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ASSOCIATION STATES OF TUBULIN IN THE PRESENCE AND ABSENCE OF MICROTUBULE-ASSOCIATED PROTEINS

ANALYSIS BY ELECTRIC BIREFRINGENCE

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Electric birefringence has been used to examine the states of association of tubulin in phosphocellulose-purified tubulin or depolymerized microtubule protein solutions at low temperature. In a high electric field (1000–4000 V/cm), tubulin could be orientated (owing to the existence of a permanent and/or induced dipole) and exhibited a positive birefringence (Δn), related to its intrinsic optical anisotropy. The analysis of the relaxation process (depending on hydrodynamic properties of molecules), by measurement of the time decay of Δn, revealed the existence of a multicomponent or polydisperse system, whatever the tubulin solution. Two relaxation times, representative of the smallest and the largest orientated species, were obtained by computer-fitting analysis. The mean values of relaxation time for phosphocellulose-purified tubulin were 0.8 and 8 μs. In microtubule protein solutions, large-sized macromolecular species with relaxation time up to 450 μs were detected. The largest species (relaxation times ranging from 50 to 450 μs) could be eliminated by centrifugation at 300000 $\times g$ for 1 h. Addition of microtubule-associated protein to either pure tubulin or high-speed centrifuged microtubule protein led to a rapid formation of large species analogous to those present in microtubule protein. Molecular dimensions of the relaxing structures were estimated using simple hydrodynamic models and values of rotational diffusion constants calculated from the relaxation times, and compared to those of the structures described in the literature. In conclusion, we have found that (a) phosphocellulose-purified tubulin is not only composed of elementary species (dimers) but also contains tubulin-associated forms of limited size (up to 7–10 dimers), (b) depolymerized microtubule protein solutions contain ring oligomers and structures very much larger, the formation of which is dependent on the presence of microtubule-associated protein.

1. Introduction

It is now well established that tubulin self-associates to form a wide variety of structures. Tubulin solutions have been extensively studied for their content in rather well defined entities: ring oligomers. Species corresponding to ring particles with sedimentation coefficients ranging from 18 to 42 S have been reported [1–7]. Rings have also been well characterized by electron microscopy [8–14]. Ring structures present in microtubule protein solutions are composed of tubulin and microtubule-associated protein (MAP). The different classes of MAP (high molecular weight proteins and tau proteins) have different roles in the polymorphism of microtubule protein [15,16]. Tubulin seems to be able to form ring-shaped structures in the absence of MAP; in this case, however, high magnesium concentrations are required [3,17,18]. Using X-ray scattering [14,19] and quasi-elastic laser light scattering [20], tubulin oligomers smaller than rings have been detected during the early events of microtubule formation. These oligomers seem to be transient and may play a role in the

nucleation phase of microtubule assembly. Weisenberg [2] has shown the formation of a 9 S boundary prior to polymerisation. Metastable tubulin aggregates sedimenting between 10 and 20 S have been evidenced by Gethner et al. [21,22]. A 19 S boundary was found together with 6 S species in phosphocellulose-purified tubulin [23]. More recently, the existence of small oligomers in phosphocellulose purified tubulin was documented using new approaches: X-ray scattering [14], ultrafiltration, non-denaturing electrophoresis and chemical cross-linking [24]. It is therefore apparent that small oligomers together with ring structures and possibly more complex aggregates [8,11,12,14] can exist in tubulin solutions.

We report here a study on tubulin association states by electric birefringence, a method which has been successfully used in the study of small- (nucleosomes [25,26]), intermediate- (spectrin [27], myosin [28]) and large- (collagen [29]) sized structures. The aim of the present work was to characterize tubulin oligomers by a new approach based on transient electric birefringence, which involves the rotational diffusion properties of molecules. Using this method, we have re-examined whether pure tubulin (purified by phosphocellulose chromatography) contains species other than dimers and how the presence of MAPs alters the tubulin oligomeric pattern.

2. Materials and methods

2.1. Preparation of protein solutions

Microtubule protein was prepared from rat brain by two cycles of temperature-dependent assembly-disassembly according to Shelanski et al. [30]. The buffer used was 100 mM Mes, 0.5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 1 mM

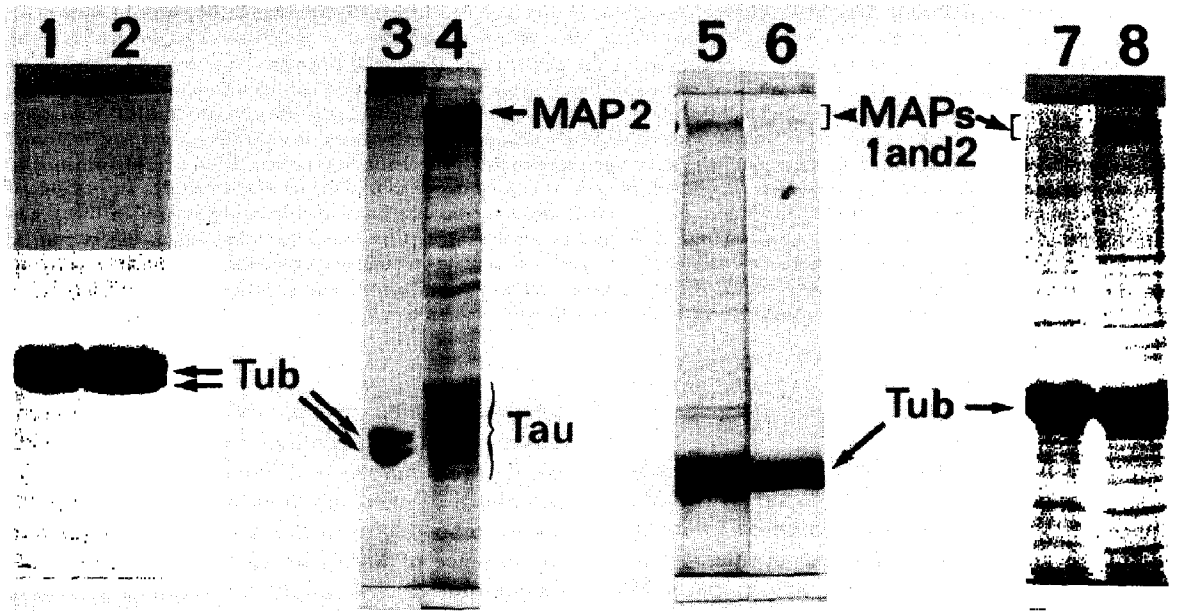


Fig. 1. Polypeptide composition of tubulin solutions analyzed by polyacrylamide gel electrophoresis in the presence of SDS. Lanes 1–3, phosphocellulose-purified tubulin (PC-tubulin) corresponding to three different preparations. Lane 4, thermostable MAP fraction. Lanes 5 and 8, microtubule protein prepared by the assembly-disassembly procedure (two cycles) corresponding to two different preparations. Lanes 6 and 7, microtubule protein after high-speed (300000 × *g*, 60 min) centrifugation (HS-tubulin). HS-tubulin of lanes 6 and 7 was prepared from microtubule protein of lanes 5 and 8, respectively.

2-mercaptoethanol, 0.5 mM GTP, pH 6.4 (buffer A). Polymerization steps were carried out in buffer A supplemented with 4 M glycerol. Microtubule protein solutions were stored at −196°C. Before use, solutions were centrifuged at 50000 × g for 20 min to remove undepolymerized material. Microtubule protein had a critical concentration for polymerization of about 0.2–0.3 mg/ml.

Tubulin (PC-tubulin) was purified by phosphocellulose chromatography [31] using buffer C: 25 mM Mes, 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.1 mM GTP, pH 6.4. The colchicine-binding activity of PC-tubulin was between 0.3 and 0.5 mol bound colchicine per mol tubulin.

MAP were prepared from microtubules assembled in vitro [32]. Microtubule pellets were resuspended in buffer E (buffer A + 3 mM dithiothreitol + 1 M NaCl) for 60 min at 0°C. The depolymerized solution was kept at 95°C for 5 min and centrifuged at 100000 × g for 60 min. The supernatant which contained MAP and no tubulin was dialyzed against buffer A. Protein solutions were stored at −196°C. Protein composition of microtubule protein, phosphocellulose-purified tubulin and MAP fraction was analyzed by polyacrylamide gel electrophoresis in the presence of SDS. Results of representative experiments are given in fig. 1. Proteins were assayed according to Lowry et al. [33] using bovine serum albumin as standard.

2.2. *Electric birefringence: principles and analysis*

Under the action of an electric field (applied as a rectangular pulse), macromolecules in solution tend to orientate, provided that they exhibit either a permanent dipolar moment or an induced polarizability. In the case of optically anisotropic molecules, the solution becomes birefringent (Kerr effect): light propagation is different along two definite directions which are parallel and perpendicular to the electric field; thus, two refractive indexes can be determined. The birefringence is the quantity: $\Delta n = n_{\parallel} - n_{\perp}$.

Three phases can be distinguished in the birefringence signal: (i) the Δn setting up depending on the optical, electric and hydrodynamic properties of orientating molecules, (ii) the steady-state birefringence (Δn_{eq}) depending on the optical and electric properties of orientated molecules, (iii) the decay of Δn depending exclusively on the hydrodynamic properties of de-orientating molecules.

According to Kerr's law, Δn_{eq} is proportional to the square of the electric field.

Benoit [34] has shown that the decay of Δn can be described by the relation:

$$\Delta n(t) = \Delta n_{eq} e^{-t/\tau}$$

The relaxation time (τ) is determined using a logarithmic plot of $\Delta n(t)/\Delta n_{eq}$ vs. time:

$$\ln \frac{\Delta n(t)}{\Delta n_{eq}} = -\frac{1}{\tau} \cdot t$$

If the solution is polydisperse, the Δn decay is described by the relation:

$$\frac{\Delta n(t)}{\Delta n_{eq}} = \sum_i a_i e^{-t/\tau_i}$$

where a_i is the contribution of the species having a relaxation time τ_i to the total birefringence. In practice, it is very difficult to determine more than two relaxation times. In this work, we have analyzed the Δn decay kinetics according to the simplified equation:

$$\ln \frac{\Delta n(t)}{\Delta n_{eq}} = \ln\left(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}\right)$$

τ_1 and τ_2 being the short and the long relaxation times, respectively. The four variables (a_1, a_2, τ_1, τ_2) were determined using a computer analysis program based on a two-step adjustment. a_2 and τ_2 are obtained from the end of the curve by linear fitting. Then, a subtractive method allows one to obtain a_1 and τ_1. In most cases, we obtained a good fitting between the experimental decay curve and the curve calculated from a_1, a_2, τ_1 and τ_2. A limited discrepancy was observed in the intermediate region of the decay curves, indicating that polydispersity was more extended than assumed for analysis.

The relaxation times are related to the rotational diffusion constants Θ of de-orientating molecules by the equation:

$$\tau = \frac{1}{6\Theta}$$

Θ can be related to the molecular dimensions of molecules, assuming simple rigid models. For revolution ellipsoids, Perrin [35] has shown the relation:

$$\Theta = \frac{kT}{6\eta V} \cdot r(p)$$

where V is the volume of molecules, η the medium viscosity, k the Boltzmann constant, T the absolute temperature and $r(p)$ a function of the axial ratio (p) of molecules, which has been calculated for $0.001 < p < 1000$ by Daune et al. [36]. For cylinders, we used the equation of Broersma [37]:

$$\Theta = \frac{3kT}{8\pi\eta a^3}\left(\ln\frac{2a}{b} - 0.8\right)$$

where a is the half length and b the radius of the cylinder.

2.3. Electric birefringence measurements

The electric birefringence apparatus has been previously described [38]. We have used electric field ranging from 1000 to 4000 V/cm and pulse length varying from 10 to 500 μs. The volume of the cell was 2 ml (5 cm path length, 0.5 cm between electrodes). Measurements were made at 10 ± 2°C in buffer C using protein solution of concentration varying from 0.2 to 1.0 mg/ml. Measurements were performed on several tubulin preparations. Since the quantitative behaviour of the protein often varied from one preparation of tubulin to another and since for a given protein solution, relaxation times were determined with an uncertainty of about 30%, the data are mostly presented as the range of observed values. The figures illustrate the results of representative experiments.

3. Results

3.1. Analysis of PC-tubulin

Tubulin exhibited positive birefringence of weak amplitude. A typical signal is reported in fig. 2A. There is asymmetry between the rise (R) and decay (D) of birefringence as revealed by measurements of hatched areas: $R/D > 1$ (fig. 2A). This indicates that the orientation process depends at least in part of the presence of a permanent electric dipole on tubulin molecules. The birefringence decay curve (fig. 2B) appeared to be multiexponential, indicating the polydispersity of the tubulin solution. Decomposition of the curve (fig. 2B) as the combination of two relaxation times gave values of $\tau_1 = 0.8$ and $\tau_2 = 4$ μs. Values obtained for different PC-tubulin preparations varied from 0.5 to 1.2 μs for τ_1 and 4 to 12 μs for τ_2. For a given tubulin preparation, relaxation times did not vary significantly with the protein concentration (from 0.2 to 0.6 mg/ml). Below 0.2 mg/ml, relaxation times could not be accurately determined because of low values of Δn. Fig. 2 (C and D) shows that a similar composition in relaxing species was obtained when the pulse length varied from 15 to 50 μs. There was a linear relationship between the steady-state birefringence (Δn_{eq}) and the squared value of the applied electric field (E^2) (fig. 3). Thus, Kerr's law is verified for PC-tubulin. This indicates that there is no deformation of tubulin components due to the applied electric field (up to 4000 V/cm).

3.2. Study of microtubule protein components

At 4000 V/cm, the orientation of the molecular species of microtubule protein solution required pulse lengths very much longer than those used for PC-tubulin. A definite steady-state birefringence could not be reached whatever the applied electric field (Fig. 4A) and pulse length (up to 500 μs). Longer pulses were not used so as to avoid a temperature rise in the measuring cell. Fig. 4B shows that the species which were orientated at different electric fields (1000–4000 V/cm) were the same: $\tau_1 = 14$–18 μs, $\tau_2 = 190$–270 μs; these variations were not considered as significant, since the relaxation times could only be determined with 30% accuracy. It is apparent that the relaxation times obtained with microtubule protein solutions are 20–30-times larger than those determined with PC-tubulin. The data reported in fig. 5 demonstrate that the high values of the relaxation time

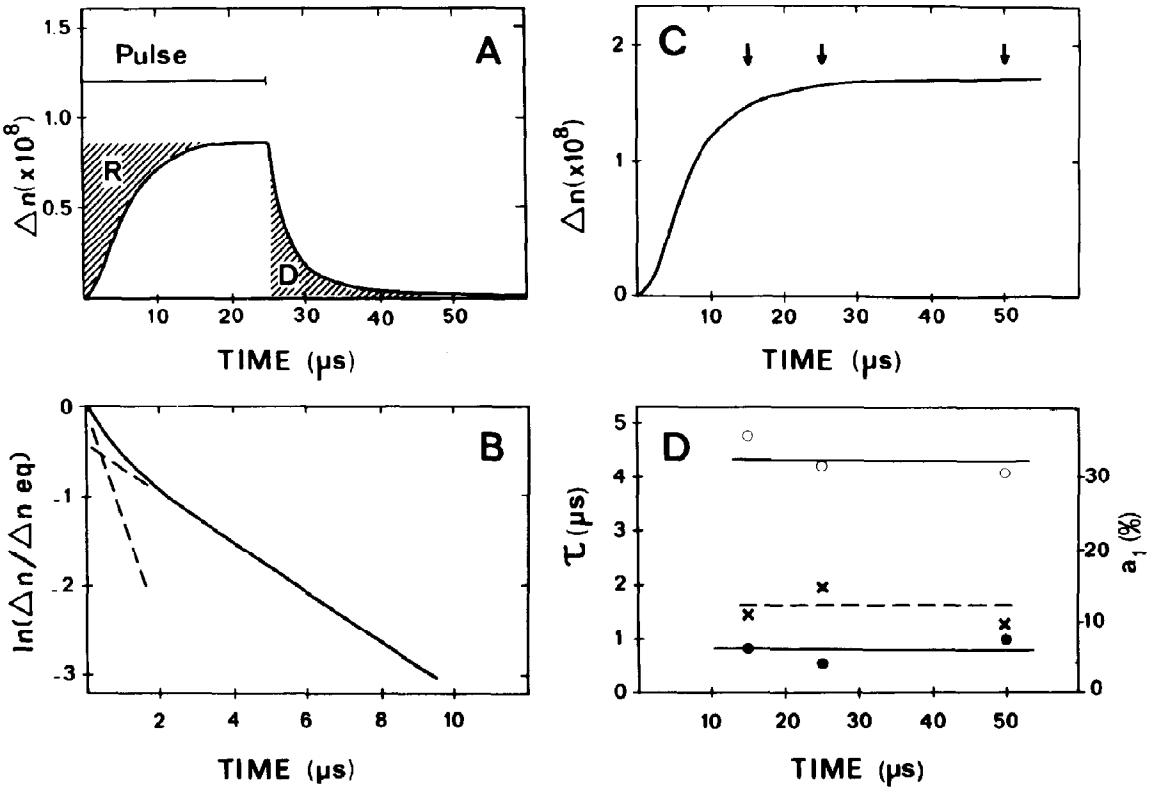
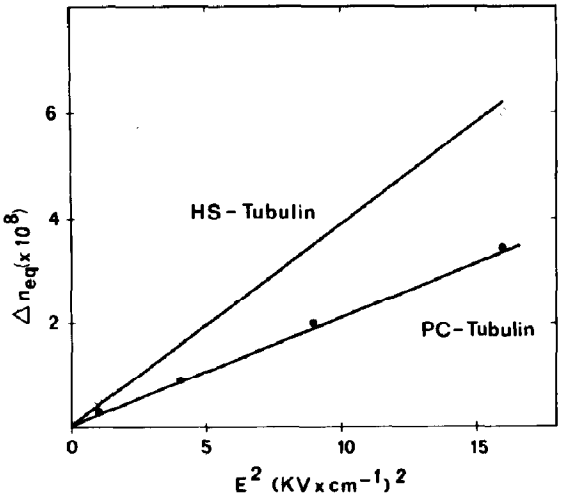


Fig. 2. Electric birefringence analysis of PC-tubulin. (A) Typical birefringence signal obtained at 4000 V/cm with a pulse of 25 μs. PC-tubulin concentration was 0.32 mg/ml. R and D, surface areas corresponding to the rise and the decay of birefringence. (B) Semilogarithmic plot of the Δn decay vs. time (conditions of panel A). The relaxation process was decomposed into two relaxation times (- - - - - -): $\tau_1 = 0.8$ μs, $\tau_2 = 4$ μs. (C and D) Effect of pulse length on the determination of relaxation times. PC-tubulin concentration was 0.46 mg/ml. In panel C, arrows indicate the shutting off of the electric field (4000 V/cm). (D) Values of relaxation times τ_1 (●) and τ_2 (○) and a_1 (×) obtained for each pulse length.



observed in microtubule protein solutions are related to the presence of large oligomeric forms of tubulin. Indeed, after high-speed centrifugation (300 000 × g, 60 min), the resulting microtubule protein solutions did not exhibit relaxation times greater than 50 μs (fig. 5B). This centrifugation procedure led to the sedimentation of about 35% of the microtubule protein (initial concentration, 2.5 mg/ml) and to the majority of MAP (MAP1

Fig. 3. Steady-state electric birefringence (Δn_{eq}) as a function of the square of the applied electric field. The electric field varied from 1000 to 4000 V/cm. Pulse length was 50 μs for PC-tubulin (●) and 250 μs for HS-tubulin (○). Protein concentration was 0.54 and 0.80 mg/ml for PC-tubulin and HS-tubulin, respectively.

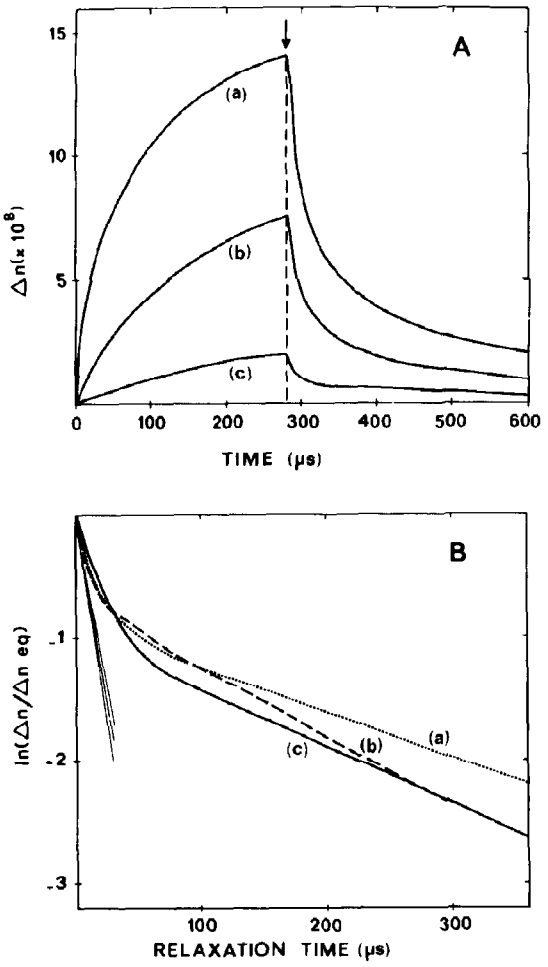


Fig. 4. Electric birefringence analysis of microtubule protein. (A) Variation of the birefringence signal with the applied electric field: 4000 V/cm (a), 2000 V/cm (b), 1000 V/cm (c). The arrow indicates the shutting off of the electric field. Protein concentration was 0.9 mg/ml. (B) Birefringence decay analysis of signals of panel A. Values of τ_1 given by the slope of the straight lines starting from the origin were 15 μs (a), 14 μs (b) and 17 μs (c). τ_2 values were 270, 190 and 230 μs for a, b and c, respectively.

and MAP2) (fig. 1). The effect of centrifugation is apparent on the birefringence signal. A steady-state electric birefringence was clearly reached with high-speed centrifuged tubulin (HS-tubulin) (fig. 5A). Consequently, the dependence of Δn_{eq} on E^2 could be studied (fig. 3). As for PC-tubulin, Kerr's

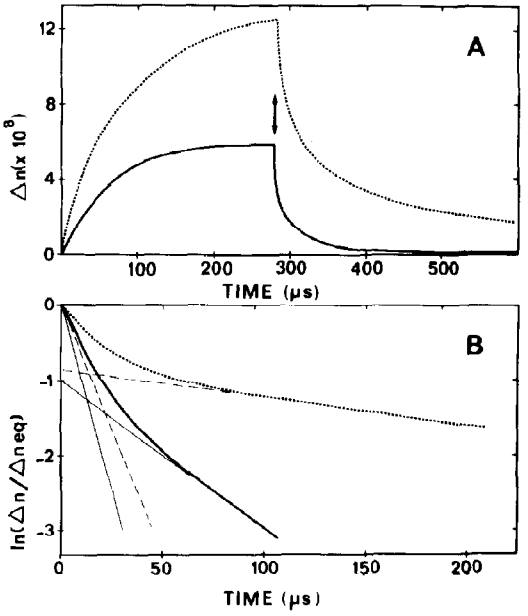


Fig. 5. Effect of high-speed centrifugation on birefringence properties of microtubule protein solution. (A) Signals obtained for microtubule protein before (·····) and after (———) centrifugation at 300000 × g for 1 h. Protein concentration was 0.8 mg/ml in both cases (HS-tubulin was obtained from a microtubule protein solution at 2.5 mg/ml, high-speed supernatant was assayed for protein and each solution diluted to obtain a final concentration of 0.8 mg/ml). The shutting off of the electric field (4000 V/cm) is indicated by the double-ended arrow. (B) Analysis of relaxation times of microtubule protein (·····) and HS-tubulin (———). Decomposition of the relaxation process into short (τ_1) and long (τ_2) relaxation times (illustrated by thin straight lines) gives the following values: $\tau_1 = 15$ μs, $\tau_2 = 280$ μs and $\tau_1 = 10$ μs, $\tau_2 = 50$ μs for microtubule protein and HS-tubulin, respectively.

Table 1

Effect of MAP on the birefringence properties of tubulin solutions

Conditions: 4000 V/cm, pulse = 250 μs. The protein concentrations for PC-tubulin, HS-tubulin and MAP were the same as those of fig. 7. Microtubule protein concentration was 0.5 mg/ml. Values of Δn were taken at the end of the pulse.

Protein solution	Δn ($\times 10^8$)	τ_1 (μs)	a_1 (%)	τ_2 (μs)	a_2 (%)
PC-tubulin	1.8	1.2	63	12	36
PC-tubulin + MAP	4.3	25	49	190	51
HS-tubulin	1.3	5	53	26	47
HS-tubulin + MAP	6.4	14	78	300	21
Microtubule protein	8.5	22	47	330	45

law was verified for HS-tubulin. The variations of the relaxation times with microtubule protein concentration were studied using two different pulse lengths: (a) a pulse of 250 μs allowing us to reach a Δn value rather close to the steady-state of birefringence, and (b) a pulse of 50 μs to search for the presence of small species possibly masked by the relaxation process of large species. Irrespective of the electric pulse conditions, Δn increased linearly with protein concentration (fig. 6). As expected, short pulse length allowed detection of the orientation-relaxation process of components ($\tau_1 = 1$–4 μs, $\tau_2 = 20$–30 μs) very much smaller than those seen with a 250 μs pulse ($\tau_1 = 15$ μs, $\tau_2 = 450$ μs). Using either short or long pulses, there were neither significant changes in the measured relaxation times nor variations in the proportion of Δn corresponding to each relaxation time when the microtubule protein concentration was increased from 0.25 to 1.0 mg/ml (fig. 6).

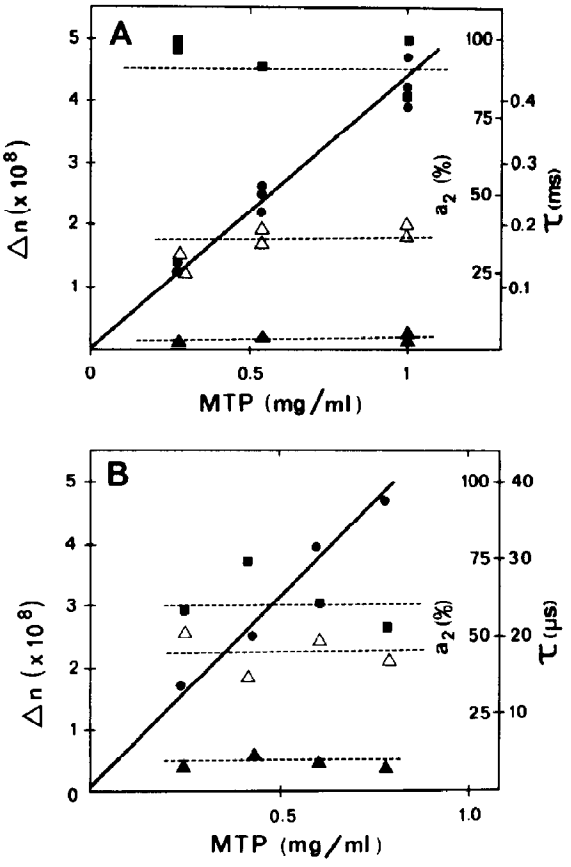


Fig. 6. Concentration dependence of the birefringence properties of microtubule protein (MTP). Conditions: (A) 2000 V/cm, pulse = 250 μs. (B) 4000 V/cm, 50 μs. Δn values at the end of the pulse (●), τ_1 (▲), τ_2 (■), a_2 (△).

3.3. *Reconstitution experiments*

We have tried to determine whether addition of the MAP fraction to PC-tubulin or HS-tubulin: (i) affects the relaxation time distribution pattern observed with these protein solutions and (ii) gives rise to relaxing species analogous to those present in microtubule protein. Experiments were carried out using low concentrations of MAP which did not exhibit any birefringence signal. The MAP/tubulin concentration ratio was between 0.2 and 0.3. Addition of MAP to PC-tubulin or HS-tubulin induced a rapid change (within 1 min) of the relaxation process (fig. 7) and a net increase in Δn (table 1). The two relaxation times (τ_1 and τ_2) were altered and reached values comparable to those in microtubule protein (table 1). Earlier measurements (within the first minute of reconstitution) could not be performed. There were no further alterations within 10 min at the working temperature of about 10°C.

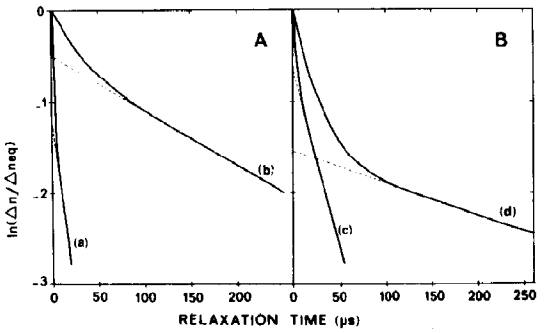


Fig. 7. Modifications of the relaxation process of PC-tubulin (A) or HS-tubulin (B) by addition of MAP. Conditions: 4000 V/cm, pulse = 250 μs. (a) PC-tubulin (0.44 mg/ml). (b) PC-tubulin (0.33 mg/ml)+MAP (0.11 mg/ml). (c) HS-tubulin (0.63 mg/ml). (d) HS-tubulin (0.52 mg/ml)+MAP (0.11 mg/ml). Measurements were made 1 min after addition of MAP to either PC-tubulin or HS-tubulin.

4. Discussion

Protein solutions containing tubulin or tubulin + MAP exhibit very different electric birefringence properties (Δn, relaxation times). These data are in agreement with the generally held belief that tubulin oligomerization largely depends on the presence of MAP. Since our results clearly indicate that tubulin solutions, whatever their protein composition, exhibit a certain degree of polydispersity, we have tried to answer two main questions: (1) what is the composition in terms of the macromolecular structure of pure tubulin solutions, and (2) do microtubule protein solutions only consist of tubulin dimers and ring oligomers?

The molecular form of pure tubulin in solution is still a matter of controversy. Ultracentrifugation shows that pure tubulin mainly consists of the 6 S species [23]. In contrast, ultrafiltration and nondenaturing electrophoresis reveal that tubulin solutions contain small oligomers and a minority of tubulin dimers [24]. Our results, based on a completely different approach, provide evidence for the existence of both dimers and small oligomers in purified tubulin solutions. Indeed, the relaxation of phosphocellulose-purified tubulin components could be resolved into two relaxation times. The smallest determined values (0.5–1.2 μs) can be ascribed to the tubulin dimer. These relaxation times are, however, very much larger than the theoretical value calculated from the molecular dimensions of the tubulin dimer. This is due to the limitations of the signal recorder. The relaxation time of the device determined with nitrobenzene, a small birefringent molecule, was 0.2 μs. That the mean relaxation time of 0.8 μs could correspond to the tubulin dimer is ascertained by measurement of the relaxation times of other globular proteins either smaller (bovine serum albumin) or larger (thyroglobulin) than tubulin. Values of 0.5 and 1.5 μs were obtained for bovine serum albumin and 19 S thyroglobulin, respectively. The experimental relaxation time of 19 S thyroglobulin corresponds to the theoretical value (1.3 μs) calculated from the molecular dimensions (30 × 15 nm) and assuming a prolate revolution ellipsoid in agreement with electron microscope observations [39]. Molecular species with relaxation times greater than 1.5 μs could thus be accurately analyzed in terms of size. Given the relaxation time measured for bovine serum albumin, it is reasonable to propose that τ_1 values, ranging from 0.5 to 1.2 μs, correspond to tubulin dimers. Variation of τ_1 between 0.5 and 1.2 μs could be due at least in part to the variation of the second relaxation time (τ_2), since the value of τ_1 is calculated from τ_2. Using the equation of Daune et al. [36] for an oblate revolution ellipsoid, we have calculated the theoretical relaxation times for three types of ring structures; about 6 μs for the single walled-single layered and single walled-double layered ring with a diameter of 40 nm [13], 11 μs for the double walled-single layered ring (diameter = 50 nm) [1]. Thus, the largest relaxing species of phosphocellulose-purified tubulin might correspond to ring oligomers. However, the variability of τ_2 from preparation to preparation from 4 to 12 μs (a variation larger than that inherent to measurements) does not argue for the presence of a defined oligomeric structure. Furthermore, ring structures are not observed on negatively strained specimens of purified tubulin at low protein concentration. The existence of a polymer, the association degree of which could vary, seems more probable. A linear polymer in which tubulin dimers associate end to end or side by side can be considered, the tubulin molecule being a cylinder 8 nm long with a radius of 2.5 nm [40]. A protofilament-like structure consisting of n end-to-end associated tubulin molecules would exhibit relaxation times ranging from 4 μs for $n = 7$ to 11 μs for $n = 10$, assuming a rigid cylindrical model. In the hypothesis of a side-side interaction, relaxation times ranging from 5 to 10 μs correspond to cylinders containing 15 to 20 tubulin molecules, respectively. The end to end association appears more probable, since such a type of tubulin-tubulin interaction is known to take place in the microtubule protofilament. Whatever the model, it predicts a rather large variation of the relaxation time for a small variation of the number of molecules in the oligomer. It is probable that any intermediate tubulin oligomer ranging from 1 to 10 (or 20) molecules can co-exist in solution, explaining the multi-exponential birefringence decay. The possibility that the multi-exponential relaxation process could be due to an electric field-induced deforma-

tion of PC-tubulin components was ruled out since Kerr's law was verified. Our method did not give direct information on the proportion of each molecular species in the tubulin solution, since the intrinsic birefringence of each oligomer can depend on its size. However, the contribution of the largest species (related to τ_2) to the total birefringence was always large enough (35–85%) to assert that oligomerization is a significant event in tubulin solutions. Our findings confirm and extend the recent observation of Kravit et al. [24].

The molecular components of phosphocellulose-purified tubulin can be converted into larger structures upon addition of an MAP fraction (weight ratio: MAP/tubulin = 0.25). The birefringence characteristics of the reconstituted species closely resembled those of the species observed in microtubule protein solution. In the presence of MAP, the birefringence decay corresponding to small species is masked by the relaxation of very large structures (relaxation time greater than 200 μs). This was apparent when the pulse time was reduced from 250 to 50 μs and therefore when large species were not orientated or only weakly so. What could be the structure of these slowly relaxing macromolecular species (200–500 μs) in a solution where ring structures are thought to represent the major species? One may consider the formation of superstructures composed of ring particles: clusters of rings such as those reported by Bordas et al. [14] or stacks of rings as observed by Olmsted et al. [11]. We have recently reported [41] that association of tubulin ring particles is a major process in the presence of basic proteins (histones). A cluster taken as a sphere with a radius ranging from 50 to 100 nm would yield relaxation times varying from 125 μs to 1 ms. A stack of rings forming a cylinder 200 nm long with a diameter of 35 nm [11] would give a relaxation time of 210 μs. Studies at different microtubule protein concentrations (0.25–1.0 mg/ml) suggest that there is no detectable interconversion between the different types of oligomers. Neither τ nor a values were affected significantly with decreasing protein concentration. The above-mentioned structures, clusters and/or stacks of rings, were no longer detected in the microtubule protein solutions after high-speed centrifugation. They were either sedimented [42] or disassembled by the effect of hydrostatic pressure. Indeed, ring oligomers have been shown to be pressure-sensitive structures [6]. The high-speed supernatant (HS-tubulin) still contains species compatible with rings and, surprisingly, aggregates larger than rings. This is in keeping with a pressure-induced dissociation-reassociation reaction as described by Marcum and Borisy [6]. On increasing the proportion of MAP in HS-tubulin, the structures removed by centrifugation reappeared, indicating that tubulin oligomerization is a dynamic process.

In conclusion, we have analyzed association states of tubulin at low temperature by measuring a parameter (relaxation time) related to the rotational diffusion constant. This parameter is very sensitive to the variation of the molecular dimensions, since it varied as the third power of the dimensions. Electric birefringence in addition to other recently reported techniques (quasi-elastic laser light scattering, synchrotron X-ray scattering) brings new insights in the nature of co-existing tubulin associated forms. We are currently trying to determine whether electric birefringence could help in the understanding of microtubule assembly.

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