Liu, R. S. H., Asato, A. E., & Denny, M. (1983) *J. Am. Chem. Soc. 105*, 4829-4830.
Liu, R. S. H., Asato, A. E., Denny, M., & Mead, D. (1984) *J. Am. Chem. Soc. 106*, 8298-8300.
Liu, R. S. H., Crescitelli, F., Denny, M., Matsumoto, H., & Asato, A. E. (1986) *Biochemistry 25*, 7026-7030.
Loufty, R. O., & Arnold, B. A. (1982) *J. Phys. Chem. 86*, 4205-4211.
Maeda, A., Ogurusu, T., Shichida, Y., Tokunaga, F., & Yoshizawa, T. (1978) *FEBS Lett. 92*, 77-80.
Maeda, A., Shichida, Y., & Yoshizawa, T. (1979) *Biochemistry 18*, 1449-1453.
Matsumoto, H., & Yoshizawa, T. (1975) *Nature (London) 258*, 523-526.
Matsumoto, H., Horiuchi, K., & Yoshizawa, T. (1978) *Biochim. Biophys. Acta 501*, 257-268.
Matsumoto, H., Asato, A. E., Denny, M., Baretz, B., Yen, Y.-P., Tong, D., & Liu, R. S. H. (1980) *Biochemistry 19*, 4589-4594.
Ottolenghi, M. (1982) *Methods Enzymol. 88*, 470-491.
Pauling, L. (1960) *The Nature of the Chemical Bond*, Cornell University Press, New York.
Rothschild, K. J., Roepe, P., Ahl, P. L., Earnest, T. N., Bogomolni, R. A., Das Gupta, S. K., Mulliken, C. M., & Herzfeld, J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 347-351.
Shichida, Y., Matuoka, S., & Yoshizawa, T. (1984) *Photobiochem. Photobiophys. 7*, 221-228.
Wald, G. (1968) *Nature (London) 219*, 800-807.
Yoshizawa, T. (1972) in *Handbook of Sensory Physiology* (Dartnall, H. J. A., Ed.) Vol. VII/1, pp 146-179, Springer-Verlag, Heidelberg.
Yoshizawa, T., & Wald, G. (1963) *Nature (London) 197*, 1279-1286.
Yoshizawa, T., & Shichida, Y. (1982) *Methods Enzymol. 81*, 333-354.
Yoshizawa, T., Shichida, Y., & Matuoka, S. (1984) *Vision Res. 24*, 1455-1463.

Microtubule Elongation and Guanosine 5′-Triphosphate Hydrolysis. Role of Guanine Nucleotides in Microtubule Dynamics

Marie-France Carlier,* Dominique Didry, and Dominique Pantaloni
Laboratoire d'Enzymologie, CNRS, 91190 Gif-sur-Yvette, France
Received December 29, 1986; Revised Manuscript Received March 11, 1987

ABSTRACT: The tubulin concentration dependence of the rates of microtubule elongation and accompanying GTP hydrolysis has been studied over a large range of tubulin concentration. GTP hydrolysis followed the elongation process closely at low tubulin concentration and became gradually uncoupled at higher concentrations, reaching a limiting rate of 35–40 s^{-1}. The kinetic parameters for microtubule growth were different at low and high tubulin concentrations. Elongation of microtubules has also been studied in solutions containing GDP and GTP in variable proportions. Only traces of GTP present in GDP were necessary to confer a high stability (low critical concentration) to microtubules. Pure GDP–tubulin was found unable to elongate microtubules in the absence of GTP but blocked microtubule ends with an equilibrium dissociation constant of 5–6 μM. These data were accounted for by a model within which, in the presence of GTP–tubulin at high concentration, microtubules grow at a fast rate with a large GTP cap; the GTP cap may be quite short in the region of the critical concentration; microtubule stability is linked to the strong interaction between GTP and GDP subunits at the elongating site; dimeric GDP–tubulin does not have the appropriate conformation to undergo reversible polymerization. These results are discussed with regard to the possible role of GDP and GTP and of GTP hydrolysis in microtubule dynamics.

It is well-known that GTP[1] hydrolysis accompanies microtubule assemby (Weisenberg et al., 1976) and that GTP is hydrolyzed by tubulin itself in this process (David-Pfeuty et al., 1977). The lack of tight temporal correlation between the time courses of tubulin spontaneous polymerization and accompanying hydrolysis of GTP indicated that these two reactions are not mechanistically coupled and GTP is hydrolyzed on microtubules following polymerization, which results in a core of subunits and stretches of GTP subunits at the ends of the growing microtubules [Carlier & Pantaloni, 1981; McNeal & Purich, 1978; for reviews see Carlier (1982) and Purich and Kristofferson (1984)]. These results together with the evidence for microtubule assembly in the presence of GMPPNP (Arai & Kaziro, 1976) showed that GTP hydrolysis was not necessary for tubulin polymerization; in contrast, GTP hydrolysis generates an unstable GDP polymer. The heterogeneity of microtubule subunits in GTP and GDP and the difference in their kinetic parameters were taken into account in a theoretical model for tubulin polymerization and GTP hydrolysis (Hill & Carlier, 1983) according to which the rate of microtubule elongation does not vary linearly vs. the concentration of dimeric tubulin, in contrast with the case of the reversible polymerization scheme described by Oosawa (1975); a sharp bend occurs at the critical concentration due to the rapid dissociation rate of GDP subunits from the polymer below the critical concentration. The theoretical predictions have been experimentally confirmed (Carlier et al., 1984a).

[1] Abbreviations: GTP, guanosine 5′-triphosphate; GDP, guanosine 5′-diphosphate; Ap_5A, P^1,P^5-bis(5′-adenosyl) pentaphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; GMPPNP, guanosine 5′-(β,γ-imidotriphosphate); BSA, bovine serum albumin; HPLC, high-performance liquid chromatography.

0006-2960/87/0426-4428$01.50/0

Similar observations have been reported independently (Farrell et al., 1983). Incidentally, in the case of actin depolymerizing in ATP, the same deviation from linearity has also been observed in the region of the critical concentration, and the same explanation has been proposed to account for the data (Carlier et al., 1984b).

In the model and theory initially proposed (Hill & Carlier, 1983), the simple hypothesis was made that GTP was hydrolyzed at random at a slow rate on all the GTP subunits of the GTP cap. This feature resulted in the existence of a GTP cap of appreciable length (a few tens of subunits) at the ends of microtubules at steady state, as indicated by Monte Carlo calculation (Hill & Chen, 1984; Hill, 1985; Chen & Hill, 1985a). The possibility was raised by Mitchison and Kirschner (1984a,b) of total loss of the GTP cap following sequential dissociation events of the terminal GTP subunits in the course of the statistical monomer–polymer exchange reactions at steady state. Such a total loss would result in the rapid dissociation of a large number of GDP subunits; therefore, at steady state, microtubules would oscillate between long phases of slow growth alternating with short periods of rapid depolymerization. Such an asymmetric dynamic behavior contrasts with the classical random walk monomer–polymer diffusional exchange usually exhibited by equilibrium polymers and was proposed as the basis for the observed "dynamic instability" of microtubules [for a review see Kirschner and Mitchison (1986)]. Recently, a consensus has been reached for the dynamic instability of microtubules in a variety of in vivo and in vitro systems (Kristofferson et al., 1986; Cassimeris et al., 1986; Horio & Hotani, 1986; Sammak & Borisy, 1986; McIntosh et al., 1986; Schulze & Kirschner, 1986). Phase transitions between the slowly growing state and rapidly depolymerizing state, as well as the total disappearance of microtubules in solution, have actually been generated by computer simulation of a population of microtubules at steady state, within the simple model of slow hydrolysis at random on all the subunits of a large GTP cap (Hill, 1985; Chen & Hill, 1985b).

An alternate model, where the rate of GTP hydrolysis is cooperatively enhanced on a GTP subunit adjacent to a GDP subunit, has also been evoked by Hill, for its analytical simplicity [see Carlier et al. (1984a) appendix], and the same view has also been proposed by Caplow et al. (1985). Such a mechanism results in practically one single GTPase site per elongating stretch of polymer and a vectorial hydrolysis toward the tip of the microtubule. We have shown previously (Carlier & Pantaloni, 1985) that the distinction between the cooperative and noncooperative GTP hydrolysis could be brought by experiments in which the time courses of microtubule *elongation* and accompanying GTP hydrolysis would be monitored simultaneously. Briefly, in the noncooperative case of slow hydrolysis on all the GTP subunits of the cap, the rate of GTP hydrolysis increases with the number of GTP subunits added to the polymer end until a steady state is reached (Carlier & Pantaloni, 1986a), where the polymer grows with a GTP cap of constant size, at a given concentration of dimeric tubulin; in this case a lag is expected in the time course of GTP hydrolysis accompanying the linear elongation process. GTP hydrolysis is uncoupled from the elongation process at all tubulin concentrations. In contrast, in the vectorial (cooperative) hydrolysis case, no lag is expected in the time course of GTP hydrolysis because GTP is hydrolyzed at a single site at the boundary between the GTP cap and the GDP core, at a rate independent of the size of the GTP cap. Therefore, in this case apparent coupling between polymerization and hydrolysis is observed when the rate of elongation is lower than the GTP hydrolysis; uncoupling between the two reactions is expected above a tubulin concentration c' at which the rate of elongation equals the rate of GTP hydrolysis. This study is presented here. The data show that GTP hydrolysis accompanying the elongation of pure tubulin onto microtubules appears tightly coupled to the elongation reaction at low tubulin concentration; uncoupling gradually increases above 7–9 μM tubulin in MES buffer, pH 6.8, containing 3.4 M glycerol and 6 mM $MgCl_2$. Elongation of microtubules is also studied in the presence of different proportions of GDP and GTP, in order to better understand the role of interactions between tubulin–GTP and tubulin–GDP in the mechanism of microtubule assembly.

Materials and Methods

Materials

Guanosine di- and triphosphate nucleotides were from Boehringer. ^3H- and ^{32}P-labeled nucleotides were from New England Nuclear. All other chemicals used in buffers were analytical grade. Tubulin was purified from pig brain by three cycles of polymerization according to Shelanski et al. (1973), followed by phosphocellulose (Whatman P11) chromatography according to Weingarten et al. (1975). The sample and elution buffer was 25 mM MES, pH 6.7, 0.25 mM EGTA, and 0.125 mM $MgCl_2$. Pure tubulin was concentrated by ultrafiltration over Diaflo PM30 by using an Amicon ultrafiltration cell, immediately brought to 50 mM MES, 0.5 mM EGTA, 0.25 mM $MgCl_2$, 100 μM GTP, and 3.4 M glycerol, and stored at −70 °C until use.

Methods

Protein and Nucleotide Assay. In the absence of free nucleotide, tubulin concentration was determined spectrophotometrically by using an extinction coefficient of 1.2 mg^{-1} cm^2 at 277 nm (Detrich & Williams, 1978) and a molecular weight of 10^5 (Valenzuela et al., 1981). When nucleotides were present, the Lowry assay was used (Lowry et al., 1951) with a correction coefficient of 0.89 vs. the BSA standard (David-Pfeuty et al., 1977).

Nucleotide Extraction and Determination. Nucleotides were extracted on ice with 4% $HClO_4$, followed by a buffering of the solution to pH 4 by addition of 0.4 M sodium acetate. Concentration of GTP and GDP in this acid solution was determined spectrophotometrically by using a molar extinction coefficient of 11200 cm^{-1} at 257 nm. Separation of nucleotides was performed by HPLC using a Synchropak X300 column (Synchrom) and isocratic elution with 0.35 M KH_2PO_4, pH 3.5, containing 1.33 M NaCl. The concentrations of GTP and GDP were determined from the areas of the corresponding peaks, calculated by an Hitachi D-2000 chromatointegrator, by using a calibration curve of the areas obtained for standards of GTP and GDP.

Microtubule Elongation Assay. Pure tubulin was deprived from unbound nucleotides by chromatography over Sephadex G-25 equilibrated in buffer A (50 mM MES, 0.5 mM EGTA, 0.25 mM $MgCl_2$, 3.4 M glycerol). The concentration of chromatographed tubulin was determined spectrophotometrically. Nucleotide extraction showed that, following that step, tubulin contained 2 mol of guanine nucleotide per mole of tubulin dimer. The HPLC determination of the respective amounts of bound GTP and GDP showed that routinely at most 10% of the total bound nucleotide was GDP. Assuming that only GTP is bound to the nonexchangeable site, this indicates that at least 80% of tubulin has GTP bound to the

exchangeable (E) site. The tubulin–GTP complex was then diluted to different final concentrations in buffer A. GTP (20 μM) was then added to the samples, which were incubated on ice for 1 h before starting the experiment. Control experiments were performed in the presence of 100 and 200 μM GTP and gave the same result. To an aliquot of tubulin (15–25 μM) were added 50 μM GTP and 5% Me_2SO. This solution was used for the preparation of seeds. Microtubule seeds were polymerized by addition of 6 mM $MgCl_2$ and incubation at 30 °C for 30 min before starting the experiment. Elongation of microtubules from seeds was monitored turbidimetrically at 350 nm by using a Cary 219 spectrophotometer equipped with a thermostated jacket. A 1-cm light path cuvette with thick lateral walls was used. The tubulin solution was prewarmed in the cuvette for 3 min. No turbidity change was observed during that time. The reaction was started by addition of 6 mM $MgCl_2$, followed by the seeds. The concentration of seeds was adjusted so that, in the whole range of tubulin concentrations studied, the initial rate of elongation could be conveniently measured; i.e., less than 15% of the tubulin present at time zero was polymerized onto the seeds within the period of time needed for the measurement (30 s–2 min). Generally, 1–2 μM polymerized tubulin was used as seeds. The final number concentration of microtubules in the elongation assay was determined from average length measurement of the microtubules of this seed solution by electron microscopy (Karr et al., 1980; Carlier, 1983). The average length was routinely 2–3 μm, and the final number concentration of microtubules in the assay was 0.2–0.3 nM.

Elongation of tubulin onto the seeds resulted in a linear increase in turbidity. Less than 20% of the dimeric tubulin polymerized onto the seeds during the period needed for the rate measurement, ensuring true initial rate conditions. The concentration of polymerized tubulin therefore varied in the 1–5 μM range in the experiments. A spontaneous nucleation control showed that no increase in turbidity was observed when no seeds were added, at least during the time course of measurement of initial rate. At very high tubulin concentration (above 15 μM) a slight turbidity increase occurred in the control, representing less than 10% of the rate observed in the presence of seeds, and was subtracted from the corresponding sample signal.

The conversion of turbidity changes into concentration of polymerized tubulin was done by using a calibration curve obtained as follows: several tubulin samples at different concentrations in the range 4–12 μM were processed for elongation onto 1 μM seeds by using the same instrument and in the same cuvette as above. The time course of elongation was monitored until the plateau was reached, and then the samples were spun in the airfuge at 30 °C for 8 min; warm air, circulating in a tubing placed in a water bath at 70 °C, was flown through the airfuge for 1 h prior to starting the experiment to ensure the maintenance of a temperature of 30 °C during the centrifugation. The concentration of tubulin in the supernatant was determined. A linear correlation was established between the extent of turbidity change and the amount of polymerized tubulin onto the seeds (Figure 1). Three independent measurements yielded an average value of 0.055 ± 0.003 absorbance unit per micromolar polymerized tubulin. This procedure, in which seeded polymerization was carried out rather than spontaneous polymerization, was used to avoid all possible problems of length-dependent turbidity changes.

GTP Hydrolysis Accompanying the Elongation Process. For this assay, two alternative methods were used and gave

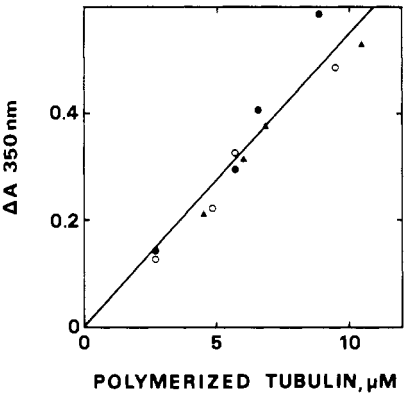

FIGURE 1: Calibration curve for the correlation between observed turbidity changes and concentration of tubulin polymerization onto microtubules seeds in the elongation assay. The experiment was performed as described under Methods. A_{350nm} represents the extent of turbidity change recorded upon elongation of tubulin onto 1 μM polymerized tubulin used as seeds. The corresponding amount of polymerized tubulin was derived from sedimentation of polymerized tubulin when the turbidity plateau was reached. The three symbols refer to three independent experiments.

the same results. In the first method, tubulin samples were prepared as in the preceding paragraph except that the 20 μM GTP was labeled with [γ-^{32}P]GTP. The specific radioactivity of GTP in each sample was calculated by knowing the concentration of free and tubulin-bound exchangeable GTP. Alternatively, pure tubulin was incubated for 60 min on ice with 100 μM γ-^{32}P-labeled GTP before Sephadex G-25 chromatography. The eluted radiolabeled tubulin–[γ-^{32}P]GTP complex was then brought to different concentrations by simple dilution in buffer A, without addition of any free GTP, and processed for the microtubule elongation and GTP hydrolysis assays. In this latter procedure, the specific radioactivity of tubulin-bound [γ-^{32}P]GTP is the same in all samples, and no assumptions have to be made about the more or less complete exchange of radiolabel on tubulin at different concentrations.

GTP hydrolysis was measured in the time course of the elongation process by taking off 50-μL aliquots from the cuvette placed in the spectrophotometer, at different time intervals following the addition of seeds, and mixing immediately in 1.5 mL of an ice-cold 10 mM ammonium molybdate solution in 1 N HCl, 4% $HClO_4$, and 0.2 mM H_3PO_4 as a carrier, followed (within 5 min) by extraction of acid-labile [^{32}P]P_i by 3 mL of cyclohexane/isobutyl alcohol/acetone/ammonium molybdate (50/50/10/1). We have checked that all [^{32}P]P_i is extracted by this method following total nucleotide hydrolysis by alkaline phosphatase.

RESULTS

Tubulin Concentration Dependence of the Rate of Microtubule Elongation. Figure 2 shows the growth was observed off microtubule seeds in a range of tubulin concentrations between 1.7 and 20 μM. In agreement with our previous report (Carlier et al., 1984a), a value of 1.7 μM was found for the critical concentration, i.e., the tubulin concentration at which the rate of elongation was zero, and the rate of growth J increased linearly with tubulin concentration up to 6–7 μM, consistent with an apparent association rate constant of 2 ± 0.2 μM^{-1} s^{-1}. However, above 7 μM, the $J(c)$ plot curved upward, and a second linear regime was clearly established, with a 2.5-fold higher apparent association rate constant. It was carefully checked that this increase in the slope of the plot was not due to spontaneous nucleation; such an artifact would in fact have resulted in a cooperative increase in the slope of the $J(c)$ plot upon increasing tubulin concentration. Because

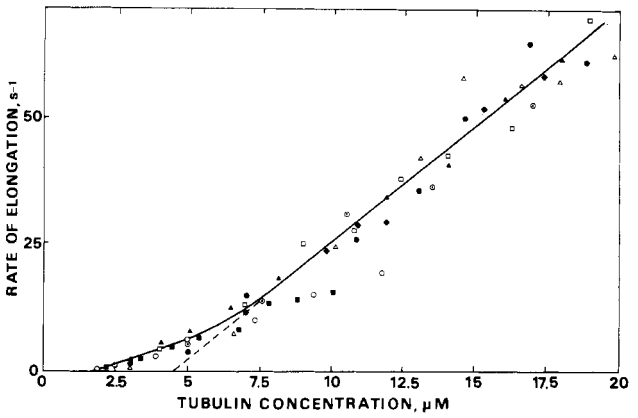

FIGURE 2: Change in the rate of microtubule elongation with the concentration of dimeric GTP–tubulin. The initial rate of turbidity increase is measured following the addition of microtubule seeds to solutions of dimeric tubulin at different concentrations. Data corresponding to eight independent experiments are superimposed and plotted in number of subunits added per second to one microtubule end vs. dimeric tubulin concentration.

the addition of up to 1–3 μM GDP necessarily accompanies the addition of seeds to the tubulin solution, we sought whether the sole addition of very small amounts of GDP could enhance spontaneous nucleation in a 15 μM tubulin solution in the presence of 25 μM GTP. Thorough measurements even in a very low range of GDP concentrations failed to demonstrate such an effect. In contrast, GDP appeared to inhibit spontaneous nucleation, as previously reported (Carlier & Pantaloni, 1978). In addition, control experiments showed that the rate of elongation, at all tubulin concentrations, was proportional to the number concentration of microtubule seeds, as further illustrated in Figure 2, which shows that superimposable plots are derived, after normalization, from experiments done with different seeds concentrations.

GTP Hydrolysis Accompanying Microtubule Elongation. In order to better understand the above result, the hydrolysis of GTP accompanying the elongation process was investigated. Figure 3 shows the simultaneous turbidimetric recordings and GTP hydrolysis measurements at different tubulin concentrations. The data show that (1) both reactions proceed linearly with time as soon as microtubule seeds are added, (2) a steady rate of GTP hydrolysis is established immediately following the addition of seeds, (3) no GTP hydrolysis can be detected when seeds are absent (and no elongation occurs), and (4) GTP hydrolysis superimposes microtubule elongation at low tubulin concentration ($c < 7$ μM) and takes place at a rate significantly lower than elongation as tubulin concentration increases. Figure 4 shows the derived plot of the initial rates of elongation (J) and GTP hydrolysis (J_H), which emphasizes the uncoupling between the two reactions. The change in the rate of GTP hydrolysis with tubulin concentration exhibits a saturation type shape and seems to reach a finite value around 35–40 s^{-1}. The downward bend in the rate of GTP hydrolysis is observed in the same range of concentration as the upward bend of the rate of elongation. The change in the ratio J/J_H with tubulin concentration is shown in the inset of Figure 4. J/J_H is equal to 1 at low tubulin concentration and increases above 7 μM, reaching a value of 2.5 at 20 μM tubulin. It should be noted that, in this experiment, pure tubulin–[γ-^{32}P]GTP complex (without any free GTP) was used, which guarantees an identical specific radioactivity of ^{32}P at all tubulin concentrations (see Methods). A control experiment done in the presence of 20 μM labeled free GTP, i.e., conditions identical with those used in Figure 2, gave the same result. This observation shows that the calculation of ^{32}P specific radioactivity within the hypothesis of complete labeled nucleotide exchange on tubulin is valid.

These data show that extent of coupling between GTP hydrolysis and microtubule elongation depends on the concentration of dimeric tubulin. At low tubulin concentration (up to 3–4-fold the critical concentration), GTP hydrolysis is concomitant with tubulin elongation and the GTP cap at the end of growing microtubules must be small; a larger GTP cap seems to exist in a higher range of tubulin concentration where microtubule growth is faster than GTP hydrolysis. In the region where the rate of GTP hydrolysis reaches a finite limit, the apparent elongation rate constant also reaches a value over 2 times higher than at low tubulin concentration.

Change in Kinetic Parameters for Microtubule Growth with Dimeric Tubulin Concentration. The fact that the rate of microtubule elongation does not vary linearly with tubulin concentration (Figure 2) implies that, as a consequence, the time course of microtubule elongation up to steady state should not obey first-order kinetics, in regions of high enough tubulin concentration. In order to test this consequence, seeded polymerization curves have been recorded at a series of tubulin concentrations, using the same amount of microtubule seeds. Turbidimetric recordings are shown in Figure 5, together with the corresponding semilogarithmic plots of the reactions. All

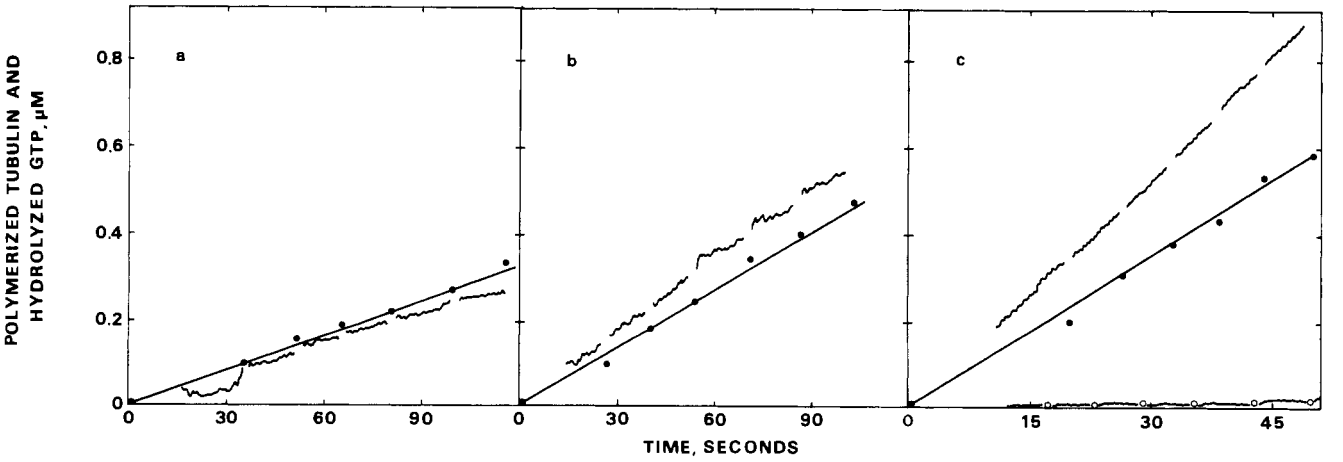

FIGURE 3: Correlation between microtubule elongation and GTP hydrolysis at different tubulin concentrations. The time courses of the turbidity change (chart recorder tracing) and hydrolyzed GTP (●) following seeds addition is shown at three different tubulin concentrations: (a) 5, (b) 7.5, and (c) 13.5 μM. The turbidity changes have been converted into micromolar polymerized tubulin by using the calibration curve shown in Figure 1. In panel c, the lower curve and open circles represent the turbidity change and correlated hydrolyzed GTP observed when no seeds were added.

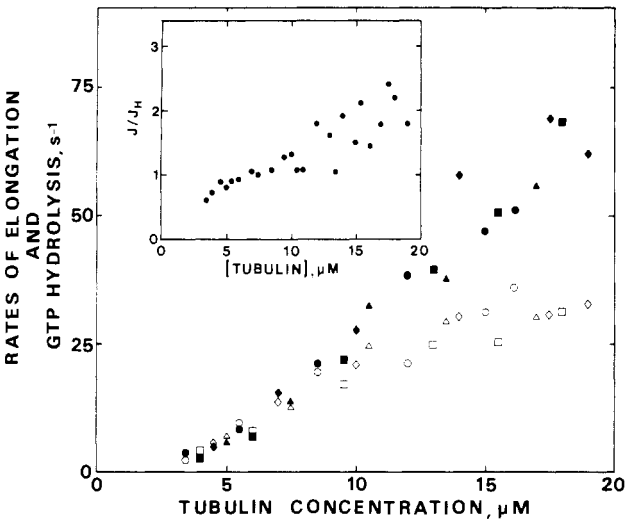

FIGURE 4: Uncoupling between microtubule elongation and accompanying GTP hydrolysis as a function of dimeric GTP-tubulin concentration. Closed symbols represent the initial rate of elongation J and open symbols the initial rate of GTP hydrolysis J_H, as derived from data such as shown in Figure 3. Different types of symbols refer to four independent series of experiments. Inset: change in the ratio J/J_H with tubulin concentration.

reactions were carried out in the range of tubulin concentrations where a linear correlation had ben established between the change in turbidity and the concentration of polymerized tubulin subunits. The data indicate that, at high tubulin concentration ($c > 7$ μM), the apparent rate constant for association of tubulin to microtubule ends has a higher value in the early times of the elongation process than at later times; in contrast, at low tubulin concentration ($c < 7$ μM), true monoexponential growth was observed, with the lower apparent association rate constant. These results, derived from the analysis of the whole elongation curve to steady state, where the concentration of dimeric tubulin varies with time, are in good agreement with the results obtained from measurements of initial rates of elongation, where the concentration of dimeric tubulin is different in each sample at $t = 0$.

Microtubule Elongation in the Presence of GDP and GTP in Varying Proportions. The role of GDP and GTP in microtubule assembly was further investigated by examining the tubulin concentration dependence of the initial rate of microtubule elongation in tubulin solutions containing both GDP and GTP in variable proportions. The free nucleotide concentration was set constant at 200 μM. Because the relative affinities of GTP and GDP toward tubulin change with Mg^{2+} ion concentration (Croom et al., 1985), the samples were preincubated with 1 mM Mg, a concentration high enough for the proportions of GTP- and GDP-tubulin to be practically the same as in the final state in the elongation assay where 6 mM Mg is present. When ratios of (GDP)/(GTP) lower than 20 were assayed, the GTP-tubulin complex was first isolated through Sephadex G-25 chromatography, diluted to different concentrations in buffer A, and adjusted to the desired concentrations of GTP and GDP. When (GDP)/(GTP) ratios of 20 and up were assayed, the GDP-tubulin complex was first prepared by a 60-min incubation of the chromatographed GTP-tubulin complex in the presence of 200 μM GDP on ice. The mixture was then again chromatographed over Sephadex G-25 to isolate the GDP-tubulin complex. The nucleotide content of GTP-tubulin and GDP-tubulin obtained following these procedures was routinely checked by HPLC of the perchloric extract. The isolated GDP-tubulin complex was then diluted to different concentrations and incubated in

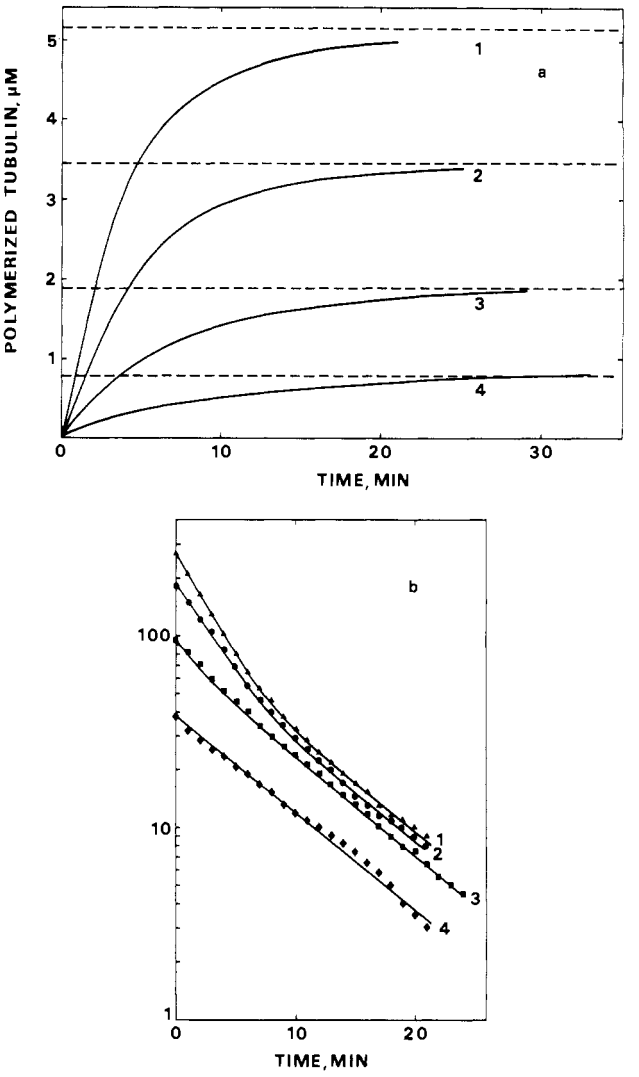

FIGURE 5: Change in kinetic parameters for microtubule elongation with concentration of dimeric tubulin. (a) Full time course of tubulin elongation onto seeds, at different tubulin concentrations: (1) 10, (2) 8, (3) 5.8, and (4) 4 μM. The same concentration of microtubule seeds was added to each sample. Turbidity changes are converted into concentrations of polymerized tubulin. (b) Semilogarithmic plot of the curves shown in panel a.

the presence of the appropriate concentrations of GTP and GDP and 1 mM Mg^{2+}.

The solution of microtubule seeds used consisted of 30–35 μM GTP-tubulin complex polymerized in the presence of 40 μM GTP. Therefore, a final maximum concentration of GTP of 2.5 μM was introduced into the elongation assay upon addition of $^1/_{16}$ volume of the assay of microtubule seeds.

Figure 6 shows a series of $J(c)$ plots obtained at different (GDP)/(GTP) ratios. Except at low ratios (1–2), where a slight upward curvature was still observed, the data obtained fitted one single straight line. The slope of the plot decreased upon increasing (GDP)/(GTP). When only GDP (200 μM) was added in the elongation assay, we estimated the (GDP)/(GTP) ratio to about 100, due to the presence of GTP in the added seeds. The values of the (GDP)/(GTP) ratio may be lower if we assume some contamination of GDP by GTP (see Figure 7). Analysis of the change in apparent critical concentration and in the slope of the $J(c)$ plot, i.e., the apparent rate constant for association of tubulin to microtubule ends, can help to understand the role of GTP and GDP in the elongation process. If, in a simple model (Bayley & Martin, 1986), only GTP-tubulin is able to elongate onto microtubule

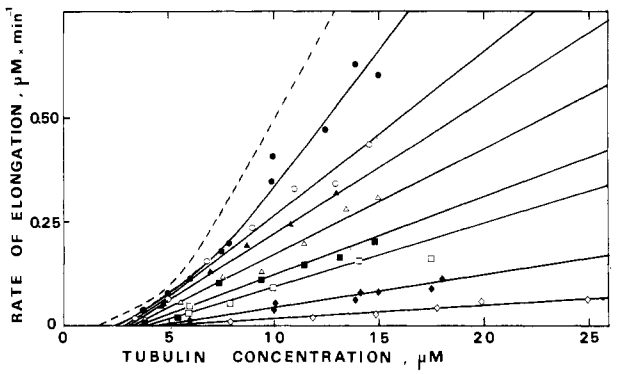

FIGURE 6: Tubulin concentration dependence of rate of microtubule elongation at different (GDP)/(GTP) ratios. Tubulin was preincubated as described in the text with 200 μM nucleotide, consisting of GDP and GTP in the following ratios: (●) 1, (○) 2, (▲) 4, (△) 8, (■) 10, (□) 20, (♦) 50, and (◇) 100. Control experiments showed that the rate of elongation in pure GTP was the same whether 20 μM or 200 μM GTP was present in the assay (the reference curve in the absence of GDP is shown by the dashed line). True elongation rates are plotted. For each series of experiments at a given (GDP)/(GTP) ratio, at least four reference points in pure GTP have been obtained. All curves have then been normalized vs. the same pure GTP reference. The average seeds concentration is 0.3 nM.

ends, and GDP solely sequesters dimeric tubulin, then the rate of elongation in the presence of GTP and GDP will be

$$J = k_+M\left(c_0\frac{\alpha}{\alpha + x} - c_c\right) \quad (1)$$

where α represents the ratio K_D/K_T of the equilibrium dissociation constants for the GDP–tubulin and GTP tubulin complexes in the elongation assay medium, x the (GDP)/(GTP) ratio, c_0 the total concentration of dimeric tubulin in the assay [c_0 = (tubulin–GTP) + (tubulin–GDP)], c_c the critical concentration for polymerization of GTP–tubulin, k_+ the rate constant for association of GTP tubulin for microtubule ends measured in the presence of pure GTP, and M the concentration of microtubule seeds in the elongation assay.

Equation 1 shows that, within this simple model, the apparent critical concentration, defined as the concentration of dimeric tubulin at which $J = 0$, increases linearly with the ratio (GDP)/(GTP):

$$c_c^{app} = c_c[1 + (x/\alpha)] \quad (2)$$

Similarly, the reciprocal of the slope of the $J(c)$ plot also varies linearly with x, according to the equation:

$$1/k^{app}M = (1/k_+M)[1 + (x/\alpha)] \quad (3)$$

The slopes of the two straight lines described by eq 2 and 3 provide an estimation of α.

Figure 7 shows that the slope of the $J(c)$ plot increases linearly with (GDP)/(GTP), indicating a value of 4–5 for the ratio $K_D/K_T = \alpha$. This value is in agreement with previous reports (Arai et al., 1975; Zeeberg & Caplow, 1979; Carlier & Pantaloni, 1978). In contrast, the change in apparent critical concentration was not linear and bent downward very strongly, showing that the critical concentration remained in the 4–5 μM range while the (GDP)/(GTP) ratio varied from 10 to 100.

Therefore, the plot in Figure 7A indicates that GDP–tubulin does not assemble into microtubules and GTP–tubulin is the only polymerizable species in the mixture; in contrast, the plot in Figure 7B shows that microtubules are fairly well stabilized, i.e., a low critical concentration is remained, when only traces of GTP are present in solution.

Therefore, additional information was needed to clarify the situation.

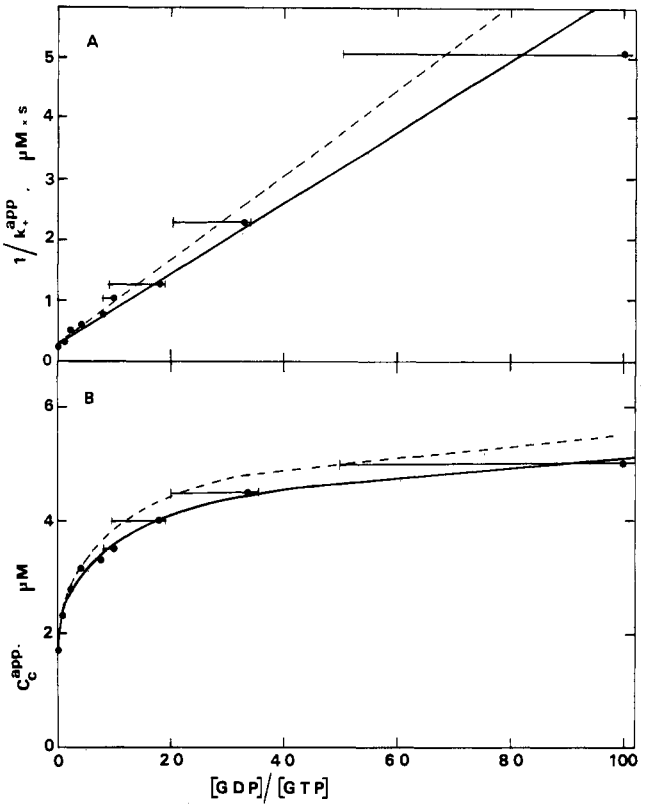

FIGURE 7: Change in elongation rate constant and apparent critical concentration with the (GDP)/(GTP) ratio. (A) The reciprocal of the slope of the $J(c)$ plots shown in Figure 6 is plotted vs. (GDP)/(GTP), according to eq 3. When the $J(c)$ plot curved upward, the higher slope was measured. The error bar and derived dashed curve account for the maximum possible uncertainty in the (GDP)/(GTP) ratio, assuming that up to 2% of GDP may be contaminated by GTP. The data are satisfactorily fitted by a straight line; the two possible extremes lines shown correspond to a 4–5-fold higher affinity of tubulin toward GTP than GDP. (B) The apparent critical concentration, as defined by the dimeric tubulin concentration at which $J = 0$ in Figure 6, is plotted vs. (GDP)/(GTP). Error bars and the two extreme curves have the same meaning as in panel A.

Characterization of the Polymer Formed upon Addition of Microtubule Seeds Formed in GTP to GDP–Tubulin. The elongation assay was performed in the presence of 200 μM GDP and 30 μM GDP–tubulin; it was checked that the increases in turbidity did reflect elongation of tubulin onto the seeds because the initial rate was proportional to the seeds concentration, and microtubules were observed to elongate both in the electron microscope and in dark-field optical microscopy; no turbidity developed in the absence of seeds. In one experiment the extent of turbidity developed after 60 min was consistent with the polymerization of 5 μM polymerized tubulin; at the same time an increase in average microtubule length from 2 to 6.6 μm was measured. Since the seeds concentration was 0.6 nM, the increase in length lets us anticipate an increase of 0.6 × 4.6 × 1640 nM = 4.5 μM polymerized tubulin, which is in agreement with the turbidimetry and microscopy measurement.

In addition, the elongation process was not at all inhibited by up to 20 μM Ap_5A, a well-known potent inhibitor of myokinase, thus eliminating the possibility of GTP generation from GDP in the medium by enzymatic catalysis. However, the elongation process was not exponential, and the critical concentration of 5 μM expected from the corresponding $J(c)$ plot was never reached; instead, after 2 h a turbidity plateau was practically reached; both turbidity and sedimentation measurements indicated that only 25–30% tubulin had po-

Table I[a]

	concentration of tubulin dimer				nucleotide concentration		
	supernatant, 30 °C	supernatant, 0 °C	microtubules = $S_{30} - S_0$	resuspended microtubule pellet	GTP in pellet	GDP in pellet	[^{3}H]GDP in pellet
concentration (μM)	18.7	27.8	9.1	17.9	16.2	16.8	6.4
% of dimeric tubulin				100	0.91	0.94	0.36

[a] GDP–tubulin at a concentration of 30 μM was prepared as described in the text. HPLC determination of the bound nucleotide showed that 48% was GDP; that is, 96% of the nucleotide bound to the E site was GDP. This complex was incubated for 2 h at 0 °C with 200 μM[^{3}H]GDP. The purity of the ^{3}H label was also checked by HPLC and was found to contain a maximum of 0.2% of radioactivity coeluting with GTP. The elongation assay was conducted by addition of $^1/_{25}$th of the total volume of a solution of GTP tubulin (27 μM) polymerized for 30 min in the presence of 40 μM GTP. After 2-h reaction the sample was divided in two parts; one was spun at 30 °C to sediment microtubules, and the other was cooled to 0 °C for 5 min and spun at 0 °C. Protein concentration in the supernatants was determined. The microtubule pellet was carefully washed, resuspended in a small volume, and processed for protein assay and HPLC determination of GDP and GTP. The concentration of [^{3}H]GDP in each fraction was derived from radioactivity measurement.

lymerized at that time, leaving 20–25 μM tubulin in the supernatant. When the solution was cooled to 4 °C, numerous rings were observed in the electron microscope, which shows that this tubulin is not inactivated and unable to assemble.

When the above experiment was conducted with [^{3}H]GDP, 35% of the tubulin that sedimented in the pellet after 2 h of the elongation process had [^{3}H]GDP bound. The data are summarized in Table I. Since nucleotide exchange is fairly complete under these conditions, we have to assume that most of the remaining polymerized tubulin (5.8 μM) had initially GTP bound before being incorporated in the polymer.

Dynamics of Microtubules in the Presence of Pure GDP–Tubulin and Total Absence of GTP. The behavior of microtubule seeds was observed in solutions of GDP–tubulin at different concentrations preincubated for 1–2 h in the presence of 200 μM GDP, 2 mM glucose, and 40 units/mL hexokinase (Boehringer) in order to ensure the total absence of GTP and its rapid disappearance from the solution upon addition of the seeds. Ap_5A (20 μM) was also added to inhibit the myokinase activity potentially contaminating the hexokinase preparation. A $J(c)$ plot was realized under these conditions and is shown in Figure 8. In the presence of GDP exclusively, microtubules were found unable to grow in solutions of tubulin up to 52 μM; essentially, depolymerization of the seeds was observed in a large range of GDP–tubulin concentrations; however, the rate of depolymerization approached zero at 20–25 μM GDP–tubulin. The data can be described by a Michaelian binding isotherm, consistent with an equilibrium dissociation constant of 5.5 μM for the binding of GDP–tubulin to microtubule ends (see the double-reciprocal plot in the inset to Figure 8). The uncertainty of the measurements when J is close to zero, however, does not allow us to eliminate the possibility of some cooperativity in the binding of tubulin–GDP. These results quantitatively document the fact that microtubules are mainly unstable in GDP and can be blocked by GDP–tubulin at concentrations above 20 μM, under these experimental conditions.

The following conclusions can be derived from the experiments described in Figures 6–8. In the presence of both GTP and GDP, essentially GTP–tubulin is incorporated into microtubules. Even when traces (<1%) of GTP are in solution with GDP, most (over 60%) of the tubulin incorporated in microtubules comes from GTP–tubulin. This proportion would be higher at lower (GDP)/(GTP) ratios as previously documented (Carlier & Pantaloni, 1982) and illustrated in Figure 7A. However, the simple scheme within which GDP–tubulin does not participate in assembly is not sufficient to account for the data. Pure GDP–tubulin does not polymerize reversibly, a finding that corroborates the quasi-absence of copolymerization between GTP– and GDP–tubulins; but GDP–tubulin binds to microtubule ends and blocks association and

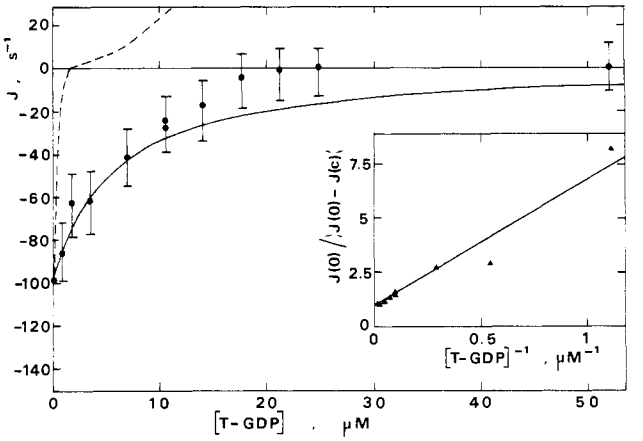

FIGURE 8: Behavior of microtubules in the presence of pure GDP–tubulin at different concentrations. Microtubule seeds were added to GDP–tubulin preincubated in the presence of 200 μM GDP, 2 mM glucose, and 40 units/mL yeast hexokinase. The initial rate of turbidity change was measured. As a reminder, the corresponding plot in GTP is shown by the dashed line. A double-reciprocal plot of the data, showing binding of GDP–tubulin to microtubule ends, is shown in the inset. The solid curve is the corresponding Michaelian isotherm consistent with an equilibrium dissociation constant of 5.5 μM for binding of tubulin–GDP to microtubule ends.

dissociation reactions. This explains why polymerization in the presence of GDP and traces of GTP proceeds until all the GTP is exhausted from the solution and stops *if* the concentration of dimeric GDP–tubulin remaining in solution is high enough to block microtubule elongation; if it is too low, spontaneous depolymerization of microtubules can be observed (Carlier & Pantaloni, 1978).

Discussion

In this work we have attempted to get a more detailed insight into the mechanism through which GTP hydrolysis is coupled to microtubule assembly and in the respective roles of GTP and GDP in the regulation of microtubule dynamics. The following conclusions have been reached: GTP hydrolysis accompanying tubulin elongation onto microtubule seeds follows closely the elongation process at low tubulin concentration and is more largely uncoupled from polymerization at high tubulin concentration. The rate of GTP hydrolysis appears to reach a finite limit of about 35 s^{-1} at high tubulin concentration. Therefore, growing microtubules have a large cap of GTP subunits at their ends at high tubulin concentration (e.g., above 7 μM under our experimental conditions) and a much shorter GTP cap in a region of lower tubulin concentration, i.e., close to the critical concentration. These results confirm our previous observations that GTP hydrolysis is not mechanistically coupled to the polymerization reaction (Carlier & Pantaloni, 1981). In that initial work, essentially the

correlation between spontaneous polymerization and accompanying GTP hydrolysis had been analyzed. On the other hand, the use of the seeded elongation assay, in the present work, circumvents the problem of the lag time due to the nucleation reaction and now enables us to better understand the molecular mechanism of GTP hydrolysis on growing microtubules, as theoretically developed previously (Carlier & Pantaloni, 1985). The fact that GTP hydrolysis proceeds without lag, that is, linearly with time at any given tubulin concentration, indicates that GTP hydrolysis is independent of the size of the GTP cap and therefore does not proceed at random at the same slow rate on all the terminal GTP subunits of the growing microtubule, as had been first proposed, on the basis of our earlier less detailed studies. Instead, a model within which GTP would be hydrolyzed at a fast rate on a GTP subunit adjacent to a GDP subunit, thus creating a GDP–GTP boundary, migrating rapidly at a constant rate (here 35 s^{-1}) toward the tip of the growing microtubule, as initially theoretically developed by T. L. Hill [Carlier et al. (1984a), Appendix] and also proposed by Caplow et al. (1985), would account better for the present data. Note that, in the model of the hydrolysis at random on any subunit of a large cap, the lag should have a value of $1/k_h$, independent of the concentration of dimeric tubulin, and the steady-state rate of GTP hydrolysis should be equal to the rate of elongation at all tubulin concentrations. Examination of Figure 3c shows that these two conditions are not fulfilled: indeed, a short lag of a few seconds could have been missed in the mixing time of the experiment, but then the identity of the steady-state rates of elongation and hydrolysis should be observed at all concentrations following this short lag, which is not the case; on the other hand, the uncoupling observed at high concentration could be attributed to the initial part of a long lag, but then the same long lag should be observed at low concentration, which is evidently not the case. Therefore, we favor the interpretation of the involvement of cooperative tubulin–tubulin interactions in GTP hydrolysis. Within this model, both GDP and GTP could be bound to the elongating sites of the microtubule at low tubulin concentration; this "D–T" configuration of the microtubule end would be characterized by a low critical concentration (1.7 μM). At higher tubulin concentration, the rate of growth increases and can overcome the rate of GTP hydrolysis, leading to a more and more represented "T–T" configuration of the elongating site, characterized by a larger critical concentration (4.5 μM), corresponding to a somewhat less stable polymer. The peculiar shape of the $J(c)$ plot, exhibiting an upward bend as a consequence of this change in growth regime, is at variance with the shape predicted by Caplow and Reid (1985) within a scheme of nonproductive binding of GTP–tubulin subunits to microtubule ends. Our results are not in disagreement with recent reports suggesting that a short GTP cap exists at steady state (O'Brien et al., 1986; Murphy & Wallis, 1986).

Our present view implies that a particular type of heterologous interaction between GDP and GTP subunits at the end of microtubules is extremely strong and maintains the stability of the otherwise unstable GDP core. The data obtained for microtubule elongation in the presence of mixtures of GTP and GDP support this view since they indicate that (1) although the apparent rate of elongation is inhibited, upon increasing (GDP)/(GTP), consistent with the model in which GDP–tubulin does not polymerize, nevertheless in the same range, the apparent critical concentration does not increase linearly with (GDP)/(GTP) but appears "buffered" and maintained at values much lower than expected within a simple model where GDP solely sequesters tubulin; (2) GDP–tubulin can be partly incorporated into microtubules as soon as only traces of GTP are present; and (3) in contrast, when all trace of GTP is eliminated, absolutely no growth of microtubule in GDP can be observed. The $J(c)$ plot obtained in the presence of pure GDP–tubulin can be best described as a binding isotherm, the GDP–tubulin complex blocking all association and dissociation processes by binding to the polymer ends with an equilibrium dissociation constant of 5–6 μM. The "nonproductive binding" (Caplow & Reid, 1985) of GDP–tubulin to microtubule ends therefore accounts for our data in pure GDP.

Discrepancies have been reported by different groups concerning the effect of GDP on microtubules, both in vitro and in vivo, some observations leading to the conclusion that microtubules depolymerize totally in GDP and that GDP–tubulin does not polymerize (Carlier & Pantaloni, 1982; Engelborghs & Van Houtte, 1981), some others indicating the existence of a "metastable state" of microtubules in GDP (Margolis, 1981; Zackroff et al., 1980) and showing the persistence of microtubules in cells blocked by azide, a situation presumably representing the GDP state (De Brabander et al., 1982). Although the situation is not yet totally analyzed, the present data may offer some reconciliation of previous observations since they show that (1) only traces of GTP can maintain the stability of microtubules and (2) although microtubules depolymerize in pure GDP in a large range of tubulin concentrations and cannot be assembled from pure GDP–tubulin, they can be blocked by GDP–tubulin above 20 μM (under our experimental conditions), which may explain the "metastable state". In cells treated by azide, the concentration of dimeric GDP–tubulin, following a partial depolymerization of microtubules, may have been high enough to block the remaining ones.

The failure of GDP–tubulin to reversibly elongate microtubules while it actually binds to microtubule ends suggested that the conformation of free GDP–tubulin is very different from that of the polymerized GDP subunit in the microtubule, once GTP hydrolysis has occurred. Therefore, some at least of the microtubular interaction areas between tubulin subunits are hindered on the GDP–tubulin dimer. They are not all hindered, though, since GDP–tubulin actively binds to microtubule ends, and double rings of GDP–tubulin are observed at 0 °C in the elongation assay medium, in agreement with Howard and Timasheff (1986).

A tentative scheme of the microtubule end in the growing state and at steady state under different GDP–GTP conditions is drawn in Figure 9 and summarizes our results. The main features of this scheme that are suggested by ours and others' results are as follows: (1) The kinetic parameters for microtubule growth in GTP are different at high concentration of GTP–tubulin where the elongating site is only composed of GTP subunits (intermediate state I) and at steady state, where interactions between GTP and GDP subunits are involved in the association–dissociation processes (intermediate state II); (2) GDP subunits, once exposed at the tip of the microtubule, depolymerize at a fast rate (intermediate state III); (3) GDP subunits dissociating from the microtubules isomerize (in the absence of GTP) into a tubulin–GDP species that can only block the ends (intermediate state IV). The relative proportions of microtubule ends in states I–IV depend on the concentrations of tubulin–GDP and GTP. Dynamic instability is linked to the transitions between states II and III. No dynamic instability is expected for microtubules in state IV (pure GDP).

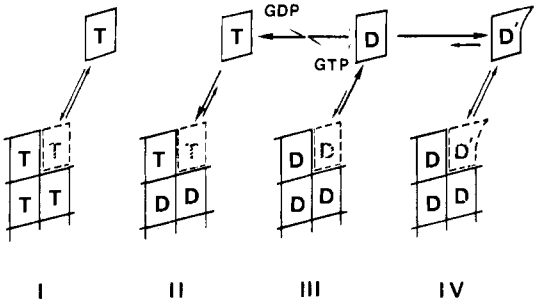

FIGURE 9: Minimum scheme showing the four different states of microtubule ends in GTP and GDP. State I represents the growing state of microtubules in GTP, at concentrations of GTP-tubulin for above the critical concentration. A cap of GTP subunits exists at the tip of the polymer. At steady state, transitions occur between state II, characterized by the very stable D–T configuration of the elongating site, and the totally uncapped state III, characterized by the fast depolymerization of the GDP subunits. Tubulin–GDP subunits that dissociate from microtubules isomerize into a species tubulin–GDP', featured with a horned shape. The equilibrium (K_{is}) is strongly toward the tubulin–GDP' conformation. This complex can bind to microtubule ends in rapid equilibrium (K_D = 5.5 μM), leading to the blocked state IV.

The most important question raised by this work and awaiting an answer is the following: the dynamic instability model proposed by Mitchison and Kirschner (1984a,b) to interpret the large fluctuations in length observed on microtubules in the region of the critical concentration relied on the hypothesis, sustained by our previous work, of a large GTP cap at the ends of microtubules. The large size of the GTP cap and the very rapid rate of depolymerization of GDP subunits were the two main reasons for the two phases (slow growing and rapid shrinking) both experimentally observed and theoretically modeled by Monte Carlo calculations. Our present work, however, shows that at steady state the GTP cap could be reduced to a very small size, maybe less than 10 subunits, e.g., one GTP subunit per elongating site. Is it still possible to generate large phase changes involving the gain and loss of only a few GTP subunits? We believe the answer may be yes, if some cooperativity exists between the different adjacent helices or corners that compose the elongating site. The fact that the stable "D–T" configuration of the microtubule end seems to persist over a large range of (GDP)/(GTP) ratios indicates that it is difficult to lose this small GTP cap and gives some credit to this idea. An analysis of the possible phase changes of a polymer with a short XTP cap has recently been developed by Hill (1987).

Finally, an interesting parallel between the microtubule and the actin system has to be drawn: with Mg–actin similar data concerning the correlation between the rates of filament elongation, monitored fluorometrically, and accompanying ATP hydrolysis has been obtained and analyzed with a similar model (Pantaloni et al., 1985a,b; Hill, 1986; Carlier et al., 1987). The rate constants for ATP hydrolysis on Mg–F-actin (13.6 s^{-1}) and for GTP hydrolysis on microtubules (35–40 s^{-1}) are of the same order of magnitude. Note that similar hydrolysis rate constant values (20–50 s^{-1}) have been found for another GTPase reaction catalyzed by EF-Tu on the binding of Phe-tRNA–EF-Tu to poly(U)-programmed ribosome (Eccleston et al., 1985). In the case of actin, reversible polymerization of ADP–actin can be observed, although with a 25-fold larger critical concentration than in the presence of ATP (Carlier et al., 1984a; Pollard, 1984; Lal et al., 1984). This difference between the microtubule and microfilament system may be due to the higher structural strains imposed to the tubulin–tubulin interactions in the microtubular lattice than to the actin–actin interactions in the microfilament. In the same respect, nucleation of actin filaments is largely enhanced in mixtures of ATP- and ADP–actin as compared to pure ATP–actin or pure ADP–actin solutions (Pantaloni et al., 1985b). In contrast, with tubulin, we have been unable to observe any increase in the rate of nucleation of microtubules by addition of GDP (even in my small amounts) to solutions of GTP–tubulin. Again, this difference suggests that the heterologous interactions between GTP–tubulin and GDP–tubulin that take place at microtubule ends to stabilize the polymer are not the same as the ones that are involved in the formation of the primary nucleus.

It should be pointed out that, in all our experiments, both microtubule ends are free; therefore, we cannot quantitatively assess the contribution of each of the two ends in the rates of elongation and GTP hydrolysis, and in the upward curvature exhibited by the $J(c)$ plot. Experiments done with an agent blocking one end should tell whether essentially the (+) end contributes, as in the case of actin (Carlier et al., 1986).

Finally, recent evidence has been obtained for the intermediate F–ADP–P_i–actin in the polymerization of ATP–actin (Carlier & Pantaloni, 1986). Similarly, we may expect that GDP–P_i–tubulin also exists as an intermediate in microtubule assembly, which would obviously complicate the analysis of microtubule dynamics.

ADDED IN PROOF

The works of Mitchison and Kirschner (1984a,b) and of Horio and Hotani (1986) have shown that microtubules depolymerize very fast at the *two* ends below their critical concentration [that is, the $J(c)$ plots bend downward below c_c at the two ends]. It is easy to figure out graphically that if the upper branch of the $J(c)$ plots was strictly linear for the two ends, the sum of the two plots would also have a linear upper branch; therefore, the upward curvature observed here when the two ends contribute must be due to an upward curvature, above the critical concentration, of the $J(c)$ plot at one of the two ends at least. This conclusion is at variance with the one proposed for actin by Pollard (1986) in a paper that appeared while the present one was being reviewed. Note that, in the case of actin too, the ATPase data (Carlier et al., 1987) confirm the elongation pattern and the implications of the previously proposed model (Pantaloni et al., 1985a,b; Hill, 1986).

ACKNOWLEDGMENTS

We are grateful to Dr. E. W. Taylor for a stimulating discussion. We thank Jean Laporte for help in the electron microscopy experiments and Christian Cibert for help in dark-field microscope observation of microtubules.

REFERENCES

Arai, T., & Kaziro, Y. (1976) *Biochem. Biophys. Res. Commun. 69*, 369–376.

Arai, T., Ihara, Y., Arai, K., & Kaziro, Y. (1975) *J. Biochem. (Tokyo) 77*, 647–658.

Bayley, P. M., & Martin, S. R. (1986) *Biochem. Biophys. Res. Commun. 137*, 351–358.

Caplow, M., & Reid, R. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3267–3271.

Caplow, M., Shanks, J., & Brylawski, B. P. (1985) *Can. J. Biochem. Cell Biol. 63*, 422–429.

Carlier, M. F. (1982) *Mol. Cell. Biochem. 47*, 97–113.

Carlier, M. F. (1983) *J. Biol. Chem. 258*, 2415–2420.

Carlier, M. F., & Pantaloni, D. (1978) *Biochemistry 17*, 1908–1915.

Carlier, M. F., & Pantaloni, D. (1981) *Biochemistry 20*, 1924–1932.

Carlier, M. F., & Pantaloni, D. (1982) *Biochemistry 21*, 1215–1224.
Carlier, M. F., & Pantaloni, D. (1985) in *Microtubules and Microtubule Inhibitors*, pp 61–69, Elsevier, Amsterdam.
Carlier, M. F., & Pantaloni, D. (1986a) *Ann. N.Y. Acad. Sci. 466*, 496–509.
Carlier, M. F., & Pantaloni, D. (1986b) *Biochemistry 25*, 7789–7792.
Carlier, M. F., Hill, T. L., & Chen, Y. (1984a) *Proc. Natl. Acad. Sci. U.S.A. 81*, 771–775.
Carlier, M. F., Pantaloni, D., & Korn, E. D. (1984b) *J. Biol. Chem. 259*, 9983–9986.
Carlier, M. F., Criquet, P., Pantaloni, D., & Korn, E. D. (1986) *J. Biol. Chem. 261*, 2041–2050.
Carlier, M. F., Pantaloni, D., & Korn, E. D. (1987) *J. Biol. Chem. 262*, 3052–3059.
Cassimeris, L. U., Wadsworth, P., & Salmon, E. D. (1986) *J. Cell Biol. 102*, 2023–2032.
Chen, Y., & Hill, T. L. (1985a) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1131–1135.
Chen, Y., & Hill, T. L. (1985b) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4127–4131.
Croom, H. B., Correia, J. J., Baty, L. T., & Williams, R. C., Jr. (1985) *Biochemistry 24*, 768–775.
David-Pfeuty, T., Erickson, H. P., & Pantaloni, D. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5372–5376.
DeBrabander, M., Genens, G., Nhydens, R., Willebrords, R., & de Mey, J. (1982) *Cold Spring Harbor Symp. Quant. Biol. 47*, 227–240.
Detrich, H. W., III, & Williams, R. C., Jr. (1978) *Biochemistry 17*, 3900–3907.
Eccleston, J. F., Dix, D. B., & Thompson, R. C. (1985) *J. Biol. Chem. 260*, 16237–16241.
Engelborghs, Y., & Van Houtte, A. (1981) *Biophys. Chem. 14*, 195–202.
Farrell, K. W., Himes, R. H., Jordan, M. A., & Wilson, L. (1983) *J. Biol. Chem. 258*, 14148–14156.
Hill, T. L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 431–435.
Hill, T. L. (1986) *Biophys. J. 49*, 981–986.
Hill, T. L. (1987) in *Linear Aggregation Theory in Cell Biology*, Chapter 8, Springer, New York.
Hill, T. L., & Carlier, M. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7234–7238.
Hill, T. L., & Chen, Y. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5772–5776.
Horio, T., & Hotani, H. (1986) *Nature (London) 321*, 605–607.
Howard, W. D., & Timasheff, S. N. (1986) *Biochemistry 25*, 8292–8300.
Karr, T. L., Kristofferson, D., & Purich, D. L. (1980) *J. Biol. Chem. 255*, 8560–8566.
Kirschner, M. W., & Mitchison, T. (1986) *Cell (Cambridge, Mass.) 45*, 329–342.
Kristofferson, D., Mitchison, T., & Kirschner, M. W. (1986) *J. Cell Biol. 102*, 1007–1019.
Lal, A. A., Brenner, S. L., & Korn, E. D. (1984) *J. Biol. Chem. 259*, 13061–13065.
Margolis, R. L. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1586–1590.
McIntosh, J. R. (1984) *Nature (London) 312*, 196–197.
McIntosh, J. R., Saxton, W. M., & Stemple, D. L. (1986) *J. Cell Biol. 103*, 270a.
McNeal, R. L., & Purich, D. L. (1978) *J. Biol. Chem. 253*, 4683–4687.
Mitchison, T., & Kirschner, M. W. (1984a) *Nature (London) 312*, 232–237.
Mitchison, T., & Kirschner, M. W. (1984b) *Nature (London) 312*, 237–242.
Murphy, D. B., & Wallis, K. T. (1986) *J. Cell Biol. 103*, 132a.
O'Brien, E. T., Voter, W. A., & Erickson, H. P. (1986) *J. Cell Biol. 103*, 132a.
Oosawa, F. (1975) in *Thermodynamics of the Polymerization of Protein*, Academic, New York.
Pantaloni, D., Hill, T. L., Carlier, M. F., & Korn, E. D. (1985a) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7207–7211.
Pantaloni, D., Carlier, M. F., & Korn, E. D. (1985b) *J. Biol. Chem. 260*, 6572–6578.
Pollard, T. D. (1984) *J. Cell Biol. 99*, 769–777.
Pollard, T. D. (1986) *J. Cell Biol. 103*, 2747–2754.
Purich, D. L., & Kristofferson, D. (1984) *Adv. Protein Chem. 36*, 133–212.
Sammak, P. J., & Borisy, G. G. (1986) *J. Cell Biol. 103*, 270a.
Schulze, E., & Kirschner, M. W. (1986) *J. Cell Biol. 102*, 1020–1031.
Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.
Valenzuela, P., Quiroga, M., Zaldivar, J., Rutter, W. J., Kirschner, M. W., & Cleveland, D. W. (1981) *Nature (London) 289*, 650–655.
Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1858–1862.
Weisenberg, R. C., Deery, W. J., & Dickinson, P. J. (1976) *Biochemistry 15*, 4248–4254.
Zackroff, R. V., Deery, W. J., & Weisenberg, R. C. (1980) *J. Mol. Biol. 139*, 641–659.
Zeeberg, B., & Caplow, M. (1979) *Biochemistry 18*, 3880–3886.