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TEMPERATURE JUMP RELAXATION STUDY OF MICROTUBULE ELONGATION IN THE PRESENCE OF GTP/GDP MIXTURES

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GDP was added to microtubules at steady state. The amount of dissociation obtained was dependent on the GDP/GTP ratio and a method was developed to extrapolate to pure GDP conditions. From this extrapolation it was concluded that in the absence of GTP no elongation events occur. It was shown that at 35°C nucleotide exchange is very fast, but at 25°C, it is rate limiting for GDP-induced dissociation. Relaxation experiments, using temperature jumps before and after the addition of GDP, show that the nucleotide composition of the ends has to be taken into account. The model accepted so far cannot explain the observations. Several model mechanisms are described and their implications for equilibrium and relaxation data are analysed. All the acceptable models predict an increase in treadmilling efficiency at high GDP concentrations.

1. Introduction

The role of the nucleotides in the assembly of microtubules has been studied extensively by many research workers. (For a review see ref. [1].) Conflicting views of the effect of GDP are, however, present in the literature.

It is generally agreed that the addition of GDP to microtubules at steady state leads to only a partial dissociation. Furthermore, addition of GDP before initiation of assembly seems to be more inhibitory.

Carlier and Pantaloni [2] conclude that GDP-tubulin is not able to nucleate, but can participate in elongation with an affinity for the ends that is 6 × lower than in the case of GTP-tubulin. These experiments were, however, done in glycerol in the absence of microtubule-associated proteins. Zackroff et al. [3] find that the stable levels of assembly found with GDP depend strongly on the moment of GDP addition. Different final states can therefore be obtained under identical final solvent conditions. These authors deduce the existence of two fractions of GDP-tubulin: a fraction that is unable to participate in assembly, and a fraction that shows a critical concentration similar to that of GTP-tubulin.

Karr et al. [4] also conclude that the critical concentration of GDP-tubulin is similar to that of GTP-tubulin. In their experiments, the final assembly state does not depend on the manner of approach to the steady state.

Jameson and Caplow [5] point to the formation of an inactive GDP-tubulin complex, the concentration of which depends on the relative nucleotide concentrations and the affinities.

We find, as do Jameson and Caplow [5], that the level of assembly obtained, in the presence of GDP, depends on the GDP concentration. We have therefore developed a method of extrapolation to zero GTP concentrations. In this way we can show that in the absence of GTP the critical concentration for GDP-tubulin is very high if not infinite. However, this does not necessarily mean that GDP-tubulin is completely inactive. The absence of elongation in pure GDP could also be due to the inactivation of the microtubule ends. These two possibilities are distinguished using tempera-

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ture jump relaxation experiments. In this way we show that the relaxation rates for elongation are considerably reduced at high GDP concentrations, which implies that saturation of the ends with GDP has to be taken into account.

The experiments of David-Pfeuty [6] also point to the importance of the composition of the ends, but no attempt was made to change this composition sufficiently.

We conclude that in the absence of GTP no elongation occurs. Moreover, the nucleotide composition of one end has to be taken into account. We cannot exclude association of GDP-tubulin with microtubule ends that bear GTP. These conclusions are valid for assembly in the presence of microtubule-associated proteins. The implications for the mechanism of treadmilling are discussed.

2. Materials and methods

Microtubule protein was purified from pig brain homogenates according to the method of Shelanski et al. [7], modified as previously described [8]. Glycerol was used only in the first polymerisation. This preparation contained about 15% of microtubule-associated proteins. It was stored in liquid nitrogen.

Protein concentration was determined by the method of Lowry [9] using bovine serum albumin (Serva) as a standard. GTP and GDP were purchased from Boehringer as lithium salts.

Microtubule protein was passed down a Sephadex G-25 column at 4°C to remove the free nucleotides. The solution was then adjusted to 0.2 mM GTP and samples of 0.25–0.40 ml were frozen in liquid nitrogen. The solution was thawed just prior to the experiment. It was found that in this way polymerisability was retained for many hours, in contrast to a rapid loss at 4°C (half-life = 4 h).

Temperature jump experiments were performed in a special cell, constructed in the laboratory [10]. The optical pathlength is 2 cm, and the temperature changes exponentially with a half-life of 1.3 s. The cell fitted in the measuring compartment of a Cary 118 spectrophotometer.

Upon a temperature jump from 35 to 25°C, the total turbidity in our experiments changed maximally by about 5%. These perturbations were thus very small and could be considered as true relaxation experiments.

The curves obtained are characterized by their relaxation time (τ), this is the time necessary for the signal to decrease to $1/e$ of its maximal value. It should be realized that for relaxation processes of microtubule elongation, the relaxation time is determined by the concentration of the microtubule ends (m) and by the on rate constant (k_+) even if the process leads to dissociation. This can simply be deduced from the following kinetic equation for elongation: At temperature T_1 (at steady state) [8]

$$-\mathrm{d}c_1/\mathrm{d}t = k_+ m c_1 - k_- m = 0,$$

where c_1 is the tubulin dimer concentration and k_- the off rate constant. After a temperature jump from T_2 to T_1:

$$-\mathrm{d}(c_1 - \Delta c_1)/\mathrm{d}t = k_+ m(c_1 - \Delta c_1) - k_- m,$$

or

$$\mathrm{d}\,\Delta c_1/\mathrm{d}t = -k_+ m\,\Delta c_1 = -\tau^{-1}\,\Delta c_1,$$

where Δc_1 is the difference between the actual dimer concentration and the critical concentration c_1 at T_1. The amplitude or maximal value for Δc_1 is determined by the difference in critical concentration between the two temperatures.

This analysis of elongation kinetics, based on turbidity measurements, has so far been done in the presence of either a large excess of GTP or a GTP-regenerating enzyme system. As a consequence, c_1 represents the concentration of tubulin in solution and m, the concentration of microtubule ends, both with their nucleotide-binding site saturated with GTP. Our strategy will be to find out how GDP influences the concentration of active ends and the tubulin concentration by the analysis of the relaxation times and amplitudes, respectively, in GTP/GDP mixtures.

All experiments were performed in a buffer of pH 6.4 with the following composition: 50 mM 4-morpholinethanesulfonic acid, 70 mM KCl, 1 mM $MgCl_2$, 1 mM NaN_3, and 1 mM ethylene glycol bis(β-aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA).

This buffer is subsequently refered to as MES buffer.

3. Results and discussion

In a typical set of experiments, 0.35 ml of microtubule protein at 7.6 mg/ml and at 0.2 mM GTP was allowed to assemble at 35°C. After 30 min, 0.65 ml of warm MES buffer was added, the nucleotide composition of which depended on the particular series of experiments. The solution was then subjected to a sequence of temperature jumps between 35 and 25°C.

In a first control series warm MES buffer was added with GTP to a final concentration of 1 mM. The temperature jumps were repeated up to 20 times, without an appreciable change in time response (fig. 1). This proves that the concentration of microtubule ends remains the same, despite a process of length redistribution that might take place.

When a similar series of temperature jumps were applied to the microtubules at a final GTP concentration of 0.2 mM, a gradual decrease in the reciprocal relaxation time was observed. This was even more pronounced when GDP was added.

For the study of the GDP effect, microtubules were again equilibrated at 0.2 mM GTP, and subjected to a down- and upward-temperature jump. Subsequently, aliquots of 1–10 μl of 100 mM GDP were added to create a varying GDP concentration. A dissociation phase was observed, and the amplitude was determined (fig. 2). Due to the time necessary for mixing, a variable part of the time course was lost. This was, however, never larger than 40% of the total amplitude, and the relaxation time of the dissociation could still be determined.

Control experiments were done with 100 mM GTP instead of GDP. These results showed that dissociation was only obtained with GDP and was therefore not due to a general nucleotide effect or to local concentration gradients. A general

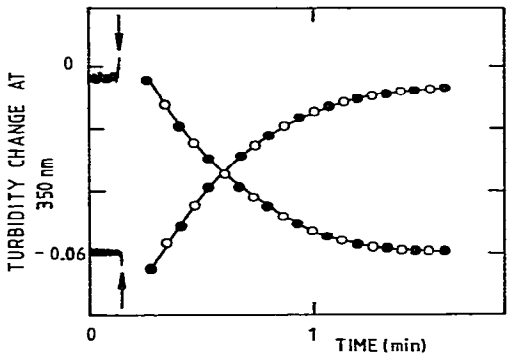

Fig. 1. Microtubules were assembled at 35°C at a concentration of 7.6 mg/ml. After reequilibration in 1 mM GTP upon a three-fold dilution, the solution was subjected to a temperature jump to 25°C (↓) and subsequently a temperature jump back to 35°C (↑). Due to the refractive index change with temperature, a sudden increase or decrease in apparent turbidity is found, which fades out with a half-life of 1.3 s. Therefore the first 10 s are not taken into account. The first two relaxation curves (●) are superimposed on the 19th and 20th (○). The optical pathway is 2 cm.

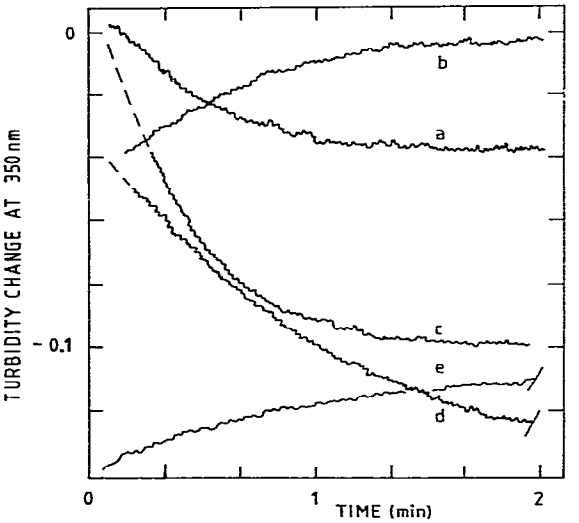

Fig. 2. As in fig. 1, microtubules were assembled at 35°C and diluted in 0.2 mM GTP. (a) Relaxation curve obtained for the first temperature jump to 25°C. (b) Relaxation curve for the first jump back to 35°C. (c) Dissociation curve obtained by the addition of GDP to 0.2 mM at 35°C. The initial part is missing due to the time necessary for mixing. (d) Dissociation curve due to the addition of the same amount of GDP at 25°C. (e) Temperature jump back to 35°C, after (d). The curves a, b, c and a, d, e are subsequent in time, but the origins are all translated to the same abscissa.

nucleotide effect was noticed by Jameson and Caplow at much higher concentrations [5].

When GTP was added up to a concentration of 1 mM, after GDP addition, the original turbidity and relaxation processes were restored, showing that no irreversible loss of microtubules had occurred as a consequence of GDP-induced dissociation.

Analysis of the relaxation times for the process of GDP-induced dissociation and for the association after a temperature jump from 25 to 35°C, showed that both relaxation times were the same. This proves that at 35°C, nucleotide exchange is fast and not rate limiting. When similar experiments were done at 25°C, GDP-induced dissociation was much slower than dissociation after a temperature jump to 25°C. Therefore, at this temperature nucleotide exchange is rate limiting for GDP-induced dissociation (fig. 2).

Analysis of the relaxation amplitudes shows that the amount of dissociation obtained upon GDP addition is dependent on the GDP concentration. This indicates that none of the final states can be interpreted in terms of a critical concentration for T-GDP (= GDP-tubulin).

Therefore, the critical concentration for T-GDP has to be determined by extrapolation to pure T-GDP concentrations. This can be done as follows: the free tubulin will be distributed as a GTP and GDP complex, according to the ratio of the free nucleotide concentrations and the respective affinities:

$$[\text{T-GTP}]/[\text{T-GDP}] = \{[\text{GTP}]/[\text{GDP}]\}K^{\text{GTP}}/K^{\text{GDP}} = \alpha, \qquad (1)$$

with $K^{\text{GTP}}/K^{\text{GDP}} = 2.8$ as determined by Zeeberg and Caplow [11], and T-GTP represents the tubulin-GTP complex. At 35°C, the establishment of this equilibrium is apparently fast, and not rate limiting for dissociation as shown in fig. 2.

From the mass balance we know that

$$[T_{tot}] = [\text{T-GTP}] + [\text{T-GDP}]. \qquad (2)$$

Therefore, combining both equations, we can write for the steady state (st):

$$(1+\alpha)/[T_{tot}]_{st} = \alpha/[\text{T-GTP}]_{st} = 1/[\text{T-GDP}]_{st}. \qquad (3)$$

The total concentration of unpolymerised tubulin at steady state $[T_{tot}]_{st}$ can be estimated from the original critical concentration and from the amplitude of dissociation, using a specific turbidity change of 0.2 OD cm^{-1} $(mg/ml)^{-1}$ at 350 nm [12]. In this way the small contribution of unpolymerised microtubule associated proteins is also interpreted as tubulin.

When our data are calculated and plotted as such, a straight line is obtained (fig. 3). The intercept is zero. This proves that at very high GDP concentrations, the equilibrium concentration of T-GDP is very high, if not infinite. From the constant slope we deduce that the equilibrium concentration for T-GTP is constant and equal to the original critical concentration (0.25 as compared to 0.23 mg/ml).

A simple interpretation would be that T-GDP is not active, and that the turbidity levels obtained after GDP addition are due to the establishment of the original critical concentration for T-GTP.

However, the question arises as to whether this is the only possibility to explain the observations. Indeed, the interpretation above does not include the nucleotide composition of the microtubule ends. In the following text we deduce a series of models that do include elongation by T-GDP and

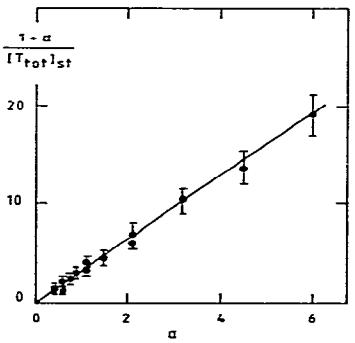

Fig. 3. The amount of dissociation obtained upon GDP addition, depends on the concentration of GDP. From the original critical concentration and from the amplitude of turbidity change, the total concentration of unpolymerised tubulin $[T_{tot}]_{st}$ at steady state is determined. According to eq. (3) an extrapolation is possible to pure GDP conditions ($\alpha=0$). The intercept is, however, zero, indicating that no elongation occurs in complete absence of GTP.

still can explain the equilibrium data, due to the fact that the nucleotide composition of the ends is taken into account.

As the zero intercept of fig. 3 means that in the complete absence of GTP no elongation occurs, all steps that are completely independent of GTP can be excluded. A priori it is, however, possible that the obligatory GTP is situated on the dimers in solution, or on the dimers in the microtubule ends. Taking this into account, several possibilities are still open. The commonly accepted picture [13–15], inspired by Wegner's model for actin polymerisation [16], assumes that at both ends association occurs by T-GTP and dissociation occurs after GTP hydrolysis, and thus as T-GDP.

Here it is also accepted that every association event is accompanied by GTP hydrolysis, however, the site of hydrolysis is specified, and the ends are distinguished (fig. 4):

(A) end 1: here the exchangeable nucleotide sites occur on the dimer in solution and they are buried in the microtubules after association. As hydrolysis occurs upon association of T-GTP, only the T-GTP/T-GDP ratio in solution is significant. Therefore we can write

$$d[T_{tot}]^1_{st}/dt = k_+^{1T} m_1 [\text{T-GTP}]_{st} - k_-^{1D} m_1, \qquad (4)$$

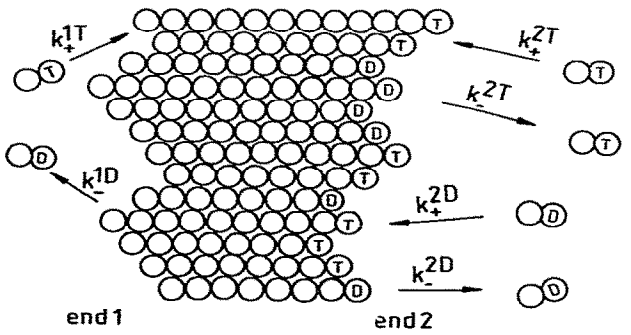

Fig. 4. Representation of the process of elongation at both ends of a microtubule (shown unfolded). At end 1, only GTP-tubulin associates and GDP-tubulin dissociates. At end 2 both GTP- and GDP-tubulin can associate and dissociate. Every association event is accompanied by hydrolysis of the GTP on the buried site. Association therefore only occurs at GTP-saturated sites. For association the reactants are shown, for dissociation the reactions products.

where the superscripts or indices 1 and 2 identify the end, while the superscripts D and T indicate GDP or GTP (saturation), respectively. m is the product of the number of microtubules and the number of binding sites per end (≈ 5).

(B) end 2: Here the exchangeable sites occur both on the dimers in solution and on the ends itself. At end 1 the GTP involved in hydrolysis was situated on the dimer in solution. Therefore at end 2 it should be found on the microtubule itself. As a consequence we have to take the GTP/GDP composition of the ends into account. It is a priori possible that both T-GTP and T-GDP can associate with and dissociate from the ends. The GTP/GDP ratio at the end can also be restored by direct nucleotide exchange. The different possibilities, for end 2, are shown in table I and fig. 4.

The assumptions made are not in contradiction with the observations of impaired dissociation with non-hydrolysable GTP analogues. Under our conditions, T-GTP is assumed to dissociate after hydrolysis of the GTP that was bound on the microtubule site (fig. 5). This hydrolysis is not impaired. The coupling of association and hydrolysis is also not absolute, due to the fact that our conditions are always close to steady state. In a large excess of unpolymerised tubulin, association could be faster than hydrolysis, as shown by Carlier and Pantaloni [17].

As a typical example, the results of model VI are calculated as follows:

(a) In the presence of a mixture of GTP and GDP, the following relation for the steady state is

Table I

Possible reaction partners in elongation at end 2.

Model No.	Association		Dissociation by
	by	to	
I	T-GTP	m_2	T-GDP
II	T-GTP	m_2	T-GDP+T-GTP
III	T-GDP+T-GTP	m_2	T-GDP
IV	T-GDP+T-GTP	m_2	T-GDP+T-GTP
V	T-GTP	m_2^T	T-GDP+T-GTP
VI	T-GDP+T-GTP	m_2^T	T-GDP+T-GTP
VII	T-GTP	m_2	T-GDP+T-GTP
	T-GDP	m_2^T	

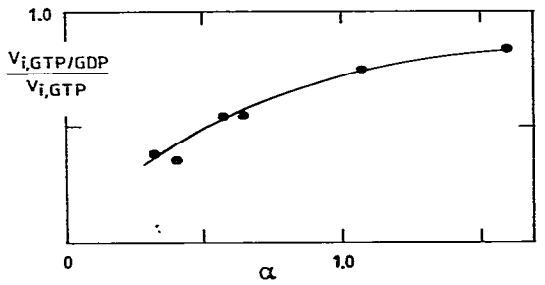

Fig. 5. The initial rate of association after a temperature jump from 25 to 35°C, in the presence of GDP/GTP mixtures ($V_{i,GTP/GDP}$) is compared to its value before GDP addition ($V_{i,GTP}$). No decrease is expected for the models that do not take the composition of the ends into account.

obtained ($d[T_{tot}]_{st}/dt = 0$):

$$[\text{T-GTP}]_{st}\left(k_+^{1T}m_1 + k_+^{2T}m_2^T\right) + [\text{T-GDP}]_{st}k_+^{2D}m_2^T = k_-^{1D}m_1 + k_-^{2T}m_2^T + k_-^{2D}m_2^D. \quad (5)$$

(b) Under pure GTP conditions, this relation reduces to

$$[\text{T-GTP}]_{st}\left(k_+^{1T}m_1 + k_+^{2T}m_2\right) = k_-^{1D}m_1 + k_-^{2T}m_2. \quad (6)$$

We assume that under these conditions m_2 is saturated with T-GTP.

As we know from the experiments that $[\text{T-GTP}]_{st}$ is the same under both conditions, the terms of (6) can be eliminated in (5). The result can be rearranged to give

$$(1+\alpha)/[T_{tot}]_{st} = \left(\alpha k_+^{2T} - \beta k_+^{2D}\right)/\left(k_-^{2T} - k_-^{2D}\right), \quad (7)$$

with $\beta = [\text{T-GTP}]/[\text{T-GDP}]$ on the end 2.

As α and β are expected to be proportional, the second part of (7) is proportional to α, which is in agreement with the experimental results. From the slope, which has to be the critical concentration in GTP, according to (3), the following relation between the ratio of α to β, and the different rate constants can be calculated:

$$\frac{\alpha}{\beta} = \frac{k_+^{2D}\left(k_-^{1D} + k_-^{2T}\right)}{k_+^{1T}\left(k_-^{2T} - k_-^{2D}\right) + k_+^{2T}\left(k_-^{2D} + k_-^{1D}\right)}. \quad (8)$$

As in this model association is coupled to hydrolysis, the process of treadmilling can occur because the net number of association events at one end does not have to be zero at steady state, but can be balanced by the net dissociation at the other end.

The flux of dimers that results from such a mechanism is equal to the net association at one end:

$$\phi = k_+^{1T}[\text{T-GTP}]_{st} - k_-^{1D}. \quad (9)$$

The efficiency of treadmilling (s) as defined by Wegner [15] is the ratio of the flux to the total number of dissociation or association events [15]:

$$s = \frac{k_+^{1T}[\text{T-GTP}]_{st} - k_-^{1D}}{k_-^{1D} + k_-^{2D}[1/(1+\beta)] + k_-^{2T}[\beta/(1+\beta)]}. \quad (10)$$

It clearly depends on the saturation of the ends with GTP. From eqs. (5) and (6), the relaxation equation for a temperature jump to 35°C can be deduced:

$$d\,\Delta[T_{tot}]/dt = [\alpha/(1+\alpha)]\,\Delta[T_{tot}] \times m\{k_+^{1T} + [\beta/(1+\beta)]k_+^{2T} + [\beta/\alpha(1+\beta)]k_+^{2D}\}. \quad (11)$$

This is done under the assumption of fast nucleotide exchange which holds at 35°C. The factor $\alpha/(1+\alpha)$ which appears in the relaxation time is a consequence of the fact that for every T-GTP that associates with a microtubule, $\alpha/(1+\alpha)$ T-GTP is reformed from T-GDP by nucleotide exchange.

Similar calculations can be done for all the models of table 1. The results are shown in table 2.

These models can be classified into two groups. The first group includes models I–IV, and does not take the composition of the ends into consideration for the association step. As the results of table 2 show, model IV can be discarded because it predicts a finite critical concentration at high GDP concentrations, which is not observed. Model III reduces to model I, so that in this group only T-GTP is active in association. Model I corresponds to the current assumptions found in the literature [13–15] and is the simplest model that accounts for the equilibrium data. For the whole of this groups the efficiency of treadmilling is

Table 2

Restrictions on the relations between the different rate constants, obtained from equilibrium calculations. Relaxation equations for a temperature jump from 25 to 35°C.

Model No.	Restrictions on the rate constants	Reciprocal relaxation time $\tau^{-1}[(1+\alpha)/\alpha m]$
I	NO	$k_+^{1T}+k_-^{2T}$
II	$k_+^{2D}=k_-^{2T}$	$k_+^{1T}+k_+^{2T}$
III	$k_+^{2D}=0$ → reduces to model I	
IV	$\frac{1+\alpha}{[T_{tot}]_{st}}=\frac{(1+\beta)k_+^{2D}}{k_-^{2T}-k_-^{2D}}$ not in agreement with the data	
V	$k_-^{2D}=\frac{k_+^{1T}k_-^{2T}-k_+^{2T}k_-^{1D}}{k_+^{1T}+k_+^{2T}}$	$k_+^{1T}+(\frac{\beta}{1+\beta})k_-^{2T}$
VI	$\frac{\alpha}{\beta}=\frac{k_-^{2D}(k_-^{1D}+k_-^{2T})}{k_+^{1T}(k_-^{2T}-k_-^{2D})+k_+^{2T}(k_-^{2D}+k_-^{1D})}$	$(k_+^{1T}+\frac{\beta}{1+\beta}k_-^{2T}+\frac{\beta}{\alpha(1+\beta)}k_+^{2D})$
VII	$\frac{\alpha}{\beta}=\frac{k_+^{2D}(k_-^{1D}+k_-^{2T})}{(k_+^{2T}+k_+^{1T})(k_-^{2T}-k_-^{2D})}$	$(k_+^{1T}+k_+^{2T}+\frac{\beta}{\alpha(1+\beta)}k_+^{2D})$

given by the following relation, and is independent of the GDP concentration.

$$s=\left(k_+^{1T}[\text{T-GTP}]-k^{1D}\right)/\left(k_-^{1D}+k_-^{2D}\right). \qquad (12)$$

The relaxation equation for this group is found to be

$$\mathrm{d}\,\Delta[T_{tot}]/\mathrm{d}t=\left[\alpha/(1+\alpha)\right]\times\Delta[T_{tot}]m\left(k_+^{1T}+k_+^{2T}\right). \qquad (13)$$

This equation shows that the reciprocal relaxation time is reduced by the factor $\alpha/(1+\alpha)$. Moreover, as all the intrinsic rate constants, including the dissociation rate constants, are the same, the amplitude is increased by the factor $(1+\alpha)/\alpha$. Therefore the initial rate should not be changed. Analyzing the initial rate provides the advantage of being independent of the exact value of α, and makes the experiments faster.

The experiments are performed as follows.

After equilibration at 35°C with GDP a subsequent temperature jump to 25°C was applied. This relation process is now biphasic and slow, due to the slow nucleotide exchange. But upon warming up to 35°C, a single relaxation process is again observed, because nucleotide exchange is again fast. Of this process the initial rate is analysed. In this case the GTP concentration is adjusted to 0.4 mM, to avoid depletion of GTP (fig. 5). The results show that the initial rate of elongation at 35°C is strongly reduced, so that the first group of models can be excluded on the basis of these relaxation experiments.

In the second group of models, the composition of the ends is taken into account. As the calculations in table 2 show, a reduction of the initial rate of association after a temperature jump is allowed and will depend on the nucleotide composition of end 2. This is in agreement with the observations. As the calculations show [eq. (10)] these models all predict that the efficiency of treadmilling will increase with increasing GDP concentrations, as it is obligatory that $k_-^{2T}>k_-^{2D}$, from the relations in table 1. Model V is the simplest of this group. Due to its simplicity it allows the calculation of k_-^{2D} from the other parameters:

$$k_-^{2D}=\left(k_+^{1T}k_-^{2T}-k_+^{2T}k_-^{1D}\right)/\left(k_+^{2T}+k_+^{1T}\right). \qquad (14)$$

If this relation is applied to the data of Bergen and Borisy [15], k_-^{2D} is found to be 2.3 s^{-1}. As the rate constant k_-^{2D} has to be a positive number and as only one combination of the rate constants of Bergen and Borisy [15] leads to a positive numerator of eq. (14), end 2 is identified as the minus end [15].

In conclusion we can say that the relaxation data clearly show that the composition of the ends has to be taken into account. This implies that

association to GTP ends must be different from association to GDP ends. From the extrapolation to high GDP concentrations, we can conclude that no elongation events occur in the complete absence of GTP. This reduces the complexity of the problem.

The necessity for GTP is, however, different for the two ends: at one end GTP is necessary on the dimer for association to occur, at the other end it is necessary on the end itself.

That T-GDP is able to elongate onto a microtubule with GTP sites, cannot be excluded, but is not necessary to explain the data. This binding equilibrium cannot strictly be described by a critical concentrations, as binding of T-GDP to a GTP-saturated site eliminates this site, until it is restored by direct nucleotide exchange.

It should be possible to determine all the rate constants necessary to identify the model by applying the same technique of elongation at flagellar fragments (Bergen and Borisy, [15]) under conditions of varying GDP/GTP ratios.

It is interesting to note that all the acceptable models predict that the treadmilling will dependent on the GDP concentration and that the efficiency parameter s will increase, as $k_-^{2T} > k_-^{2D}$. This could be a possible way of control of treadmilling in the cell with all its implications for the different transport mechanisms that are based on it.

It is clear that the exact values of the parameters depend on the concentration of MAPS present, and that these results cannot necessarily be extrapolated to the situation in glycerol.

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Note added in proof

While this paper was being submitted, the study of Margolis (Proc. Natl. Acad. Sci. USA 78 (1981) 1586) appeared. In this work the effect of GDP is analyzed by complete hydrolysis of GTP using the enzyme phosphofructokinase and a large excess of fructose 6-phosphate. When complete hydrolysis is obtained in the presence of microtubules, a transient dissociation is observed which levels off long before complete dissociation. This result seems to be in conflict with our prediction of complete dissociation. However, the end state in the experiments of Margolis was observed to be a metastable state, as further dissociation after cooling cannot be reversed by warming, only by GTP addition. So here too, the final state in pure GDP should be complete dissociation. That this final state is not reached can only be due to very slow dissociation in pure GDP which is not excluded by our data. In fact, in another study (M.F. Carlier and D. Pantaloni, Biochemistry 20 (1980) 1918) it was shown that GTP-tubulin dissociates faster than GDP-tubulin. Margolis also observed that treadmilling efficiency increases with decreasing GTP concentration, in accordance with our results.

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