ACKNOWLEDGMENTS

We thank Joel E. Morgan for very helpful discussions and Professor G. T. Babcock for permission to discuss the results of his research prior to publication.

REFERENCES

Artzatbanov, V. Y., Konstantinov, A. A., & Skulachev, V. P. (1978) *FEBS Lett. 87*, 180–185.

Blair, D. F., Bocian, D. F., Babcock, G. T., & Chan, S. I. (1982) *Biochemistry 21*, 6928–6935.

Copeland, R. A., Smith, P. A., & Chan, S. I. (1987) *Biochemistry 26*, 7311–7316.

Ellis, W. R., Jr. (1986) Ph.D. Thesis, California Institute of Technology, Pasadena, CA.

Gelles, J., Blair, D. F., & Chan, S. I. (1986) *Biochim. Biophys. Acta 853*, 205–236.

Goodman, G. (1984) *J. Biol. Chem. 259*, 15094–15099.

Hartzell, C. R., & Beinert, H. (1974) *Biochim. Biophys. Acta 368*, 318–338.

Nilsson, T., Gelles, J., Li, P. M., & Chan, S. I. (1988a) *Biochemistry 27*, 296–301.

Nilsson, T., Copeland, R. A., Smith, P. A., & Chan, S. I. (1988b) *Biochemistry* (submitted for publication).

Scholes, C. P., & Malmström, B. G. (1986) *FEBS Lett. 198*, 125–129.

Wang, H., Blair, D. F., Ellis, W. R., Jr., Gray, H. B., & Chan, S. I. (1986) *Biochemistry 25*, 167–171.

Wikström, M. (1978) in *The Proton and Calcium Pumps* (Azzone, G. F., et al., Eds.) pp 215–226, Elsevier/North-Holland, New York.

Wikström, M., & Sigel, E. (1979) in *Membrane Biochemistry* (Carafoli, E., & Semenza, G., Eds.) pp 82–91, Springer-Verlag, New York.

Wikström, M., & Casey, R. P. (1985) *J. Inorg. Biochem. 23*, 327–334.

Wikström, M., Krab, K., & Saraste, M. (1981) *Cytochrome Oxidase, A Synthesis*, Academic, New York.

Stabilization of Microtubules by Inorganic Phosphate and Its Structural Analogues, the Fluoride Complexes of Aluminum and Beryllium†

M. F. Carlier,*,‡ D. Didry,‡ R. Melki,‡ M. Chabre,§ and D. Pantaloni‡

Laboratoire d'Enzymologie, CNRS, 91198 Gif-sur-Yvette, France, and Laboratoire de Biophysique Moléculaire et Cellulaire, CENG, Boite Postale 85, 38041 Grenoble, France

Received March 1, 1988

ABSTRACT: In order to elucidate how the elementary reactions of GTP cleavage and subsequent inorganic phosphate (P_i) release, which accompany microtubule assembly, regulate microtubule dynamics, the effect of P_i and of its structural analogues AlF_4^- and BeF_3^- on the stability of GDP–microtubules has been investigated. Inorganic phosphate binds to microtubules with a low affinity (K_D = 25 mM) and slows down the rate of GDP–subunit dissociation by about 2 orders of magnitude. AlF_4^- and BeF_3^- exhibit phosphate-like effects with 1000-fold higher affinity. Evidence has been obtained for direct binding of BeF_3^- to microtubules with a stoichiometry of 1 mol of BeF_3^- per mole of GDP–subunit and an equilibrium dissociation constant of 12–15 μM. AlF_4^- and P_i compete for this site. Phosphate analogues abolish oscillatory polymerization kinetics and slow down microtubule turnover at steady state. In view of these results, we propose that P_i and its structural analogues bind to the site of the γ-phosphate of GTP in the E site and reconstitute a GDP–P_i–microtubule, from which tubulin subunits dissociate very slowly. We therefore understand that, following GTP cleavage on microtubules, P_i release in the medium is accompanied by a structural change resulting in a large destabilization of the polymer. A cap of slowly dissociating GDP–P_i–subunits prevents depolymerization of the microtubule GDP–core at steady state. The similarity with the actin system [Carlier, M.-F., & Pantaloni, D. (1988) *J. Biol. Chem. 263*, 817–825] is underlined.

Microtubules behave in a paradoxical fashion with regard to classical thermodynamics. While systems generally evolve toward a greater stability, microtubules form from GTP–tubulin, GTP is hydrolyzed following the incorporation of tubulin, P_i[1] is released into the medium (Weisenberg et al., 1976; Carlier & Pantaloni, 1981), and the resulting GDP–microtubules are unstable and rapidly lose subunits upon dilution; in contrast, in a regime of growth, the dissociation rate constant of terminal subunits is very low (Hill & Carlier, 1983; Carlier et al., 1984). The loss and rebuilding of a putative GTP–cap has been, to date, the only proposed (Mitchison & Kirschner, 1984) and successfully modeled (Chen & Hill, 1985) hypothesis accounting for the reported dynamic instability features of microtubules (Horio & Hotani, 1986; Cassimeris et al., 1986; Sammak et al., 1987). If some of the kinetics (Johnson & Borisy, 1977) of microtubule assembly are well accounted for by models of reversible polymerization, the dynamic instability behavior is most likely explained by the absence of microreversibility, due to the involvement of GTP hydrolysis, in some step of the assembly process.

† The CNRS group is supported in part by the Ligue Nationale Française contre le Cancer.

‡ CNRS.

§ CENG.

[1] Abbreviations: P_i, inorganic phosphate; MES, 4-morpholineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

0006-2960/88/0427-3555$01.50/0

To understand in greater detail how the elementary steps of GTP cleavage and subsequent P_i release affect microtubule dynamics, we have investigated the effects of inorganic phosphate on microtubule assembly. This work was triggered by our recent finding [Carlier & Pantaloni, 1987, 1988; for a review, see Korn et al. (1987)] that, in the closely related actin system, P_i release following ATP cleavage on actin filament regulates F-actin dynamics by destabilizing the filament. The complexes of aluminum and beryllium with fluoride, AlF_4^- and BeF_3^- (Brosset & Orring, 1943; Goldstein, 1964; Mesmer & Baes, 1969), affect the activity of other G-proteins (Bigay et al., 1987; Sternweis & Gilman, 1982) and have recently been proposed to act as structural analogues of inorganic phosphate, mimicking the γ-phosphate of GTP in its binding site (Bigay et al., 1985). Therefore, the effects of these compounds on microtubule assembly have also been investigated.

Materials and Methods

All usual chemicals were of Merck analytical grade. MES was from Calbiochem; GTP and EGTA were from Sigma; aluminum nitrate and beryllium sulfate (Gold label compounds) came from Aldrich; ^7Be, [^{3}H]GTP, and [γ-^{32}P]GTP came from Amersham.

Pure dimeric tubulin was purified from pig brain by three assembly cycles (Shelanski et al., 1973) and phosphocellulose chromatography (Weingarten et al., 1975). Polymerization was monitored turbidimetrically at 350 nm or by sedimentation in the airfuge driven by a stream of heated air. Essentially two polymerization buffers were used. PG buffer consisted of 50 mM MES, pH 6.8, 3.4 M glycerol, 6 mM $MgCl_2$, and GTP as indicated. PM buffer was made of 100 mM MES, pH 6.8, 12 mM $MgCl_2$, 40 mM potassium acetate, and GTP. The absence of glycerol in PM buffer ensures better conditions for observation of dynamic instability (Carlier et al., 1987a).

The effect of inorganic phosphate on the rate of microtubule depolymerization upon dilution was measured by using the glass fiber filter (GFC, Whatman) assay as described by Job et al. (1985) with the following modifications. Tubulin (25 μM) was incubated for 2 h on ice in PG buffer containing 50 μM ^{3}H-labeled GTP, polymerized to steady state at 37 °C and diluted 50-fold at time 0 into PG buffers containing 50% sucrose and increasing concentrations, in the range 0–150 mM, of inorganic phosphate (from a 500 mM phosphate solution adjusted to pH 6.8). A constant ionic strength equivalent to 150 mM P_i, pH 6.8, was maintained by adding the appropriate amount of sodium sulfate to each dilution buffer. The addition of sucrose only helped to slow down the depolymerization process, thus providing an easier measurement of the initial rate of depolymerization; the effect of P_i itself was otherwise unaffected by sucrose. Aliquots of each diluted sample were taken off at different times following dilution, fixed with 0.5% glutaraldehyde, and processed for filtration, washing, and determination of the amount of microtubules still present at time t by measurement of the ^{3}H nucleotide radioactivity trapped on the filter.

Binding of beryllium fluoride to microtubules was measured by using ^7Be in a sedimentation assay. Tubulin (40–60 μM) in 50 mM MES, pH 6.8, 3.4 M glycerol, 6 mM $MgCl_2$, 0.5 mM GTP, 5 mM NaF, and ^7Be was split into a series of 150-μL airfuge tubes, each containing a different known amount of unlabeled beryllium. The samples were brought to 37 °C for 20 min and spun at 175000g for 5 min in the airfuge. The amount of beryllium bound to microtubules was determined from measurements of total ^7Be radioactivity present in solution before centrifugation and radioactivity

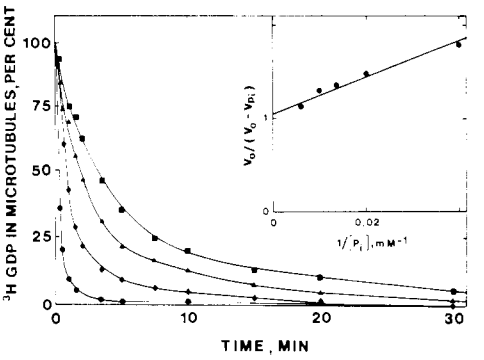


FIGURE 1: Effect of inorganic phosphate on the depolymerization of microtubules upon dilution. Microtubules were polymerized from pure tubulin (30 μM) in PG buffer containing 50 μM [^{3}H]GTP at 30 °C. At time 0, microtubules were 50-fold diluted at 30 °C (see Materials and Methods) in the presence of inorganic phosphate at the following millimolar concentrations: (●) 0; (♦) 50; (▲) 100; (■) 150. The time course of depolymerization was monitored by the filter assay (see Materials and Methods). Inset: double-reciprocal plot of the change in initial rate of depolymerization vs phosphate concentration.

remaining in the supernatant of pelleted microtubules. Control experiments were carried out in the absence of NaF and in the absence of tubulin. Because carrier-free ^7Be is obtained by electron bombardment of an aluminum alloy, it contains Al^{3+} ions (information from Amersham) that can compete with Be for binding to microtubules; the determination of the equilibrium dissociation constant K_{Be} for microtubules was carried out by using several different amounts of ^7Be in the assay described above. The change of the dissociation constant K_{Be} with the amount of ^7Be was compared to a calibration curve of the change of K_{Be} with exogenous Al^{3+}, allowing both the derivation of K_{Be} extrapolated to zero concentration of ^7Be (i.e., Al = 0) and the amount of Al^{3+} contaminating the solution of carrier-free ^7Be, which was estimated to be 0.57 mM. Usually less than 10 μM Al^{3+} coming from ^7Be was present in solution. ^7Be radioactivity was measured with a liquid scintillation spectrometer (Packard 2000 CA).

Measurements of GTP hydrolysis were carried out by using [γ-^{32}P]GTP and phosphomolybdate extraction in hydrochloric acid solution as previously described (Carlier et al., 1987b).

Results

Effect of Inorganic Phosphate and Its Structural Analogues on the Rate of Microtubule Depolymerization upon Dilution. When diluted to below the critical concentration for assembly, microtubules depolymerize, losing GDP–subunits at a fast rate. Figure 1 shows that the presence of inorganic phosphate in the dilution buffer specifically decreased the rate of depolymerization. The P_i concentration dependence of the initial rate of GDP–subunit loss from microtubule ends indicated that the effect of P_i was mediated by its rapid binding to GDP–tubulin in the microtubules with an equilibrium constant of 25 mM (Figure 1, inset). At saturating P_i, the rate of depolymerization was about 2 orders of magnitude slower than the rate of dissociation of GDP–subunits measured in the presence of Na_2SO_4 in the place of P_i.

Stabilization of Microtubules by Inorganic Phosphate and Analogues in the Absence of Free GTP. In the absence of free GTP, GTP–tubulin (1:1 complex) polymerizes into microtubules that spontaneously depolymerize once GTP has been exhausted (Carlier & Pantaloni, 1978). The presence of GTP is necessary to maintain microtubules at steady state.

When the tubulin–GTP complex was polymerized in the absence of free GTP and in the presence of 25 mM P_i, the rate

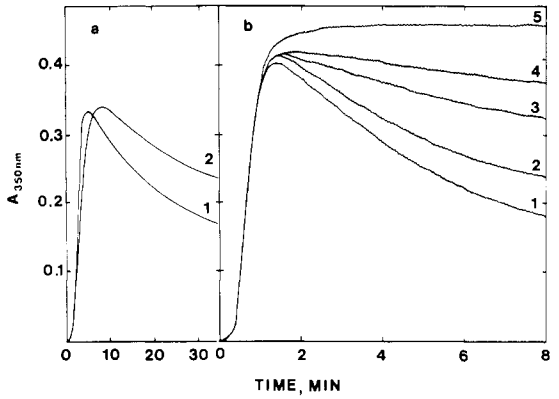


FIGURE 2: Stabilization of microtubules in the absence of GTP by inorganic phosphate and its analogues AlF_4^- and BeF_3^-. The 1:1 GTP–tubulin complex (52 μM) was isolated free of unbound GTP by Sephadex G-25 chromatography and polymerized in PG buffer in the absence of free GTP and with additions as indicated. Polymerization was measured turbidimetrically at 350 nm. Panel a: 20 mM Na_2SO_4 (curve 1) or 25 mM sodium phosphate, pH 6.8 (curve 2), was present during polymerization. Panel b: GTP–tubulin was polymerized in the presence of 25 μM Al^{3+} (1), 1 mM NaF (2), 1 mM NaF and 1 μM Al^{3+} (3), 1 mM NaF and 10 μM Al^{3+} (4), and 1 mM NaF and 25 μM Al^{3+} (5). Identical data were obtained when Al^{3+} was replaced by Be^{2+}.

of spontaneous depolymerization following hydrolysis of GTP was twice as slow as in the Na_2SO_4 control (Figure 2a). Because increasing ionic strength greatly inhibits microtubule assembly, it was difficult to assay higher concentrations of P_i. However, in this assay fluoroaluminates exhibited a phosphate-like effect with 3 orders of magnitude higher affinity. Figure 2b shows that microtubules did not depolymerize at all, following hydrolysis of GTP, in the presence of 2 mM NaF and either Be^{2+} or Al^{3+} at concentrations in the 10^{-6}–10^{-5} range, i.e., of the same order of magnitude as tubulin. The polymerization process itself was unaffected by Al or Be in the presence of NaF. NaF alone had only a very slight stabilizing effect, probably due to its reported chelation of Al^{3+} ions, which contaminate all solutions especially GTP solutions (Sternweis & Gilman, 1982). Neither Be^{2+} alone nor Al^{3+} alone showed any effect. The same stabilization was observed whether Al^{3+} and NaF were present in the tubulin solution from time 0 of the polymerization process, incubated with tubulin before polymerization, or added to microtubules when the maximum in turbidity was reached.

Only microtubules were formed in the presence of AlF_4^- or BeF_3^-, and they displayed the same appearance as regular microtubules when observed in the electron microscope (Figure 4, inset).

Microtubules formed in the presence of NaF and Al^{3+} or Be^{2+} (or to which these compounds were added at the polymerization plateau) were found to depolymerize at a slower rate, upon cooling the solution, than the controls run in the absence of either Be or NaF or both.

Binding of ^{7}Be-Labeled Beryllium Fluoride to Microtubules. In the presence of 5 mM NaF, ^{7}Be-labeled Be bound to microtubules with a stoichiometry of 1.02–1.1 mol of Be/mol of α,β-tubulin, with an apparent equilibrium dissociation constant of 12–15 μM in 50 mM MES, pH 6.8, 3.4 M glycerol, 6 mM $MgCl_2$, 1 mM GTP, and 5 mM NaF (Figure 3a). No binding of Be was observed in the absence of NaF. Aluminum ions and inorganic phosphate inhibited the binding of Be competitively in the presence of NaF, with equilibrium constants of 22 μM and 30 mM, respectively (Figure 3b). Binding of BeF_3 was unaffected by the presence of 100 μM taxol.

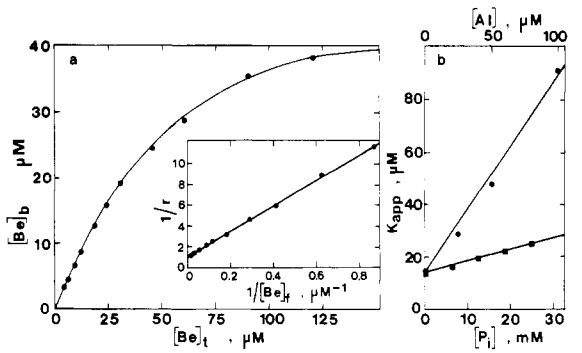


FIGURE 3: Binding of BeF_3^- to microtubules. Tubulin (42 μM) was polymerized in PG buffer containing 5 mM and NaF and ^{7}Be-labeled beryllium (see Materials and Methods). Panel a shows the amount of bound Be to sedimented microtubules (●). The solid curve is the calculated isotherm for binding of BeF_3^- to a single category of sites (40 μM) and an equilibrium dissociation K_{Be} constant of 13 μM. Inset: double-reciprocal plot of the data, with r representing the amount of Be bound per polymerized tubulin. Panel b shows the linear increase in K_{Be} with Al ions (●) or inorganic phosphate (■). The upper abscissa scale gives the concentration of total Al^{3+}, which leads to an overestimation of K_{Al}. The data collected in the presence of P_i were obtained at constant ionic strength (see Materials and Methods).

These data show that AlF_4^- and BeF_3^- bind to the same site as P_i on polymerized tubulin, presumably to the site of the γ-phosphate of GTP in the E site, and that the tubulin–GDP–P_i–subunits dissociate from microtubule ends very slowly and therefore constitute a stabilizing structure.

Effect of Inorganic Phosphate and Its Analogues on Some Dynamic Instability Features of Microtubules. The loss of a stabilizing GTP–cap at microtubule ends has been proposed as the basis for the dynamic instability behavior of microtubules (Mitchison & Kirschner, 1984). The above experiments suggest that P_i release following GTP cleavage on microtubules, or loss of slowly dissociating GDP–P_i–subunits at microtubule ends, leads to the exposure of rapidly dissociating GDP–subunits at microtubule ends and therefore generate dynamic instability. The effect of AlF_4^- and BeF_3^- on the dynamics of microtubules has been explored in the following experiments.

Under conditions of high dynamic instability, i.e., in buffers such as PM buffer containing no glycerol and Mg^{2+} ions at concentrations in the 10^{-4} M range, oscillatory polymerization kinetics have recently been reported (Carlier et al., 1987a; Pirollet et al., 1987). We have proposed that oscillations were generated by the synchronous depolymerization of a large part of the population of GDP–microtubules, following GTP hydrolysis and loss of a putative GTP–cap. Figure 4 shows that these oscillations can be totally abolished in the presence of saturating amounts of AlF_4^-. No effect was observed in the presence of either Al^{3+} or NaF alone. Decreased amplitude (but no change in the period) was observed at subsaturating concentrations of AlF_4^- or BeF_3^-.

The steady-state GTPase activity of microtubules results from the cycle of monomer–polymer exchange reactions in which GDP–subunits dissociate from microtubules over long distances, exchange medium GTP for GDP once in the dimeric state, and repolymerize again with subsequent hydrolysis of GTP. The rate of the GTPase activity therefore provides a measurement of the turnover and dynamics of microtubules. In the presence of 60 mM P_i, the steady-state GTPase was inhibited by 40% (data not shown). Fluoroaluminates acted similarly but with higher affinity, allowing 80% inhibition of the GTPase rate at 1000-fold lower concentrations (Figure 5). Fluoroaluminate did not affect the pre-steady-state (burst) rate of GTP hydrolysis accompanying the polymerization

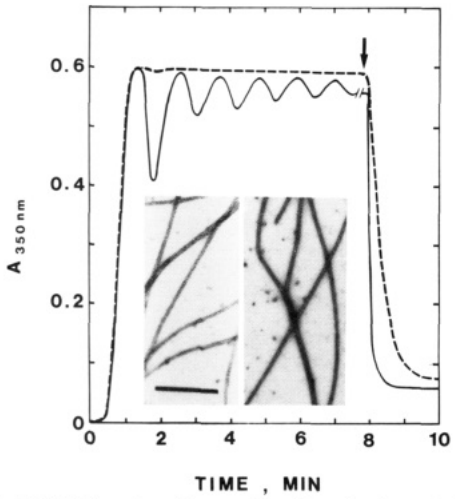


FIGURE 4: Inhibition of oscillatory kinetics of microtubule polymerization by AlF_4^-. Tubulin (200 μM) was polymerized in PM buffer containing 2 mM GTP and either no addition, 200 μM Al^{3+} alone, or 2 mM NaF alone (solid curve) or 200 μM Al^{3+} plus 2 mM NaF (dashed curve). Among several metal cations assayed, only Be^{2+} had the same effect as Al^{3+} in the presence of NaF. At time indicated by the arrow, microtubules were depolymerized by cooling to 4 °C. Note the slow rate of depolymerization in the presence of AlF_4^-. The inset shows the electron micrographs of negatively stained microtubules observed in the absence (right) and presence (left) of AlF_4^-. Bar = 1 μm.

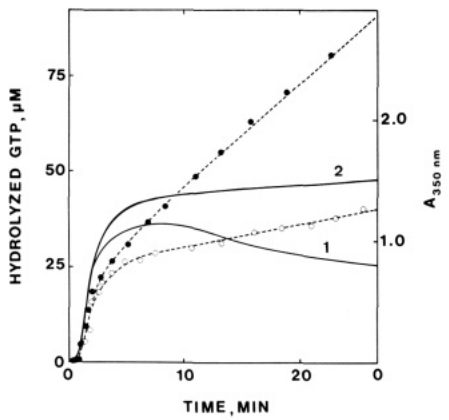


FIGURE 5: Inhibition of steady-state GTPase activity of microtubules by AlF_4^-. Tubulin (34 μM) was polymerized under the buffer conditions of Figure 2 in the presence of 145 μM γ-^{32}P labeled GTP and either 100 μM Al^{3+} (curve 1, ●) or 100 μM Al^{3+} and 10 mM NaF (curve 2, ○). Solid curves: turbidimetric recordings. Symbols: GTP hydrolysis. Note that a stable turbidity plateau is maintained in the presence of AlF_4^-, while a slight decrease in turbidity accompanies the large steady-state consumption of GTP observed in the absence of AlF_4^-.

process. We conclude that phosphate and structural analogues of $H_2PO_4^-$ inhibit microtubule turnover by decreasing specifically the rate of one step in the cycle: the dissociation of tubulin subunits from microtubules.

Under buffer conditions where dynamic instability has been observed [Figure 5 in Mitchison and Kirschner (1984)], one can notice that a large proportion (40%) of tubulin is not polymerized at steady state, leading to a slope lower than 1 for the plot of concentration of polymerized tubulin (c_w) versus concentration of total tubulin (c_t). The same observation has been made under conditions where oscillatory kinetics are observed (PM buffer). We had shown that the unpolymerizable tubulin is not active, and we hypothesized (Carlier et al., 1987a) that it consists of GDP–tubulin, liberated in large amounts by the rapid depolymerization processes and not immediately restored as polymerizable GTP–

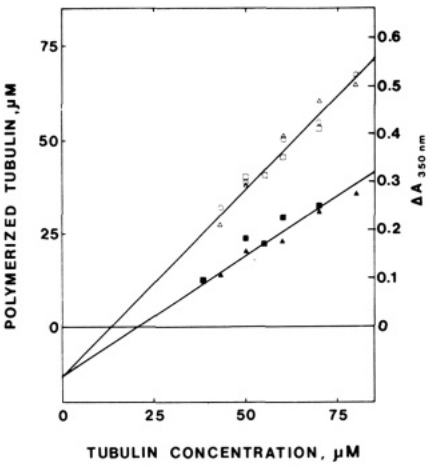
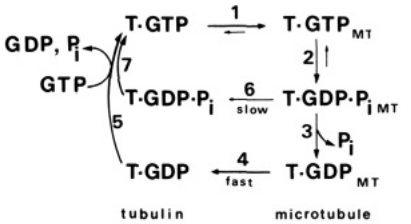


FIGURE 6: Phosphate analogues cause a decrease in the amount of nonpolymerized GDP–subunits at microtubule steady state. Critical concentration plots were obtained in PM buffer in the presence of 100 μM Al and in the absence (closed symbols) or the presence (open symbols) of 10 mM NaF. Consistent data were obtained from turbidity measurements (triangles) and sedimentation measurements (circles and squares).

Scheme I



tubulin. If the above proposition is true, one can expect that slowing down the depolymerization process by binding AlF_4^- or BeF_3^- to GDP–subunits in microtubules should cause a decrease in the amount of transient GDP–tubulin coming from microtubule depolymerization at steady state. Figure 6 shows that this is indeed the case. In the presence of AlF_4^- more microtubules are formed in PM buffer; the slope of the plot c_w vs c_t was increased up to the value of 1. However, the ordinate intercept of the plot was practically the same in the presence and absence of AlF_4^-, consistent with very little change, if any at all, in the true critical concentration.

Discussion

Use of inorganic phosphate and its high affinity analogues to reconstitute the kinetic intermediate (tubulin–GDP–P_i) in microtubule assembly has been helpful to elucidate how the chemical energy of GTP hydrolysis is involved in the regulation of microtubule dynamics. As in the actin system (Carlier & Pantaloni, 1988), cleavage of the γ-phosphate of the tightly bound nucleotide on the polymer results in a transient XDP–P_i–polymer, which is very stable and does not display dynamic instability. The subsequent P_i release in the medium plays a key role in actin and microtubule dynamics because it is linked to a major destabilization of the polymer. The stability of microtubules at steady state is ensured by a cap of slowly dissociating GDP–P_i–tubulin subunits. This conclusion is in agreement with the close coupling observed, at low tubulin concentration, between the processes of microtubule elongation and GTP hydrolysis (Carlier et al., 1987b). Dynamic instability results from the loss of the GDP–P_i–cap via subunit dissociation or P_i liberation. The data reported here can be summarized as shown in Scheme I.

Microtubule assembly and turnover at steady state take place via the two cycles 12345 (fast turnover) or 1267 (slow

turnover) according to whether microtubule terminal subunits are GDP–P_i–tubulin or GDP–tubulin. The extent of dynamic instability depends on the balance between these two cycles. We suggest that in vivo dynamic instability may be regulated by agents affecting the rate of P_i release from GDP–P_i–microtubules. Further work is needed to determine whether microtubule GDP–P_i–subunits are energetically similar to GTP–subunits.

Preliminary experiments (Carlier et al., unpublished results) show that BeF_3^- acts as a high-affinity analogue of P_i on actin too; BeF_3^- also inhibits the Ca–ATPase activity of myosin S_1 (Carlier, unpublished observations). Tubulin, actin, and myosin therefore add to the list of enzymes (Lange et al., 1986; Robinson et al., 1986) on which, in addition to the G-proteins, AlF_4^- and BeF_3^- act as high-affinity analogues of $H_2PO_4^-$, suggesting that these compounds may be of general interest to probe the mechanism of other ATPases, in particular translocation enzymes, in which a phosphate group transfer is linked to energy transduction.

REFERENCES

Bigay, J., Deterre, P., Pfister, C., & Chabre, M. (1985) *FEBS Lett. 191*, 181–185.

Bigay, J., Deterre, P., Pfister, C., & Chabre, M. (1987) *EMBO J. 6*, 2907–2913.

Brosset, C., & Orring, J. (1943) *Sven. Kem. Tidskr. 55*, 101–116.

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. (1986) *Biochemistry 25*, 7789–7792.

Carlier, M.-F., & Pantaloni, D. (1988) *J. Biol. Chem. 263*, 817–825.

Carlier, M.-F., Melki, R., Pantaloni, D., Hill, T. L., & Chen, Y. (1987a) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5257–5261.

Carlier, M.-F., Didry, D., & Pantaloni, D. (1987b) *Biochemistry 26*, 4428–4437.

Cassimeris, L. U., Wadsworth, P., & Salmon, E. D. (1986) *J. Cell Biol. 102*, 2023–2032.

Chen, Y., & Hill, T. L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4127–4131.

Goldstein, G. (1964) *Anal. Chem. 36*, 243–244.

Hill, T. L., & Carlier, M.-F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7234–7238.

Horio, T., & Hotani, H. (1986) *Nature (London) 321*, 605–607.

Job, D., Pabion, M., & Margolis, R. L. (1985) *J. Cell Biol. 101*, 1680–1689.

Johnson, K. A., & Borisy, G. G. (1977) *J. Mol. Biol. 117*, 1–31.

Korn, E. D., Carlier, M.-F., & Pantaloni, D. (1987) *Science (Washington, D.C.) 238*, 638.

Lange, A. J., Arion, W. J., Burchell, A., & Burchell, B. (1986) *J. Biol. Chem. 261*, 101–107.

Mitchison, T., & Kirschner, M. (1984) *Nature (London) 312*, 237–242.

Pirollet, F., Job, D., Margolis, R. L., & Garel, J. R. (1987) *EMBO J. 6*, 3247–3252.

Robinson, J. D., Davis, R. L., & Steinberg, M. (1986) *J. Bioenerg. Biomembr. 18*, 521–531.

Sammak, P. J., Gorbsky, G. J., & Borisy, G. G. (1987) *J. Cell Biol. 104*, 395–409.

Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.

Sternweis, P. C., & Gilman, A. G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4888–4891.

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.