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Inhibition of Microtubule Elongation by GDP

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Summary: GDP reduces both the rate and amplitude of GTP-induced assembly of microtubules from tubulin dimer or from microtubule protein, and promotes disassembly from microtubules at the steady state. One interpretation postulates that added GDP modifies microtubule ENDS so that tubulin-GTP, the species involved in steady state elongation of microtubules, cannot bind to a microtubule END containing tubulin-GDP. This concept has been used in subsequent models of assembly which treat the 'dynamic instability' of microtubules.

We question this interpretation on the basis of the published experimental data and the results reported here. Using a relatively simple model for microtubule assembly, we show by numerical simulation that the quantitative effects of GDP on the rate and amplitude of microtubule assembly and inhibition of steady state GTPase activity are well accounted for by the nucleotide exchange equilibrium of tubulin-GDP and tubulin-GTP. We therefore conclude that the effect of added GDP on elongation of MAP-containing microtubules and on steady state GTPase activity does not indicate modification of the activity of microtubule ENDs but depends on the tubulin-GTP/tubulin-GDP equilibrium. Additional evidence argues that microtubule ENDS containing GDP can indeed accept elongation by tubulin-GTP.

Theory: We aim to show that the principal effects of added GDP upon microtubule assembly can be accounted for by a relatively simple mechanism. The presence of GDP during GTP-supported elongation of microtubules from microtubule protein reduces both the rate and extent of assembly (1-8). The same extent of assembly is reached whether GDP is added during elongation, or at steady state (4,8). The effect of GDP on the rate of assembly has been interpreted on the basis that tubulin-GTP cannot elongate a microtubule with a terminal tubulin-GDP (8,9). This principle has been adopted in recent detailed models of microtubule assembly (10-12). It was found (8) that the dependence upon [GDP] of k_{obs}, the observed rate constant for microtubule elongation, followed a double-reciprocal relationship between $(k_{obs})_{GDP}$ and [GDP] of the form:

$$1 / ((k_{obs})_0 - (k_{obs})_{GDP}) = (1 + \text{Constant}/[GDP]) / (k_{obs})_0 \quad (1)$$

Abbreviations: GTP, guanosine-5'-triphosphate; GDP, guanosine-5'-diphosphate; EGTA, ethylene glycol bis(β-aminoethylether)-N,N,N',N'-tetra-acetic acid, MAPs, microtubule associated protein; tubulin-GTP, tubulin with GTP bound; tubulin-GDP, tubulin with GDP bound.

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where subscripts refer to GDP concentration. It was concluded that GDP inhibited the elongation by competition at the elongation site, implying (Eqs. (9),(10); Ref 8) that the fraction of 'active' terminal GDP-dimer would be reduced to $f = x/(1+x)$, with x related to the association constant, K, for nucleotide binding to (terminal) dimer and to the free nucleotide concentration by $x = K_{GTP}[GTP] \,/\, K_{GDP}[GDP]$.

However, an alternative mechanism, due to Jameson and Caplow (6), also considers the nucleotide exchange equilibrium of tubulin-GTP and tubulin-GDP which must occur when both GTP and GDP are present.

(A) Tu-GDP + GTP $\underset{k_4}{\overset{k_3}{\rightleftharpoons}}$ GDP + Tu-GTP (Scheme 1)

(B) Tu-GTP + END $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ END : **(C)** MT-GTP $\xrightarrow{k_h}$ MT-GDP

Engelborghs and Van Houtte (7) had shown for part of this scheme (Steps A + B) that the rate for a small amplitude relaxation at steady state in the absence of GDP:

$$(1/tau)_0 = k_1[END] \quad (2a)$$

is reduced in the presence of GDP by the same factor, $f = x/(1+x)$

$$(1/tau)_{GDP} = k_1 . f . [END] \quad (2b)$$

This is because the addition reaction of tubulin-GTP at the END is coupled to the rapid nucleotide exchange reaction in Scheme 1, and consequently the relaxation rate is a function of f, and hence of the [GTP]/[GDP] nucleotide ratio as defined above.

Since the elongation step of Scheme 1 is first-order, the kinetics for the full elongation are the same as for the relaxation process (7,13), i.e. in the absence of GDP

$$(1/tau)_0 = (k_{obs})_0 = k_1[END] \quad (3a)$$

and in the presence of GDP:

$$(1/tau)_{GDP} = (k_{obs})_{GDP} = k_1 . f . [END] \quad (3b)$$

Equation (3b) is identical to Eq. (10) of Ref 8. The intuitive interpretation suggests that the concentration of 'active' ENDs appears to be reduced by the factor f in the presence of added GDP; however it is the effective concentration of Tu-GTP which is reduced by f due to the

coupled equilibrium with Tu-GDP. Substituting Eqs. (3a) and (3b) in Eq. (1) reproduces the linear relationship of the experimental double reciprocal plot, with the Constant = x.[GDP] = [GTP].K_{GTP}/K_{GDP}. Thus Scheme 1 apparently accounts for the elongation kinetics of Carlier & Pantaloni (8).

Although these kinetics formally resemble enzyme inhibition, the reduction of rate and amplitude is, in fact, due to the coupled nucleotide exchange process, and not to inhibition of the elongation site. Since this coupling reduces the steady state rate of addition of tubulin-GTP and hence the amount of the tubulin-GTP in the polymer it should reduce the steady state GTPase activity proportionally, as found experimentally (8). Thus neither the assembly kinetics nor the steady state GTPase activity apparently require the postulate of reduction in reactivity of microtubule ENDs by GDP exchange into terminal positions.

Simulation: In order to establish that the nucleotide exchange reaction between tubulin-GTP and tubulin-GDP does indeed account for the observed effect of GDP on assembly kinetics and GTPase activity, we have used computer simulation to model the kinetics of scheme 1; the addition reaction of tubulin-GDP is excluded as energetically unfavourable, i.e. C_c(Tu-GDP) $\gg$ C_c(Tu-GTP). The Scheme is detailed for one end of a microtubule only; it is readily extended to include addition and dissociation events occurring at both ends with different affinities. The simulation (see Fig. 1a) was performed with [GDP] = 0 to 16 mM and k_1 = 5 x 10^6 M^{-1} s^{-1}, k_2 = 5 s^{-1}, k_3 = 5 x 10^4 M^{-1} s^{-1}, k_4 = 1 x 10^5 M^{-1} s^{-1}; The function of k_3 and k_4 is to give rapid nucleotide exchange, the ratio of these constants is fixed by the relationship, $k_4/k_3 = K_{GTP}/K_{GDP}$.

Figure 1a shows the progressively decreased rates and amplitudes of assembly in the presence of increasing [GDP]; these rates follow Eq. (3b) as expected. Thus the computer simulation confirms that the effects of GDP observed experimentally (8) can be accounted for without postulating the inability of GTP-dimer to elongate on a GDP-END. This result is implicit in the treatment of Engelborghs and Van Houtte (7) once the identity of the elongation reaction and the relaxation process is recognised.

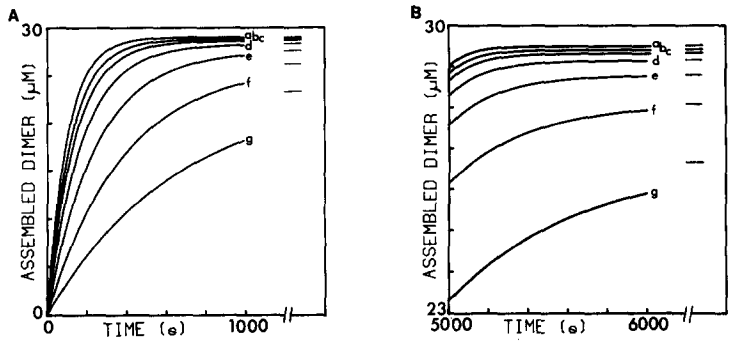

Figure 1 - Computer simulation of Scheme 1 (Steps A + B).
[Tubulin] = 30 μM, [END] = 2 nM, [GTP] = 1 mM and [GDP] = 0 (**a**), 0.5 (**b**), 1.0 (**c**), 2.0 (**d**), 4.0 (**e**), 8.0 (**f**) and 16.0 mM (**g**).

(A) Full assembly from low temperature to temperature T_1.
The simulation was performed for 1000 seconds, the horizontal lines on the right hand side of the figure are plateau values at 5000 seconds (**a-g**, top to bottom).

(B) Subsequent temperature jump from T_1 to T_2.
The simulation was performed for 1000 seconds, the horizontal lines on the right hand side of the figure are the plateau values at 10000 seconds (a-g, top to bottom).

These authors (7) also derived equations indicating that in a temperature jump from T_1 to T_2, the initial rate (i.e. the slope of the assembly curve at t=0) should be constant, independent of the [GTP]/[GDP] ratio. This condition is readily simulated by decreasing C_c(Tu-GTP) at the higher temperature. (e.g. changing k_2 from 5 s^{-1} to 2.5 s^{-1}). Figure 1b confirms that for these values the initial slope should indeed be constant, although for the small amplitude involved this would be difficult to establish unequivocally from real experimental data. In fact, Engelborghs and Van Houtte (7) reported sytematic variation in this initial rate with [GTP]/[GDP], and concluded that GDP could indeed modify the reactivity of microtubule END's for elongation by tubulin-GTP. An experimental investigation of this point is presented elsewhere (14). Our analysis indicates an alternative explanation in the temperature dependence of affinities of GTP and GDP for tubulin without necessitating modification of the reactivity of microtubule ENDS by GDP.

Figure 2 shows the time dependence of the elongation reaction and illustrates the presence at steady state of a constant proportion of tubulin-GTP in the microtubule ([MT-GTP]). This occurs so long as tubulin-GDP is lost from the polymer at the same rate as net tubulin-GTP addition,

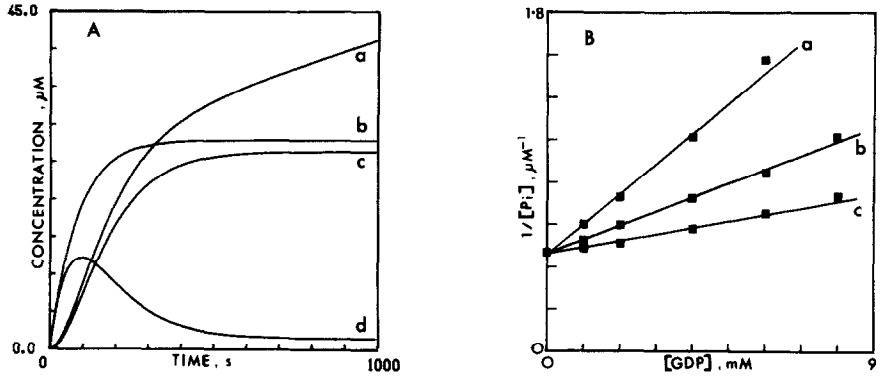

Figure 2 - Computer simulation of Scheme 1 (Steps A,B,C). GTP hydrolysis is included as a first-order rate constant, k_h for MT-GTP ⟶ MT-GDP.

(A) Simulated time course for phosphate (**a**), Dimer in microtubules (**b**), Tubulin-GDP in microtubules (**c**) and tubulin-GTP in microtubules (**d**).

(B) Influence of GDP on the rate of phosphate release. [Pi] is the increase in phosphate concentration for 100 s of simulation at steady state (4000 - 4100 s in each case). [GTP] = 0.25 (**a**), 0.50 (**b**) and 1.0 mM (**c**).

in order to maintain constant polymer mass (i.e steady state). The steady state GTPase activity is given by k_h.[MT-GTP]. In the presence of increasing [GDP], the amount of tubulin-GTP in the microtubule at steady state is reduced. Figure 2b shows that the derived plot reproduces the GDP inhibition effects observed by Carlier and Pantaloni (8). Thus, the experimental effects are accounted for without postulating modification by GDP of the reactivity of the microtubule END.

Discussion: These results firstly clarify the identity between the assembly process i.e. the 'full' assembly from the disassembled state to steady state at elevated temperature (8) and the 'relaxation process', i.e. a relatively small amplitude perturbation by, for example, temperature, from one steady state at T_1 to another at T_2 (7). The presence of added GDP introduces a coupled nucleotide exchange equilibrium (6) which affects both rates and amplitudes of these in parallel, reducing the rate by x/(1+x) where x depends on nucleotide concentrations and affinities. This exchange reaction is also shown to affect the steady state GTPase as observed experimentally. Both the theoretical arguments and the computer simulations show that the effects of GDP are accounted for without postulating that the elongation site at the end of a microtubule is inhibited by the binding of GDP. The reported disassembly effects of

added GDP (4-8) differ somewhat from results indicating a potential metastability of microtubules in the absence of GTP (15-17). While these differences are unresolved, it is interesting that recent work by Caplow and coworkers provides evidence that a tubulin-GDP microtubule END can indeed accept the productive addition of tubulin-GTP (18-19). Also the observation that shearing of steady state MAP-containing microtubules does not lead to spontaneous disassembly, although GDP-ENDS must be exposed; stabilisation of these by tubulin-GTP would also require a GDP-END to accept addition of tubulin-GTP as does the use of such sheared seeds in promoting elongation by tubulin-GTP in many studies of microtubule assembly.

These results are directly relevant to current models for microtubule assembly which treat the question of 'dynamic instability' of microtubules (20,21). In the formulation of Hill and Carlier (9-12) the condition that tubulin-GTP does not add to a microtubule END containing tubulin-GDP ($\alpha_{1D} = 0$) is a principle contributary mechanism in producing a succession of GTP-subunits, i.e. a 'GTP-cap' at an elongation site. The arguments advanced here negate setting $\alpha_{1D} = 0$ in the steady state theory (8) and this questions the numerical value derived for the GDP-dimer dissociation rate constant ($\alpha_{2D,T}$). The condition for generation of a 'GTP-cap' requires the rate of incorporation of tubulin-GTP exceeds its removal by hydrolysis or dissociation. Scheme 1 thus allows 'GTP-cap' formation with the size of the cap strongly determined by the assumptions made on the hydrolysis rate constant relative to the effective elongation rate.

In this scheme, the elongation kinetics are determined predominantly by the nature of the elongating species rather than the microtubule END (c.f. actin polymerisation (22), although a more recent mechanism does consider the actin END composition (23)). By contrast, the nucleotide composition of the microtubule END dictates absolutely the nature of the species dissociating. The dissociation rate constant for GDP-tubulin should become the controlling factor for disassembly below the critical concentration for tubulin-GTP, induced by isothermal dilution, low temperature or addition of tubulin-GDP. Tubulin-GDP dissociation processes do not appear to be 'catastrophic' in several studies of microtubules

containing MAPs (1,4-6,8,15,17-19). However, dissociation reactions are also complicated by questions of solution conditions, MAP content and protein composition (8,17), exchange reactions (15) and non-productive complex formation (18,19).

It is interesting that although the scheme does not implicitly include the oligomeric species known to be present in microtubule protein, the starting material for most of these studies (1,3-8) it is evidently able to explain the experimental data for the effect of GDP on the assembly kinetics of this material (3,6-8). Oligomeric forms (related to ring-like species) are undoubtedly involved in elongation ((24) and references therein) and these predominantly tubulin-GDP containing species may be incorporated directly (25). These results are consistent with the analysis given here, on the assumption that opening of the ring-like oligomer (in which the nucleotide is known to be non-exchangeable (26)) allows at least partial exchange of GTP into the oligomeric fragment, thus promoting the elongation reaction.

We therefore conclude that the postulate that tubulin-GDP in the terminal position of a microtubule inhibits elongation by tubulin-GTP is not a valid deduction from the effects of added GDP on microtubule elongation and steady state GTPase reaction. The involvement of tubulin in the nucleotide exchange reaction between tubulin-GTP and tubulin-GDP accounts quantitatively for the experimental observations, without assuming that the presence of GDP modifies the reactivity of microtubule ENDS.

References

1 Weisenberg, R.C., Deery, W.J. and Dickinson, P.J. (1976) Biochemistry, 15, 4248-5254
2 Carlier, M.F. and Pantaloni, D. (1978) Biochemistry 17 1908-1915
3 MacNeal, R.K. and Purich, D.L. (1978) J. Biol. Chem., 253, 4683-4687
4 Karr, T.L., Podrasky, A.E. and Purich, D.L. (1978) Proc. Natl. Acad. Sci. 76, 5475-5479
5 Zackroff, R.V., Weisenberg, R.C. and Deery, W.J.(1980) J. Mol. Biol., 139, 641-659
6 Jameson, L. and Caplow, M. (1980) J. Biol. Chem. 255, 2284-2292
7 Engelborghs, Y. and Van Houtte, A. (1981) Biophys. Chem., 14, 195-202
8 Carlier, M. and Pantaloni, D. (1982) Biochemistry 21, 1215-1224
9 Carlier, M. (1982) Mol. Cell Biochem., 47, 97-113
10 Hill,T.L. and Carlier, M.(1983) Proc. Natl. Acad. Sci. 80, 7234-7238
11 Chen, Y.D. and Hill, T.L. (1983) Proc. Natl. Acad. Sci., 80, 7520-7523

12 Hill, T.L. and Carlier, M. (1984) Proc. Natl. Acad. Sci., 81, 5772-5776
13 Bernasconi, C.F. (1976) Relaxation Kinetics. Academic Press, New York
14 Martin, S.R. and Bayley, P.M. (1986) Submitted.
15 Zeeberg, B.V. and Caplow, M. (1981) J. Biol. Chem., 256, 12051-12057
16 Margolis, R.L. (1981) Proc. Natl. Acad. Sci. 78 1586-1590
17 Lee, S.H., Kristofferson, D. and Purich, D.L. (1982) Biochem. Biophys. Res. Commun., 105, 1605-1610
18 Caplow, M. and Reid, R. (1985) Proc. Natl. Acad. Sci., 82, 3267-3271
19 Caplow, M., Shanks, J. and Brylawski, B.P. (1985) J. Biol. Chem, 260, 12675-12679
20 Mitchison, T. and Kirschner, M.W. (1985) Nature, 312, 232-237
21 Mitchison, T. and Kirschner, M.W. (1985) Nature, 312, 237-242
22 Pollard, T. (1984) J. Cell Biol., 99, 769-777
23 Pantaloni, D., Hill, T.L., Carlier, M.F. and Korn, E.D. (1985) Proc. Natl. Acad. Sci. 82 7201-7211
24 Bayley, P.M., Butler, F.M.M., Clark, D.C., Manser, E.J. and Martin, S.R. (1985) Biochem. J., 227, 439-455
25 Manser, E.J. and Bayley, P.M. (1985) Biochem. Biophys. Res. Commun., 131, 386-392
26 Caplow, M. and Zeeberg, B. (1981) Arch. Biochem. Biophys., 203, 404-411