The Non-tubulin Component of Microtubule Protein Oligomers

EFFECT ON SELF ASSOCIATION AND HYDRODYNAMIC PROPERTIES*

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We have investigated the association of non-tubulin microtubule proteins with tubulin to form the ring-shaped oligomeric structures found in microtubule preparations. We have found that the two oligomeric species present in our preparations of microtubule protein (s_{20,w} = 18 and 30 S) each require non-tubulin factors for their formation. Two types of non-tubulin protein, the high molecular weight proteins (HMW) and the tau proteins, were found to be active in ring formation. The HMW proteins promoted the formation of the 30 S oligomer, while the tau proteins promoted the formation of an oligomer of s_{20,w} = 20 S. Analysis of the 30 S oligomer by gel filtration chromatography showed that the ratio of HMW proteins to tubulin was about twice that in the microtubule. The HMW proteins could be destroyed by exposure to trypsin, resulting in a marked increase in the sedimentation coefficient of the 30 S oligomer to 39 S. The 20, 30, and 39 S species were identified as rings by electron microscopy. The identity of the 18 S oligomer was once again brought into question. Our data indicate that the 20 S species is a single ring and that the 30 S oligomer is a two-layered ring bearing HMW projections which contribute substantial hydrodynamic drag to the particle. We compare the organization of tubulin subunits and HMW molecules in the 20 S ring with the organization of these components in the microtubule and suggest that the organization in the ring is conserved in the microtubule.

Two types of components identified in preparations of microtubule protein purified from brain tissue have been implicated in the control of microtubule assembly. The presence of ring-shaped tubulin-containing oligomers in samples of depolymerized microtubules has been correlated with the capacity for efficient microtubule assembly (Borisy and Olmsted, 1972; Kirchner and Williams, 1974). Assembly in vitro has also been found to be controlled by the presence of non-tubulin protein components in microtubule preparations. At least two classes of non-tubulin species have been identified, the high molecular weight proteins (Murphy and Borisy, 1975) of subunit molecular weight 271,000 to 346,000 (Borisy et al., 1975), which are the predominant non-tubulin components in our preparations, and tau, a group of proteins of M_r ~ 60,000 to 70,000 (Weingarten et al., 1975; Penningroth et al., 1976) prominent in microtubule protein prepared by the method of Shelanski et al. (1973). The non-tubulin factors were found to stimulate both the initiation of microtubule assembly as well as the elongation phase of microtubule growth (Sh lokale et al., 1976; Murphy et al., 1977). The latter effect appears to be due to the stabilization of internuclear bonds along the entire length of the microtubule (Murphy et al., 1977b), and, indeed, the distribution of the high molecular weight non-tubulin component, observed as a lateral projection, over the entire surface of the microtubule has been directly demonstrated by electron microscopy (Murphy and Borisy, 1975; Dentler et al., 1976; Amos, 1977).

The precise role for the non-tubulin components in the initiation of microtubule assembly is not known. These components have been shown to promote the formation of the ring-shaped tubulin oligomers (Murphy and Borisy, 1975; Weingarten et al., 1975; Keates and Hall, 1975), suggesting a structural as well as functional relationship between these two elements. The incorporation of non-tubulin components into rings is also suggested by structural studies of the tubulin-containing oligomers. Our preparations of microtubule protein are characterized by the presence of oligomers of sedimentation coefficient 18.6 S and 30 S (Marcum and Borisy, 1978). Theoretical calculations indicated that the sedimentation coefficient of the 30 S species was inconsistent with values for ring-shaped structures composed of tubulin alone, suggesting that the 30 S oligomer might contain additional protein components (Marcum and Borisy, 1978). We report here the results of an investigation into the assembly and structure of the 18 S and 30 S oligomers with particular emphasis on the role of the non-tubulin factors. These investigations have revealed the HMW proteins to be an integral component of the 30 S ring, the more prominent oligomeric species present...
under solvent conditions that favor microtubule assembly. With new information regarding the effect of the HMW proteins on the hydrodynamic properties of the 30 S oligomer, we present a model for its structure and for the possible relationship of this structure to that of the microtubule.

EXPERIMENTAL PROCEDURES

Procedures for Preparing Protein - Microtubule protein was purified by the reversible assembly method described by Borisy et al. (1975). The protein was stored frozen at -80° as the pellet of microtubules obtained after two cycles of assembly and disassembly and was prepared for use by a third cycle of assembly and disassembly in a polymerization buffer consisting of 0.1 M Pipes (pH 6.94 at 25°), 0.1 mM MgSO₄, and 1.0 mM GTP. Conditions for sedimenting microtubules have been described by Johnson and Borisy (1975).

A non-tubulin protein fraction was separated from tubulin by ion exchange chromatography as previously described (Murphy et al., 1977a). Microtubules (50 to 100 mg) were sedimented after the third polymerization step, depolymerized without resuspension by addition of NaCl to 0.25 M at 0°, and applied in this condition to a column (1.5 x 2.3 cm) of DEAE-Sephadex A-50 pre-equilibrated with the polymerization buffer described above supplemented with 0.25 M NaCl. Under these conditions the non-tubulin proteins are bound to the column and are eluted virtually without dilution at a concentration of 5 to 7 mg/ml. The resulting fraction typically consisted of 65% proteins of high molecular weight, 10% tubulin, 10% tau protein, 5% non-tau proteins (Weingarten et al., 1975), and 10% other bands found in trace amounts in microtubule preparations (Murphy et al., 1977a). Tubulin was eluted from the column at 0.5 M NaCl in the polymerization buffer. The tubulin obtained by this procedure was >99% pure and was incapable of polymerizing without added factors (Murphy et al., 1977a). The non-tubulin and tubulin fractions were further prepared for use by passage over columns of Sephadex G-25 pre-equilibrated with the buffer to be used in the ensuing experiment.

Subfractionation of the nontubulin components to isolate HMW and tau proteins was accomplished as described (Murphy et al., 1977a) by chromatography of the nontubulin ion exchange fraction on Bio-Gel A-15m. (Bio-Rad, Richmond, Calif.).

Proteolytic Modification - Microtubules devoid of their lateral projections were prepared by limited exposure to trypsin (Vallee and Borisy, 1977). When required, the modified microtubules were sedimented and subjected to a further cycle of assembly and disassembly to remove unbound protein fragments.

Analytical Ultracentrifugation - Analytical ultracentrifugation was performed out with a Beckman Spinco model E analytical ultracentrifuge equipped with an electronic speed control. Schlieren optics were used to monitor sedimentation, and sedimentation coefficients were derived from the rate of migration of boundary maxima. The positions of the boundary maxima were determined from photographs of schlieren patterns with the use of a Gaertner microcomputer (Gaertner Scientific Corp., Chicago, Ill.). Sedimentation data were fitted by a least squares method to a straight line determined by Equation 1 (Schachman, 1959)

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s = \frac{c (\lambda - \alpha)}{1 - gc}
\]

where c is concentration in mg/ml. The parameter \(g\) reflects the extent of deviation from ideal solution conditions and is strongly affected by deviation in shape of the sedimenting particle from spherical symmetry (Creeth and Knight, 1965). The relative concentrations of species observed in the ultracentrifuge were estimated from the areas under the peaks, determined by planimetry, and were corrected for radial dilution.

Electrophoresis - Electrophoresis in the presence of sodium dodecyl sulfate was carried out according to the procedure of Shapiro et al. (1967). Samples were run in 7% polyacrylamide gel plates, stained with Coomassie brilliant blue R according to the procedure of Fairbanks et al. (1971). Destaining in a diffusion destainer was minimized (~24 h) to prevent loss of stain from minor bands. Gels were scanned at 570 nm in a spectrophotometer and the area under each peak was determined by weighing. Protein was applied to the gel in the range of concentrations that has been found to give a linear response of stain intensity to band size (Borisy et al., 1976).

Electron Microscopy - Negative staining of oligomers was performed in general by brief adsorption of the sample in the experimental buffer at the experimental temperature to a carbon and Formvar-coated grid. The sample was placed with a solution of polyt (lysine) (Mazia et al., 1974) for 1 min were used when it was desired to ensure quantitative adsorption of oligomers. Other modifications are noted in the text. Magnification was determined with the use of a carbon replica grating.

Protein Determination - Protein determination was accomplished using the method of Lowry et al. (1951) as modified by Schachterle and Pollack (1973).

RESULTS

Requirement for Non-tubulin Factors in Oligomer Formation - The starting material in these experiments was microtubule protein purified by the procedure of Borisy et al. (1975). This material has been characterized previously by analytical ultracentrifugation at 5° as a mixture of 6 S tubulin dimers and oligomeric species of 18.6 S and 30.6 S (Marcum and Borisy, 1978).

To examine the requirement for cofactors in the formation of the oligomers, microtubule protein was separated into two fractions by ion exchange chromatography on DEAE-Sephadex. One fraction, containing virtually all of the nontubulin electrophoretic species observed in the unfractionated protein, was eluted unbound from the column. A second fraction consisting of 99% pure tubulin was eluted by increasing the concentration of NaCl to 0.5 M as reported previously (Murphy et al., 1977a). Neither fraction showed the presence of rings in the electron microscope under solution conditions where rings were found in great numbers in the unfractionated protein. The two fractions were examined for the presence of oligomeric components by analytical ultracentrifugation, and Schlieren patterns from this experiment are shown in Fig. 1.

Both samples were observed to be devoid of material sedimenting at a position corresponding to the 18.6 or 30.6 S oligomers. The tubulin fraction alone showed a single peak sedimenting at 6.2 S, corresponding to the tubulin dimer (Weisenberg et al., 1968). The non-tubulin fraction showed a major peak which was found to have a sedimentation coefficient in the range 3.4 to 3.8 S in a series of experiments performed over a range of concentration from 1 to 5 mg/ml. A more rapidly sedimenting shoulder may also be observed, which has been seen as a peak sedimenting at 6.5 to 8 S in preparations other than that shown here.
Upon recombination of the tubulin and non-tubulin fractions, numerous rings were observed by electron microscopy and oligomeric species were observed by analytical ultracentrifugation. The effect of an increasing amount of the non-tubulin fraction on formation of the oligomeric species is shown in Fig. 2a. It can be seen that two rapidly sedimenting peaks are formed as the result of addition of the non-tubulin fraction to the purified tubulin. Determination of the sedimentation coefficients of the two rapidly sedimenting species showed these species to correspond to the 18 S and 30 S oligomers. Thus, it is clear that the formation of both the 18 S and 30 S species is stimulated by the addition of the non-tubulin components.

To obtain a quantitative estimate of the effect of the non-tubulin proteins on ring formation, the areas of the subunit and oligomer peaks were determined and are plotted in Fig. 2b as a function of the concentration of non-tubulin proteins added to the purified tubulin. It may be seen that the total amount of oligomer showed an almost linear dependence on the concentration of non-tubulin proteins. At low concentrations of non-tubulin fraction the 18 S species predominated, while in the higher range of concentration examined, the 30 S oligomer became the more prominent species and itself increased with added non-tubulin protein. The subunit peak, which represents 6 S tubulin dimers as well as non-tubulin proteins not incorporated into the oligomer species, remained almost constant, indicating that the depletion in 6 S tubulin was almost offset by an increase in noninteracting material of low sedimentation coefficient with increasing non-tubulin protein. We estimate the activity of the non-tubulin proteins to be approximately 75% of that in unfractionated microtubule protein.

Although it appeared that the concentration of the 30 S oligomer was dependent on the concentration of non-tubulin factors, a similar conclusion could not be drawn from the results of Fig. 2 for the 18 S species. To determine the effect of the non-tubulin proteins on 18 S oligomer formation in the absence of the 30 S species, a reconstitution experiment was carried out under conditions of ionic strength and pH where only the 18 S and 6 S peaks were observed (Marcum and Borisy, 1978). Fig. 3a shows a schlieren pattern from this experiment, and Fig. 3b shows a plot of the concentrations of each species as a function of the amount of non-tubulin protein added: □, low S; ○, 30 S; ●, 18 S species; ▼, total oligomer. The low S peak runs at about 6 S and corresponds to the tubulin dimer plus non-tubulin protein not incorporated into rings. Buffer and centrifugation conditions as in Fig. 1.

**Enhancement of Oligomer Formation in Unfractionated Microtubule Protein**—The results of Marcum and Borisy (1978) indicated that unfractonated preparations of microtubule protein were limited in the amount of oligomeric species that could be formed. In view of the dependence of oligomer formation on non-tubulin factors, we considered it of interest to determine whether oligomer formation would be enhanced by the addition of the non-tubulin fraction to unfractionated microtubule protein. It was found that addition to unfractionated material of the nontubulin proteins at a concentration resulting in a 60% increase in the amount of HMW led to a 50% enhancement of the 30 S concentration. This result indicates that the level of oligomer formed from microtubule protein is indeed normally limited by the amount of nontubulin protein present despite the adequacy of the normal complement of nontubulin protein for quantitative microtubule formation (Murphy et al., 1977b).

**Subfractionation of Non-tubulin Proteins and Stimulation of Oligomer Formation by Purified Factors**—The most prominent electrophoretic species other than tubulin in our preparations are the HMW proteins. It has been shown that these proteins stimulate microtubule assembly and are responsible for approximately 60% of the stimulatory activity in our preparations (Murphy et al., 1977a). A number of less prominent species are also seen in gel electrophoretic patterns of microtubule protein and were found to account for the remainder of the assembly-promoting activity. The most prominent of these species are a group at Mr. = 65,000 to 80,000, which appear to correspond to the bands referred to as tau in other laboratories (Weingarten et al., 1976; Penningroth et al., 1978).
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1976). In view of the variety of factors present in microtubule preparations we examined the relative ability of the factors to promote ring formation.

The non-tubulin protein fraction was subfractionated by chromatography on Bio-Gel A-15m as described previously (Murphy et al., 1977a). Protein from the major peak was characterized by a partition coefficient, \( K_m = 0.34 \) and corresponded to the peak position of the major HMW doublet, whereas protein from the position at \( K_p = 0.66 \) corresponded to the tau peptides. Samples of these proteins were respectively recombined with purified tubulin and the samples examined for oligomeric species by electron microscopy and analytical ultracentrifugation. Both samples showed the presence of rings in the electron microscope, but because of the low concentrations of non-tubulin factor available from the column, and perhaps because of loss of activity of the factors during preparation, only small oligomer peaks were discernible in the ultracentrifuge.

In order to concentrate the recombined protein samples as well as to select for active protein, the reconstitution experiment was repeated with the following variations. Dithiothreitol was included in all buffers beginning with the ion exchange separation of tubulin from the non-tubulin components in an attempt to preserve activity. After combination with purified tubulin of protein from the HMW- and tau-containing fractions of the A-15m column, the samples were incubated at 37°C for 0.5 h to allow microtubules to form. The microtubules were collected by sedimentation at 37°C, resuspended and depolymerized at 0°C, and any remaining aggregates were removed by centrifugation at 4°C. The final ratios of HMW and tau to tubulin in the reconstituted samples were found by sodium dodecyl sulfate-gel electrophoresis to be, respectively, 0.76 and 1.2 times that found in unfractionated microtubule protein (see also data in Table II).

Both samples, HMW + tubulin and tau + tubulin, now showed numerous rings in the electron microscope. The samples were examined in the ultracentrifuge at a series of concentrations. Fig. 4 shows schlieren patterns from this experiment along with sedimentation coefficients (Fig. 4a) and mass fractions (Fig. 4b) of the oligomeric components. At all concentrations examined, the samples showed a single peak in the ultracentrifuge in addition to the 6 S peak (schlieren pattern insets). This is in contrast to the result obtained with microtubule protein not subjected to purification beyond that obtained with the method of reversible reconstitution.
polymerization (cf. Fig. 1, Marcum and Borisy, 1978). In the latter case, two oligomer peaks were observed under the buffer conditions employed in the present experiment, a peak of $s_{20,w} = 30.6 \pm 1.4$ at concentrations below approximately 3.5 mg/ml, and an additional peak of $s_{20,w} = 18.6 \pm 0.5$ at concentrations above this value. The single oligomer peak obtained with the purified HMW + tubulin samples was found to have an $s_{20,w} = 31.4$ S with the parameter $g$ (Equation 1) = 0.058 ml/mg. The oligomer peak obtained with the purified tau + tubulin samples was found to have an $s_{20,w} = 205$ with $g = 0.016$ ml/mg. It appeared, therefore, that HMW was responsible for stimulating the formation of the 30 S oligomer, whereas tau stimulated the formation of a more slowly sedimenting oligomer.

Is tau-induced 20 S Oligomer Identical to 18 S Oligomer Present in Preparations of Unfractionated Microtubule Protein?—The results of the previous section suggested an apparently simple explanation for the presence of two oligomeric species in preparations of unfractionated microtubule protein; namely, that 18 S particles contained tau, whereas the 30 S particles contained HMW. However, this explanation was not sufficient to account for all of the data. First, the sedimentation coefficient of the tau-induced species differed somewhat from that of the 18 S oligomer found in unfractionated microtubule protein. Second, the tau-induced species was present at quite low protein concentrations (Fig. 4b) in contrast to the 18 S species, which was not observed at concentrations below approximately 3.5 mg/ml. Finally, we found that with increasing ionic strength (up to 25 mM NaCl added; cf. Marcum and Borisy, 1978) the 30 S species formed from tubulin and HMW (Fig. 4c) gave rise to a second oligomeric species of sedimentation coefficient between 6 S and 30 S. Thus, it appears that a species probably equivalent to the 18 S oligomer may be formed in the absence of tau from HMW and tubulin alone. Therefore, the contributions of the non-tubulin proteins in promoting oligomer formation are not simply additive, and the relationship between the various oligomeric species may be complex. The possibility of structural differences between the 18 S and tau-induced 20 S oligomers is discussed below.

Composition of 30 S Oligomer—To determine the composition of the oligomers directly, we attempted to isolate the oligomeric component of microtubule protein by chromatography on agarose A-15m (Weingarten et al., 1974). To determine the protein composition of the 30 S oligomer species, we subjected microtubule protein to column chromatography on Bio-Gel A-15m employing solution conditions (Marcum and Borisy, 1978) under which only the 6 S and 30 S species were seen in the ultracentrifuge over a wide range of protein concentration (0 to 12 mg/ml). Microtubules purified by three cycles of polymerization and depolymerization were depolymerized at 0°C, and aggregated protein was removed by centrifugation at 4°C. The resulting preparation was applied at a concentration of 0.1 mg/ml to Bio-Gel A-15m, pre-equilibrated with 0.050 M Pipes, pH 7.41, containing 0.1 mM MgSO$_4$, and 0.1 mM GTP, at 4°C. Under these conditions, microtubule protein showed a single 30 S oligomer peak in the ultracentrifuge. (a) Absorbance at 280 nm, showing two protein peaks (I and 2), and a GTP peak; (b) tubulin; (c) the major HMW bands at $M_r = 271,000$ and 286,000; (d) the minor HMW bands at $M_r = 345,000$, derived from peak areas of densitometric tracings of gels of the column fractions stained with Coomassie brilliant blue R in the range of linear stain/protein response (Borisy et al., 1975); $V_0$, void volume. b, cylindrical polyacrylamide gels (3.7% acrylamide) of the column fractions for which densitometric data is shown in a. Also shown is a gel of the sample (S) before application to the column. Values for $M_r$ are in thousands.

Electrophoretic patterns of the column fractions are shown in Fig. 5b. The concentrations of the major electrophoretic species were determined for each column fraction by densitometry and are plotted in Fig. 5c. Tubulin, the predominant electrophoretic species, was present in both chromatographic peaks. Peak 2 consisted of essentially pure tubulin, although some bands of molecular weight in the range 65,000 to 80,000 were also visible, eluting slightly in advance of the tubulin peak. Peak 1 consisted of tubulin and non-tubulin components. Both major groups of HMW bands were found in this peak and were present in a nearly constant ratio to tubulin throughout the peak. The average values for the mass ratio of the major HMW bands (HMW 1 and 2, Murphy et al., 1977a) to tubulin, from measurements of four peak fractions was found to be 0.60. Using a value for the molecular weight of the major HMW bands of 286,000, the molecular weight of the more prominent band (Borisy et al., 1975), and a molecular weight for the tubulin dimer of 110,000 (Lee et al., 1973), we arrived at a molar ratio of 0.23 HMW peptide chains per tubulin dimer. For microtubule protein not subjected to chromatography, the value for the mass ratio of the HMW doublet...
to tubulin was 0.26, corresponding to a molar ratio of 0.10 HMW peptide chains per tubulin dimer. Thus, the oligomer peak, peak 1, was enriched by a value somewhat greater than 2 (2.3) in the ratio of the major HMW bands to tubulin. For the less prominent group of HMW bands with a major band at $M_r = 346,000$ (Borisy et al., 1975) the following values were obtained. In peak 1, the mass ratio to tubulin was 0.21 and the molar ratio was 0.065; in unfractonated microtubule protein the mass ratio was 0.073 and the molar ratio was 0.023. This represents an enrichment in the minor HMW bands in peak 1. All of the HMW proteins together were enriched 2.4-fold in peak 1 versus unfractonated protein.

The absolute value obtained in this experiment for the ratio of HMW to tubulin in unfractonated microtubule protein was at the upper end of a range of values that we obtain from gel electrophoresis of microtubule samples. In a separate experiment, the mass ratio of HMW to tubulin in the microtubute was lower, 0.25 versus 0.33 in the experiment just described. Nevertheless, the enrichment of the HMW proteins in peak 1 was about the same in the two determinations, 2.1-fold versus 2.4-fold, as described above. Thus, it appears that relative concentration values determined in a given experiment are of greater reliability than the absolute values derived from gel electrophoresis.

That the enrichment of HMW in peak 1 represents an intermolecular association between tubulin and the HMW proteins was indicated by the observation that the non-tubulin proteins eluted at different positions in 30 S-containing samples and in samples in which the 30 S oligomer was depolymerized by an increase in ionic strength. The elution positions of the non-tubulin proteins were similarly changed when the non-tubulin fraction alone was subjected to chromatography (see previous section and Murphy et al., 1977a). Thus, our results indicate that an oligomer containing HMW and tubulin at about twice the ratio of HMW to tubulin observed in the microtubule is present in samples characterized by the presence of the 30 S sedimentation species.

In addition to the HMW proteins, other minor species corresponding to a wide range of molecular weights were detected in peak 1. Only one of the tau bands, at $M_r = 75,000$, was enriched in peak 1. The other tau bands eluted at a position near peak 2, indicating that at least a portion of the tau proteins was not strongly associated with the 30 S oligomer. Preliminary experiments suggested that the band at $M_r = 75,000$ may represent a fragment of the HMW proteins, while the other tau bands do not.

Composition of 18 S Oligomer—We also attempted to determine the composition of the 18 S oligomer by chromatography on agarose A15m under conditions favoring this oligomeric form. The conditions employed were 0.05 M Pipes, pH 7.43, plus 60 to 80 mM NaCl, 0.1 mM MgSO4, 0.1 mM GTP (Marcum and Borisy, 1978). Analytical ultracentrifugation confirmed the existence of the 6 S and 18 S material. However, under these conditions, no oligomer peak was observed by chromatography. Inclusion of 2 mM CaCl2, or removal of GTP were found to enhance the extent of 18 S formation as determined by analytical ultracentrifugation as has been reported for 36 S ring formation (Weingarten et al., 1974) and, therefore, these modifications of the solution conditions were also used for chromatography. Under these conditions a skewing of the tubulin distribution toward elution positions in advance of the dimer was observed but a distinct oligomer peak was not seen.

* R. B. Vallee, unpublished data.

We attribute the failure of the 18 S material to show a separate peak by column chromatography to the weak association constant for this species that has also been evidenced by its behavior in the ultracentrifuge (Marcum and Borisy, 1978).

Alteration of Oligomer Properties by Exposure to Trypsin—The sedimentation coefficient obtained for the 30 S oligomer corresponds neither to that calculated for a single ring nor for a double ring composed solely of tubulin dimers, but, rather, is midway between these values (Marcum and Borisy, 1978). Our results indicate that the 30 S oligomer contains a significant proportion of a second protein component, HMW, in addition to tubulin. This component would be expected to have some influence on the sedimentation properties of the 30 S particle and might account for the failure of the measured sedimentation values to correspond to the calculated values. Recently, we have found that by controlled digestion with trypsin it is possible to remove 90% of the mass of the HMW molecule from the surface of the microtubule (Vallee and Borisy, 1977). This treatment results in the disappearance of the lateral projections from the microtubule surface but preserves the capacity for microtubule and ring formation. To determine the influence of the HMW proteins on the sedimentation properties of the oligomers, we examined the oligomers produced from microtubules exposed to trypsin.

Following the procedure we have previously described, microtubules were exposed to trypsin for periods of time sufficient to destroy the HMW electrophoretic bands partially or completely. After depolymerization at 0-4°C, samples were examined by analytical ultracentrifugation under solution conditions that normally favored the formation of the 30 S oligomer. Fig. 6 shows the effect of trypsin hydrolysis on the sedimentation properties of the 30 S oligomer. Exposure of microtubules to trypsin resulted in an increase in the sedimentation rate of the oligomer species present at 5°C as indicated by the photograph of the schlieren pattern of microtubule protein before (Fig. 6, bottom pattern) and after brief exposure to trypsin (Fig. 6, top pattern). More extensive exposure to trypsin resulted in a further increase in sedimentation rate which leveled off when the 11MW electrophoretic bands had been completely destroyed. The relative concentration of oligomer was observed to decline with increasing time of exposure to trypsin, consistent with the slow loss of microtubule assembly-promoting activity previously reported (Vallee and Borisy, 1977).

To determine the intrinsic sedimentation properties of the final oligomeric product containing no intact HMW peptides, microtubules that had been exposed to trypsin were sedimented and purified free of nonbinding protein fragments by a cycle of reversible disassembly and reassembly. The resulting preparation was examined in the ultracentrifuge at a series of concentrations and the sedimentation coefficients determined for the oligomer are plotted in Fig. 6 along with values for the 30 S species. $s_{20w}$, for the newly formed oligomer was found to be 39.0 ± 0.6 S, an increase of 27% relative to the 30 S particle. From the line fit to the sedimentation data we determined a value for $g$ (Equation 1) of 0.063 ml/mg for the 30 S oligomer (cf. Marcum and Borisy, 1978) and 0.008 ml/mg for the newly formed 39 S oligomer. Thus, it appears that, despite the mass of the HMW molecules, the net effect of these proteins is to decrease the rate of sedimentation of the tubulin oligomer. When the influence of the HMW proteins is eliminated, the sedimentation coefficient of the oligomer that is observed, 39 S, falls in the range predicted for a double-ring structure composed of tubulin alone (see Marcum and Borisy.
fied oligomers. Microtubules were exposed to trypsin (0.16 pg/ml) at 30° for 25 min, sufficient time for complete destruction of the HMW electrophoretic bands, and the reaction was then stopped with soybean trypsin inhibitor. The microtubules were sedimented at 37°, resuspended and depolymerized in 0.1 M Pipes, pH 6.5, containing 0.1 mM MgSO₄ and 1.0 mM GTP at 4°, and carried through an entire additional cycle of polymerization and depolymerization, before examination in the analytical ultracentrifuge. Also shown are data for an untreated sample in 0.05 M Pipes, pH 6.5, containing 0.1 mM MgSO₄ and 1.0 mM GTP. Conditions for centrifugation were 44,000 rpm, 5°.

Our results appear to indicate, therefore, that digestion of the HMW component of the 18 S oligomer yields a structure similar in appearance to rings described by Weingarten et al. (1974) and Frigon and Timasheff (1975), corresponding, respectively, to 36 S and 42 S oligomers. To obtain a quantitative estimate of the relative proportion of the two ring types in our samples, the dimensions of a large number of rings were determined and the thickness of the wall in the plane of the grid was calculated. Data are shown in Fig. 8 and Table I for the outer diameter of the rings and for the thickness of the ring wall in the plane of the grid, values for the latter derived from the difference between outer and inner diameters.

It may be seen that the outer diameters of the rings fell in distributions with means of approximately 39 nm for the four types of ring for which data is presented. The distributions in hydrodynamic properties among the oligomers examined, it was of interest to correlate these properties with an examination of the morphology of the oligomers. Protein samples containing oligomers were prepared for electron microscopy by negative staining. These samples showed three types of particles: 1) small particles probably representing tubulin dimers or, possibly, small aggregates of dimers; 2) ring-shaped structures; and 3) large aggregates of variable and irregular morphology, probably representing denatured protein or non-microtubule contaminants in the preparation. In general, the rings were very abundant even when sample adsorption to grids was brief (1 s) or when the protein concentration or relative oligomer concentration as judged by analytical ultracentrifugation was low.

This was not the case for samples containing the 18 S oligomer where rings were observed that were indistinguishable in morphology from those seen in other samples (see below), but in quite low number even when a large oligomer peak was detected in the ultracentrifuge. To obtain a quantitative estimate of the correspondence between ring number and oligomer peak size, electron microscopy was performed in the following manner, in which possible sources of error in the ring count were minimized. To freeze the association/dissociation equilibrium between oligomers and subunits, samples of 18 S and 30 S oligomers were exposed briefly to glutaraldehyde under conditions that are sufficient to fix the 30 S oligomer (Scheele and Borisy, 1978). To insure reproducible adsorption of samples to grids, solution conditions were made equivalent before adsorption, and grids were precoated with poly(L-lysine) (Mazia et al., 1974), which resulted in a strikingly uniform and reproducible distribution of particles (Fig. 7). After identical periods of adsorption, the number of rings in the 18 S sample (Fig. 7b) was still found to be low in comparison with the 30 S sample (Fig. 7a). The ratio of values of ring number per unit of oligomer peak area for the 18 S to the 30 S samples was 1:24. Examination of the fixed samples in the ultracentrifuge indicated that oligomeric material was preserved during fixation. However, the sedimentation properties of this material were considerably altered after fixation, and it could not be established with complete certainty that the 18 S oligomer survived the fixation process. Nevertheless, our data support most strongly the possibility that the 18 S oligomer is not a ring.

We do not have similar reservations regarding the identity of rings of the 20 S oligomer formed from tau plus tubulin, or the 30 S and 39 S particles, which all showed numerous rings in the electron microscope. The rings observed were predominantly thin-walled, appearing identical to rings shown in several published reports (Borisy et al., 1975; single rings in Erickson, 1974b; in Doenges et al., 1976; and in Bryan, 1976). Some rings with concentric layers of subunits were observed, similar in appearance to rings described by Weingarten et al. (1974) and Frigon and Timasheff (1975), corresponding, respectively, to 36 S and 42 S oligomers. To obtain a quantitative estimate of the relative proportion of the two ring types in our samples, the dimensions of a large number of rings were determined and the thickness of the wall in the plane of the grid was calculated. Data are shown in Fig. 8 and Table I for the outer diameter of the rings and for the thickness of the ring wall in the plane of the grid, values for the latter derived from the difference between outer and inner diameters.

Morphology of Oligomers—In view of the wide variation in
Non-tubulin Component of Microtubule Protein Oligomers

FIG. 7. Fixed 30 S and 18 S oligomers. Microtubule protein at 6 mg/ml was prepared in 0.05 M Pipes, pH 7.45, containing 0.1 mM MgSO₄ and 1.0 mM GTP. This material showed only 6 S and 30 S peaks in the ultracentrifuge, the latter accounting for 54% of the total protein. An aliquot of this sample was made to 80 mM in NaCl, resulting in the disappearance of the 30 S peak and the appearance of an 18 S peak representing 23% of the total protein. Glutaraldehyde was added to each of the samples, which were then incubated at 15°C for 5 min (Scheele and Borisy, 1978). The fixed samples were diluted to 1 mg/ml and the final concentration of all solution components was made identical. Samples were applied to poly(L-lysine)-coated grids for 10 s, displaced with water, and negatively stained with uranyl acetate. x 51,000. a, 30 S sample; b, 18 S. Some distortion was noted in the appearance of the 30 S rings as the result of fixation, but most appeared thin-walled as was observed for unfixed rings (Fig. 8). The 18 S material showed a few rings, but mostly particles smaller in diameter than rings. These particles presumably represent subunits and, perhaps, oligomers. The ratio in the number of rings observed in the 30 S and 18 S samples was 58:1.

samples with oligomeric components of $s_{20,w} = 30$ S and 39 S were broad, possibly reflecting some variation in the number of subunits per ring as well as some extent of distortion of the rings on the grid.

The distribution of measurements of wall thickness, outside diameter minus inside diameter, confirmed that most rings were thin-walled. The means for the four classes of ring were in the range 8.6 to 9.6 nm (Table I), corresponding to values for the wall thickness of 4.3 to 4.8 nm. Based on the dimensions deduced for the tubulin subunits from electron microscopic examination of microtubules, the value for the ring wall thickness was close to the lattice dimensions for the tubulin monomer, approximately 4.0 × 5.0 nm (Amos and Klug, 1974; Erickson, 1974a). Thus, most rings appeared to have a wall close in dimensions to that expected for a structure only one tubulin monomer in thickness. In our preparations multiple-walled structures represented no more than a small fraction of the total population of rings (6% of the 30 S rings, 2% of the total measured). Therefore, we identify the 20 S, 30 S, and 39 S oligomers observed by ultracentrifugation as rings with walls no thicker than one monomeric subunit in the radial direction. The implication of this finding in conjunction with the hydrodynamic properties of the rings will be discussed below.

FIG. 8. Dimensions of rings with different hydrodynamic properties. Ring diameters were measured at × 258,000 enlargement. The difference between the outer and inner diameters, equal to twice the ring wall thickness, was determined, and the distribution of these values as well as those for the outer diameter are shown. a, 30 S rings from unfractionated microtubule protein; b, 30 S rings produced from HMW + tubulin; c, 20 S rings from tau + tubulin; d, 39 S rings produced from microtubules exposed to trypsin, pH = 6.5.

### Table I

| Parameters of ring distributions |
|---------------------------------|
| Sedimentation coefficient | Protein content | Mean$a$ of outer diameter values | Mean$a$ of wall thickness values$^b$ |
|------|-----------------|-----------------|-----------------|
|       | $n$             | $\mu m$          | $\mu m$          |
| 30 S  | Unfractionated microtubule protein | 78 | 40.5 ± 3.1 | 4.8 ± 1.3 |
| 30 S  | HMW + tubulin   | 67 | 39.1 ± 3.4 | 4.6 ± 0.7 |
| 20 S  | tau + tubulin   | 92 | 39.4 ± 2.1 | 4.7 ± 0.8 |
| 39 S  | Trypsin-modified | 83 | 37.6 ± 3.2 | 4.3 ± 0.9 |
| Total |                 | 320 | 39.1 ± 3.1 | 4.6 ± 1.0 |

$^a$ Standard deviation.

$^b$ Wall thickness values are one-half the values for outer minus inner ring diameter shown in Fig. 8.

### DISCUSSION

The results presented here indicate that the oligomers present in preparations of microtubule protein require protein factors other than tubulin for their formation. Both the 18 S and the 30 S oligomers required these factors, and more than one factor was active in promoting tubulin association. The HMW proteins promoted the formation of the 30 S oligomer (Fig. 4) and were directly incorporated into this structure (Fig. 5), which was seen to correspond to a ring-shaped particle when observed by electron microscopy (Fig. 7). Digestion of the HMW molecules with trypsin resulted in a marked change in the sedimentation properties of the 30 S ring (Fig. 5).
relationship of the 18 S and 30 S oligomers observed in our preparations are discussed below.

Structure of Oligomers—Single versus Double Rings—Ring-shaped particles were observed in abundant quantity in preparations in which the 20 S, 30 S, and 39 S oligomers were the only oligomeric species detected in the ultracentrifuge. These particles showed a wall thickness corresponding to that expected for a single monomeric tubulin subunit (Fig. 8). From this observation and the diameter of the ring of 39 S in comparison with values determined for the dimension of the tubulin dimer in the microtubule lattice (Amos and Klug, 1974; Erickson, 1974a) we calculate that there may be no fewer than 13 dimers arranged end-to-end in a ring. Calculation of the sedimentation properties of such a particle has been made (Marcum and Borisy, 1978), resulting in an expected sedimentation coefficient for a single ring of at least 22 S. This value is close to that observed for the ring composed of tubulin (Fig. 4) and we conclude, therefore, that this structure represents a single ring (Fig. 9). A similar model has been proposed (Doenges et al., 1976) for the 20 S species observed in microtubule protein prepared by a modification of the procedure of Shelanski et al. (1973). The HMW proteins are less prominent components in these preparations (Weingarten et al., 1975; Penningrulit, et al., 1976; Scheele and Borisy, 1976) and tubulin may play a more significant role in ring formation under these conditions. Thus, we consider it likely that the particle described in the present paper is equivalent to that described by Doenges et al. (1976).

The sedimentation coefficient of the 30 S particle is too high to correspond to a single ring structure composed of tubulin dimers arranged end-to-end (Marcum and Borisy, 1978). Our data indicate that the 30 S ring does not consist solely of tubulin dimers, but that the HMW proteins are also a prominent component (Figs. 4 and 5). The presence of the HMW molecules would be expected to affect the sedimentation properties of the ring but, with no clear evidence regarding the number, location, and dimensions of the HMW molecules, it is impossible to predict the direction of this effect. We have shown (Fig. 6) that removal of the portion of the HMW molecule that projects from the surface of the microtubule (Vallee and Borisy, 1977) results in a marked increase in the sedimentation coefficient of the 30 S oligomer to 39 S. This value is in the range predicted for a double-ring structure (Marcum and Borisy, 1978). We conclude that the 39 S particle, and, therefore, the 30 S particle as well, consist of two ring layers (Fig. 9). The two rings must be superimposed in the axial view available in the electron microscope to result in the thin appearance of the ring wall (Fig. 8). This conclusion is reinforced by electron microscopic measurements of the height of the 30 S ring (Scheele and Borisy, 1978).

Since the 30 S and 39 S rings have the same planar dimensions as the single 30 S ring, we would expect the same number of subunits per layer. For a double ring of 13 subunits/layer, the sedimentation coefficient has been calculated to be 42.2 S (Marcum and Borisy, 1978), close to the value of 39 S experimentally determined for the modified 30 S ring. While the calculated sedimentation coefficients for the double and single rings are somewhat higher than those observed, it is important to note that the ratio of calculated values for single and double rings for any number of subunits per layer is nearly constant (1.90 to 1.92) and corresponds almost exactly to that obtained for the 39 S and 20 S rings (1.95). In view of the possible uncertainties in the calculation of the sedimentation coefficients, the ratio of values for double rings and single rings may well be the parameter of greater significance.

Based on the conversion of the 30 S oligomer to the 39 S species by cleavage of the HMW proteins, we must conclude that the predominant effect of the HMW molecule is to increase the frictional drag of the particle to which it is attached. This results in a decreased sedimentation coefficient from that which would be observed for a structure consisting of tubulin dimers alone. The HMW molecule itself appears to have a sedimentation coefficient that is quite low for a protein of its molecular weight, indicating that the molecule is highly extended (expected sedimentation coefficient if globular = 12 to 14 S, depending on extent of hydration, compared with a measured sedimentation coefficient of 3 to 4 S, Fig. 1). Its frictional properties must be largely preserved in the 30 S ring in order to affect the sedimentation coefficient of this structure in the manner observed. Thus, we conceive of the HMW molecule as protruding from the ring much as in the microtubule, with only 10% of the molecule directly bound to the tubulin dimers (Vallee and Borisy, 1977). The protrusion of the HMW projections is also suggested by another aspect of the sedimentation data, the parameter g (Equation 1). This quantity has been found to be at a minimum for spherically symmetrical particles and to increase with increasing degree of asymmetry (Creeth and Knight, 1965). It is evident from Fig. 8 that g decreases dramatically with the removal of the HMW projections from the 30 S ring, indicative of a reduction in asymmetry.

In view of the effect of the HMW proteins on the sedimentation properties of the 30 S oligomer, we have assembled data for other tubulin-containing oligomers to determine whether a more general correlation between HMW content and sedimentation parameters might be made. In Table II we summarize data for the HMW content of microtubule protein under a number of conditions of preparation or experimental modification, compared with the measured sedimentation coefficients of the oligomers and the values determined for g. It may be seen that the values of g decrease with decreasing HMW content. The sedimentation coefficients for rings formed in preparations lacking HMW appear to fall in two categories corresponding approximately to 20 S and 40 S. We suggest that these categories represent single and double tubulin
Scheele and Borisy (1976); fast al. (1976) and R. B. Scheele (unpublished results).

The possible relatedness of the 18 S and the 20 S particle is primarily a reflection of the presence of tau, it might further be assumed that the presence of the tau-induced ring could account for the formation of the 18 S oligomer. Second, very few rings were observed in 18 S preparations, even after fixation with glutaraldehyde under conditions that result in the stabilization of the 30 S particle (Scheele and Borisy, 1978). While this result could reflect the weak association of the 18 S particle, it may indicate that the 18 S oligomer is, in fact, not a ring at all. We therefore suggest that the 18 S species could be a small tubulin oligomer of some different shape. A more nearly globular structure of perhaps four to six dimers could show a sedimentation coefficient of about 18 S. One or more HMW molecules might also be incorporated into the structure, in view of the requirement for non-tubulin proteins (Fig. 3). Such a structure might, indeed, be predicted as an intermediate in the formation of the 30 S ring, perhaps representing a segment of the ring wall (or the microtubule lattice—see below) composed of an HMW molecule and those dimers in direct contact with its tubulin binding site. If this suggestion is correct, it indicates that the 18 S and 20 S oligomers are not closely related structures. In this case the 20 S structure observed after exposure of microtubules to trypsin would not be a modified form of the 18 S oligomer but would be formed de novo, perhaps as the result of subtle changes in the content of stimulatory factors. In support of this possibility, preliminary observations have revealed the presence of numerous rings in samples containing the trypsin-derived 20 S species.

HMW-to-tubulin Stoichiometry—The number of HMW molecules per 30 S ring is indicated by the data of Fig. 5 in which the HMW proteins were shown to co-chromatograph with a fraction of tubulin in the sample. Our data indicate that the ratio of HMW to tubulin in the ring is increased relative to the ratio in the microtubule by a factor of 2.1 to 2.4. Recently, Amos (1977) has estimated the HMW content of microtubules to be 1 HMW molecule to 12 tubulin dimers based on electron microscopic observation of microtubules and symmetry considerations. While we have found some variability in the absolute value for the ratio of HMW to tubulin in microtubule preparations as determined by electrophoresis, the values we obtain are close to that estimated by Amos. Assuming a ratio of HMW to tubulin of 1:12 in the microtubule, we obtain for the ratio of HMW to tubulin in the 30 S ring values of about 1.5 to 1.6.

The increased stoichiometry of HMW to tubulin in the 30 S ring may explain the finding of Marcum and Borisy (1978) that no more than about 65% of microtubule protein is capable of assembly into 30 S rings. Since the ratio of HMW to tubulin in the ring is about twice that in the microtubule, we would calculate the maximum possible concentration of rings to be equal to half the tubulin concentration plus the total concentration of HMW. We calculate this value to be 58% of the total protein, a value close to that actually measured. Further evidence that the extent of 30 S formation is limited by the amount of HMW protein in our preparation was provided by increasing the amount of HMW, which resulted in an increase in the amount of oligomer (see "Results").

We may now write the reaction scheme for ring formation as follows:

\[ nH + mT \rightarrow P \]  

(2)

where \( H \) is HMW, \( T \) is tubulin, and \( n \) and \( m \) are the numbers of molecules per double 30 S ring. \( m \) is at least 26, judging from the dimensions of the ring measured by electron microscopy and its double nature (see preceding discussion). The exact value for \( m \) may be somewhat higher than 26 (Scheele and Borisy, 1978). Based on our measurements of the ratio of \( n \) to \( m \) in the 30 S ring of 1.5 and 1.6, we calculate that there

Table II

| History of protein | HMW content | Fast oligomer | Slow oligomer |
|-------------------|-------------|---------------|---------------|
|                    | \( s_{H\text{M}} \) | \( m/\text{mg} \) | \( g \) | \( s_{H\text{M}} \) | \( m/\text{mg} \) | \( g \) |
| Reversible assembly | 17 - 23 | 30.6 | 0.054 | 18.6 | 0.034 |
| Purified tubulin + compound | 17 | 31.4 | 0.058 | ( ) | ( ) |
| HMWb | 9 | 36 | 0.020 - 0.030 | 20 | 0.017 |
| Purified tubulin + | 2 | 20 | 0.016 |
| tau b | 0 | 30 | 0.006 | 20 | 0.001 |
| Reversible assembly | 0 | 42 | 0.019 |

a Method of Borisy et al. (1975); sedimentation data of Marcum and Borisy (1978).

b Data of this paper.

c Weingarten et al. (1974) modification of method of Shelanski et al. (1973); HMW content from Scheele and Borisy (1976); fast oligomer data, Weingarten et al. (1974), slow oligomers, Doenges et al. (1976), and R. B. Scheele (unpublished results).

Method of Weisenberg et al. (1968); conditions and sedimentation data of Frigon and Timasheff (1975).
must be 4 or 5 HMW molecules/double ring.

Relationship of 30 S Oligomer to Microtubule Dimer and HMW Protein Lattices—The increased density of HMW to tubulin in the 30 S ring may have its basis in the structure of the microtubule. Tubulin dimers are arranged in the microtubule in a helically symmetrical fashion (Amos and Klug, 1974; Erickson, 1974a). The complete pattern of dimers can be described by a 5-start right-handed group of helices of 40 nm pitch, an 8-start left-handed group of helices of 64 nm pitch, or by a 13-start group of helices of pitch too great to measure, corresponding to the microtubule protofilaments (Borisy et al., 1976). Recent work by Amos (1977) has shown the HMW protein lattices in microtubules prepared from brain tissue. Examination of the correspondence of the projections with the dimer lattice reveals that the projections are sparsely distributed along the 5- and 13-start (protofilament) dimer helices, but are populous, occurring with every third dimer, along every fourth 8-start dimer helix.

We believe it likely that this dense association of dimers and HMW molecules is reflected in the 30 S ring, and a schematic diagram depicting this relationship is shown in Fig. 10. The HMW molecules are shown to lie between two helical arrays of dimers in the microtubule. We envision this arrangement to be preserved in the 30 S ring, the two layers of the ring being derived from two helical arrays of dimers with the HMW molecules retaining their binding site in the ring. The ring can be generated from an appropriate length of dimer double helix by collapsing the helix along the microtubule axis. Because of the steepness of the 8-start helix, the center-to-center distance of dimers in the helix (7.0 nm) is greater than the interpretotofilament distance in the microtubule (5.0 nm; Amos and Klug, 1974; Erickson, 1974a) resulting in a ring of greater circumference and diameter than the microtubule, as is observed (39 nm for the ring versus about 25 nm for the microtubule). The exact number of subunits in the ring is discussed in the following paper (Scheele and Borisy, 1978).

A number of properties of the products of tubulin self-association may be explained by the structural relationship between the 30 S ring and the microtubule that we have outlined. First, the predominance of the two-layered 30 S ring under most solution conditions over single- and multiple-layered structures (Marcum and Borisy, 1978) might be explained by the location of the HMW molecules between rows of dimers. The interaction of the HMW molecules with both layers could add a measure of increased stability to the double ring. Second, according to our scheme, half of the tubulin dimers in the microtubule would be in contact with HMW molecules. Therefore, only half would be involved in the formation of 30 S rings, as appears to be the case (see above discussion; Marcum and Borisy, 1978; and Fig. 5). Third, the stoichiometry of HMW to tubulin in the ring would be a direct reflection of its density along the double 8-start helix of dimers in the microtubule, which is one HMW every third dimer pair. Therefore, the ratio of n to m in Equation 2, the ratio of HMW molecules to tubulin dimers in the 30 S ring, would be expected to be 1:6, although other considerations suggest a further modification of this value (Scheele and Borisy, 1978).

Why such a limited extent of binding should occur, with a large number of apparently equivalent tubulin sites available in the 30 S ring and in the microtubule, is not clear. It may be the case that each HMW molecule binds to as many as three adjacent pairs of dimers in the ring and in the double 8-start dimer helix via an extended binding site (Vallee and Borisy, 1977), thereby filling all available dimer sites. The fragment of the HMW molecule that appears to remain bound to the microtubule after exposure to trypsin is of sufficient size (M, = 31,000 to 35,000; Vallee and Borisy, 1977) to span the distance involved. Thus, the basic unit of HMW-tubulin dimer association might be the HMW molecule plus the dimers that are in direct contact with it in the 30 S ring and in the microtubule. We may, in fact, observe such a particle in the form of the 18 S oligomer, although further information on this species is required to determine whether this is the case.

Finally, recent work (Bryan, 1976; Johnson and Borisy, 1977; Murphy et al., 1977) has indicated strongly that the 30 S ring is not an obligatory intermediate for microtubule elongation. However, whether the 30 S ring is an obligatory precursor in microtubule initiation, providing segments of preformed 8-start double helix onto which free dimers may accrete has not been established. It appears that multiple pathways to the formation of microtubules may exist, depending on the protein composition of the preparation and possibly on the nature of the oligomers present. In our own preparations the 30 S oligomer, enriched in the HMW proteins, is the predominant oligomeric form present under solution conditions favorable for microtubule assembly. We consider it possible, therefore, that in the presence of the HMW proteins,
segments of the double S-start helix, whether derived from 30 S rings directly or formed de novo from tubulin and HMW, may represent intermediates in the initiation of microtubule assembly.

Note Added in Proof—Until recently, the term "tau" has been associated with a group of proteins of molecular weight slightly greater than that of tubulin ($M_r = 58,000$ to $70,000$; Cleveland et al. (1975) J. Cell Biol. 64, 12a; Witman et al. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4070-4074; Connolly et al. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2437-2440). The term "tau-I" (Penningroth et al. (1976) Cold Spring Harbor Conference on Cell Proliferation 3, 1233-1257; Cleveland et al. (1976) Proc. Natl. Acad. Sci. U. S. A. 74, 207-225) has now been introduced to refer to a series of four closely spaced electrophoretic bands in this range of molecular weight, $M_r = 55,000$ to 62,000, that possess microtubule assembly promoting activity. "tau-II" refers to the other active non-tubulin factors present in microtubule protein. The fraction we have referred to as "tau-I" contains as its most prominent component a band of $M_r = 70,000$. We have now found, using a gel method published after submission of this manuscript (Cleveland et al. (1976) J. Mol. Biol. 116, 207-225) that the peak we have referred to as tau also contains the series of four closely spaced bands that has been referred to as tau-I. Therefore, the fraction of non-tubulin proteins that we have found to be active in promoting 20 S ring formation may be referred to as the tau-containing fraction, or more accurately, the tau-I-containing fraction.

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