where the predominant species were thought to be single and double rings, respectively. Higher sedimentation values, which could correspond to the quadruple rings, have not been reported, even though the conditions under which Frigon and Timasheff [14] obtained the value of 42S are close to those in which we observed the quadruple rings by electron microscopy It may be that one of the two types of association is very pressure sensitive and therefore destroyed in the centrifugation, or that there are differences in the preparation that we do not yet understand.

The curved filament appears to be rather stiff, since the curvature is smooth and well defined even when it is not closed to form an intact ring [10, 12, 26]. Nevertheless, there is sufficient flexibility to permit the formation of rings whose outside diameters vary from 350 Å to 460 Å in the radially double rings, and occasionally 570 Å in the radially triple rings. If the interface between the subunits in the filament spans a distance of 20–30 Å in the radial direction the change in curvature from small to large rings could be effected with a maximum displacement of atoms at this interface of about 1 Å. This is within the range of movements commonly observed within protein molecules, so this variation in diameter can be attributed to the normal flexibility of the protein molecules. The transformation from the curved to the straight protofilament, on the other hand, must involve a much more significant rearrangement in the packing of atoms at this interface or at another hinge region, and is therefore considered to be a major conformational change.

The Bonding Patterns Between Filaments in Multiple Rings

The curved protofilaments associate and stick to each other in two distinct modes to produce rings that are doubled either by axial contact (a stack of two rings, each of the same diameter) or by radial contact (a small ring inside a larger one). It is reasonable to suppose that the bonds holding the rings together are highly specific complementary patches on the subunit surfaces [32]. The axial association is then easily understandable,

since any contact between subunits on the two rings will be repeated exactly for all subunits in the ring. Thus, even a weak interaction between individual subunits will be multiplied by the number of subunits in the ring to produce a strong association of the complete rings.

The radial association is more difficult to rationalize. The identification of the rings as protofilaments, as well as our measurements of the angular spacing of the subunits, suggests that the subunits are about 40 Å apart in both the inner and outer rings. Since the inner ring contains fewer subunits than the outer, the radial contacts between subunits in the two rings cannot be identical for all subunits.

An attractive model that provides a rational pattern of repeating bonds in the radially double ring can be constructed if the number of subunits is 32 in the outer ring and 24 in the inner ring (Fig. 9). In this case every third subunit in the inner ring will be in identical register with every fourth subunit in the outer ring, and a favorable radial contact between subunits will be repeated eight times. A radially triple ring could be constructed by adding a ring with 40 subunits on the outside. Every fifth subunit of the ring would be in register to make to favorable contact with every fourth subunit of the middle ring. A smaller ring containing 16 subunits could be placed inside the double ring, also repeating the pattern of eight contacts. Examples of this have been seen occasionally in crystals (Pantaloni, Edelstein and Fram, personal communication, and our own observations).

The strongest argument in support of this model is that it is the only arrangement consistent with the measured angular separation of subunits and diameter of the rings that provides a number of identical radial contacts between subunits in the inner and outer rings. The values of 34 and 26 subunits obtained directly from the angular measurements are within experimental error of the 32 and 24 subunits required for the model. The 40 Å separation of subunits that is found in protofilaments in the microtubule wall would occur at diameters of 407 Å and 307 Å – ie, approximately in the middle of the 55 Å thick filament of the outer and inner rings. It should be noted that if alternate subunits on the filaments are identified as alpha and beta tubulin, four of the radial contacts must be between like subunits, say alpha–alpha, and the other four between unlike subunits, alpha–beta. The logic of the model suggests that the regions of contact are identical or very similar in the two types of subunits, so that all eight of the in-register radial contacts contribute to the bond energy holding the two rings together.

Solution Conditions Favoring Rings or Microtubules

It is interesting to note that the solution conditions in which we obtain the rings and crystals of rings are very similar to those that favor assembly of microtubules from purified tubulin. A higher concentration of sulfonic acid buffer (0.1 M PIPES (piperazine-N-N′-bis(2-ethane sulfonic acid)] instead of 0.05 M MES [8], or addition of 3.4 M glycerol [6], or 10% dimethyl sulfoxide [7], would result in the tubulin forming microtubules instead of rings. GTP, which is normally required for microtubule assembly, is apparently not essential for formation of rings or crystals. Colchicine, which is a powerful inhibitor of microtubule assembly, does not block assembly of rings and actually enhances the formation of crystals.

The control mechanism that determines which form is assembled may involve the relative strength of interfilament association (axial and radial contacts in rings, and lateral bonds between protofilaments in the microtubule wall) or the preference for the curved over the straight conformation specified by the intrafilament (longitudinal bond) contacts. Thus, GTP might act either by promoting a change from the curved to the straight conformation of the filament or by enhancing the formation of lateral bonds. Colchicine

could block microtubule assembly either by locking the filament into the curved conformation or by blocking lateral bond formation.

ACKNOWLEDGMENTS

We are grateul to Mr. Burton Scott for assistance with the experimental work. Dr. Erickson gratefully acknowledges the hospitality and collaboration of Dr. D. Pantaloni, Laboratoire d'Enzymologie, Gif-sur-Yvette, France, in the initial phase of this work and in the discovery of conditions for obtaining the crystals of tubulin rings. This work has been supported by National Institutes of Health Program Project 9 P01 GM NS 23911 and NSF grant #PCM 7723592.

Note added in proof: Recently we have discovered buffer conditions (purified tubulin, 10 mM $MgSO_4$ and 30% glycerol or 50% sucrose) that enhance the formation of radially double rings that are usually not axially doubled. Such rings may correspond to the 42S species seen in sedimentation studies [14].

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