Microtubules and nucleoside diphosphate kinase

Comparison of kinetics of GTP- and CTP-induced assembly

Khalid ISLAM* and Roy G. BURNS

Biophysics Section, Department of Physics, Imperial College of Science and Technology, London SW7 2BZ, U.K.

The kinetics of assembly of MAP2–tubulin microtubule protein were examined as a function of the GTP concentration in order to test the hypothesis that CTP-induced assembly results from the generation of GTP by nucleoside diphosphate kinase. These studies show that (a) there is no assembly below a minimum GTP concentration and that this represents a nucleation requirement, (b) the rate of elongation is inconsistent with a single assembly-species, and (c) the elongation rate increases markedly as the GTP concentration is raised, although GTP is not absolutely required for elongation. These assembly kinetics have been compared with those with increasing CTP concentrations, by using microtubule protein containing a very low nucleoside diphosphate kinase activity of known substrate specificity. Neither nucleation nor the observed rates of elongation can be attributed to the formation of GTP, either (a) in terms of the generation of free GTP and subsequent binding to tubulin or (b) by the direct charging of GDP bound to the tubulin exchangeable site. The results show that nucleoside diphosphate kinase is not required for CTP-induced microtubule assembly, and suggest that CTP directly effects microtubule assembly.

INTRODUCTION

Microtubule assembly *in vitro* requires GTP or non-GTP nucleoside triphosphates. Although GTP can bind to the exchangeable site on the β -tubulin subunit, the assembly induced by non-GTP nucleotides has been postulated to result from the charging of tubulin:E-site GDP by NDP kinase (e.g. Penningroth & Kirschner, 1977). In the present paper, we examine the minimal GTP requirements for microtubule assembly, and whether the known NDP kinase content of purified microtubule protein and the concentration of GDP are adequate to account for the observed assembly kinetics with CTP.

The kinetics of microtubule assembly are governed by a kinetically unfavourable nucleation step, followed by a rapid elongation phase, which, for the assembly of tubulin dimers, conforms to the linear condensation model (Johnson & Borisy, 1977). The evidence that assembly is absolutely dependent on the presence of GTP is contentious, and the differing results appear to reflect the composition of the protein studied (Lee *et al.*, 1982). For instance, GDP has been reported to promote slow elongation, but not nucleation (Carlier & Pantaloni, 1978; Zackroff *et al.*, 1980), and to inhibit the assembly of unfractionated microtubule protein (Jameson & Caplow, 1980), whereas the addition of excess GDP to MAP–tubulin microtubules does not result in their disassembly (Zackroff *et al.*, 1980). By contrast, no assembly is observed with GDP with pure tubulin (i.e. without MAPs; e.g. Herzog & Weber, 1977; MacNeal & Purich, 1978; Hamel *et al.*, 1981; Huitorel *et al.*, 1984), and indeed exposure of a tubulin:E-site GDP subunit at a microtubule end leads to catastrophic disassembly (Carlier *et al.*, 1984; Mitchison & Kirschner, 1984).

Similar discrepancies have been reported for the assembly induced by non-GTP nucleoside triphosphates. No assembly is observed of pure tubulin in 4 M-glycerol by ATP unless NDP kinase is also present (Huitorel *et al.*, 1984). By contrast, there is extensive assembly of unfractionated (i.e. tubulin + MAPs) microtubule protein (e.g. Arai *et al.*, 1975; Penningroth & Kirschner, 1977) or partially purified tubulin (Kuriyama & Sakai, 1974), although the fraction of the total protein polymerized is lower with ATP than with GTP (Islam & Burns, 1984b).

Such differences in the assembly of pure tubulin and unfractionated microtubule protein may reflect the involvement of MAP–tubulin oligomers as direct assembly intermediates. Such oligomers necessitate the inclusion of additional terms in the linear condensation model describing the oligomer concentration, the association and dissociation rate constants, and their GTP-occupancy (Burns & Islam, 1985; Islam & Burns, 1985a).

The current study has used unfractionated chick brain microtubule protein so that the analysis, necessitating consideration of the role of such MAP2–tubulin oligomers in the assembly induced by non-GTP nucleotide triphosphates. We have examined (a) the effect of varying the exogenous GTP concentration on both the rate and the extent of microtubule assembly, (b) the identity of the assembly intermediates, and then (c) compared the observed assembly with that with non-GTP nucleoside triphosphates. As the properties of the chick brain NDP kinase have been characterized (Islam & Burns, 1984a, 1985b), the rate of GTP generation can be calculated. We show that the NDP kinase activity present in purified preparations of MAP2–tubulin microtubule protein is insufficient to account for the observed assembly with CTP.

Abbreviations used: MAP2, microtubule-associated protein 2; NDP kinase, nucleoside diphosphate kinase (EC 2.7.4.6); tubulin:E-site GTP or E-site GDP, tubulin with GTP or GDP bound to the exchangeable nucleotide-binding site.

* Present address: Dipartimento di Biologia, Università di Milano, Via Celoria 26, 20133 Milano, Italy.

METHODS

Preparation and assembly of microtubule protein

Microtubule protein was prepared from 1-day-old chick brains (Burns & Islam, 1981; Islam & Burns, 1985b) through three cycles of assembly/disassembly, the assembled protein after the third cycle being pelleted through a 30% (w/v) sucrose cushion. The reassembly buffer consisted of 0.1 M-Mes, 2.5 mM-EGTA, 0.5 mM-MgCl₂, 0.1 mM-EDTA and 1 mM-dithiothreitol, adjusted to pH 6.4 with KOH. Methods for monitoring microtubule assembly at 30°C by light-scattering and for seed preparation have both been previously described (Burns *et al.*, 1984).

Determination of NDP kinase activity

This was done by the pyruvate kinase/lactate dehydrogenase coupled assay (Islam & Burns, 1985a). A unit of NDP kinase activity is defined as the amount of enzyme that catalyses the reaction of 1 μ mol of ATP or 1 μ mol of TDP/min at 25°C.

Protein determination

Protein concentrations were determined (Hartree, 1972) with bovine serum albumin as the standard.

Nucleotide determination

The nucleotide content was determined by the A_{257} after precipitation of the protein with 5% HClO₄ (Islam & Burns, 1985a). GTP and GDP were separated by loading the neutralized HClO₄ supernatant on to a 0.9 cm $\times$ 6 cm column of polyethyleneimine-cellulose and eluting with a linear 0–4 M-LiCl gradient in 20 mM-potassium phosphate, pH 4.6.

Materials

All biochemical reagents were purchased from Sigma, except for pyruvate kinase (Calbiochem-Behring Corp.) and lactate dehydrogenase (Boehringer). The GTP was stored in liquid nitrogen until use to minimize decomposition. All other chemicals were of Fisons A.R. grade.

RESULTS

Microtubule protein

The microtubule protein, purified through three cycles of assembly and disassembly and analysed by quantitative sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, consists of 80% tubulin and 20% MAP2, with a trace amount of tau, and closely resembles the twice-cycled protein previously described (Burns & Islam, 1984b,c). Approx. 50% of the total tubulin is eluted as the MAP2-tubulin oligomer, after mild glutaraldehyde fixation and gel-exclusion chromatography (Burns & Islam, 1984a), the remainder consisting of free tubulin dimers and monomers. The NDP kinase activity is 0.015–0.020 unit/mg of microtubule protein, representing a molar contamination of approx. 1 mol of NDP kinase hexamer/3700 mol of tubulin dimer. The protein contains approx. 2 mol of nucleotide/mol of tubulin dimer, and fractionation on a polyethyleneimine-cellulose column shows that the tubulin dimer contains 1 mol of GTP (released from the N-site) and 1 mol of GDP (released from the E-site).

The maximum GDP concentration will equal the tubulin content, i.e. 8 μ M for assembly at 1 mg of

microtubule protein/ml. The free GDP concentration will be significantly lower, and will depend on the oligomer/dimer equilibrium and the respective GDP-binding constants to the free tubulin dimers and to the tubulin-MAP2 oligomers. In the study below of the role of the NDP kinase, the maximum GDP concentration will be used in computing the rate of GTP generation. Consequently, the calculated rates of GTP generation will be overestimated if the NDP kinase cannot utilize GDP bound to tubulin or the tubulin-MAP2 oligomers.

Effect of GTP concentration on microtubule assembly

The kinetics of assembly (1.34 mg of protein/ml) have been examined as a function of the GTP concentration. After a short lag period, polymerization was rapid until a final plateau value was attained. Electron microscopy revealed that the observed increase in turbidity resulted from the assembly of microtubules.

The maximum extent of polymerization was dependent on the GTP concentration. There was no detectable assembly below 4 μ M-GTP, and complete assembly was achieved at 10–15 μ M-GTP (Fig. 1). The maximum extent was slightly depressed at higher GTP concentrations, in part owing to the phosphorylation of MAP2 by the GTP affecting the critical concentration (Burns & Islam, 1984b; Burns *et al.*, 1984).

The maximum rate of elongation as a function of the GTP concentration was more complex, and no assembly occurred below 4 μ M, but as the GTP concentration was raised the maximum rate increased sharply until approx. 20 μ M-GTP. Slightly higher GTP concentration had little effect, but above about 100 μ M-GTP there was a second rapid increase in the rate (Fig. 1).

The assembly kinetics at low GTP concentrations (20 μ M) are consistent with the addition of a single subunit species (tubulin dimers), whereas those at high GTP concentrations (1 mM) indicate the addition of both tubulin dimers and MAP2-tubulin oligomers (Burns &

Fig. 1. Kinetics of microtubule assembly as a function of the GTP concentration

Microtubule protein (1.34 mg/ml) was assembled at 30°C with increasing concentrations of GTP. The maximal rate ($\Delta A_{350}/\text{min}$: ○) and the final plateau value (ΔA_{350} : ●) were determined.

Fig. 2. Microtubule elongation in the presence and absence of exogenous GTP

Assembly at 30°C of microtubule protein (1.0 mg/ml) in the (a) presence and (b) absence of 10 μM exogenous GTP was initiated by the addition of 200 nM microtubule seeds. The assembly was monitored at 350 nm.

Islam, 1984a). The increase in the rate above 100 μM (Fig. 1) is due to a change in the oligomeric association rate constant and reflects the increasing GTP occupancy of these oligomers (Burns & Islam, 1985; Islam & Burns, 1985a).

The assembly of microtubule protein (1 mg/ml) seeded with 200 nM seeds was determined without adding exogenous GTP (i.e. 8 μM -GDP) and on adding 10 μM -GTP (Fig. 2). As assembly was observed in the absence of added nucleotide (Fig. 2), the minimum concentration of 4 μM -GTP required for unseeded assembly (Fig. 1) must represent the threshold for nucleation. Elongation was observed for seeded assembly of both the GTP- and GDP-tubulin (Fig. 2), but the maximum rate of elongation with GTP was 17 times that with GDP, in agreement with previous observations (Zackroff *et al.*, 1980).

Effect of non-GTP nucleoside triphosphates on unseeded microtubule assembly

In analysing the effect of non-GTP nucleoside triphosphates on the maximum rate of elongation and the plateau level of assembly, it is important to recognize the following.

(i) Irrespective of the nucleoside triphosphate concentration or the NDP kinase activity, the maximum rate cannot, apart from a complication concerning oligomer utilization, which will be considered below, exceed that observed with a GTP concentration equal to the total tubulin-dimer concentration if there is an absolute requirement for NDP kinase.

(ii) As the chick brain NDP kinase functions via a 'Ping-Pong' mechanism, the rate of GTP synthesis will depend on (a) the NDP kinase specific activity, (b) the donor nucleotide concentration, and (c) the acceptor nucleotide concentration (equal to the tubulin-dimer concentration). The NDP kinase specific activity was 0.015–0.02 unit/mg and the enzyme preferentially uses purine nucleoside triphosphates (activity with CTP is 3% that with ATP and 5% that with GTP; Islam & Burns, 1985b).

Fig. 3. Kinetics of microtubule assembly as a function of the CTP concentration

Microtubule protein (1.0 mg/ml) was assembled at 30°C at increasing CTP concentrations, and both the maximum rate ($\Delta A_{350}/\text{min}$; $\circ$) and the final plateau value (ΔA_{350} ; $\bullet$) were determined. A control sample in which assembly was initiated with 10 μM -GTP yielded a maximum rate of 0.0062 A_{350} unit/min and a final plateau value of 0.149 A_{350} unit.

(iii) A minimum of 4 μM -GTP must be generated to induce the nucleation of unseeded preparations (Fig. 1).

The polymerization with CTP (1 mg of protein/ml, 8 μM -tubulin) exhibits the characteristic lag period followed by elongation to the final plateau value. The lag period (6 min) was independent of the CTP concentration (50–500 μM). No assembly was observed below 12 μM -CTP, and, as the concentration was increased, the maximum extent of assembly was constant for all concentrations above 50 μM -CTP (Fig. 3), and was equal to that in the control samples assembled with 10 μM -GTP. The maximum rate of assembly was, above the threshold of 12 μM -CTP, proportional to $\log[\text{CTP}]$.

These observations permit an evaluation of the role of NDP kinase in the promotion of microtubule assembly by CTP. First, the maximum rate observed with 500 μM -CTP (0.0175 A_{350} unit/min at 6 min) is 2.8-fold greater than that of the 10 μM -GTP control (0.0062 A_{350} unit/min) (Fig. 3), even though 8 μM is the maximum GTP concentration that could be generated with GDP as the phosphoryl acceptor. The concentration of GTP generated can be calculated from the CTP and GDP concentrations (500 μM and 8 μM respectively), the enzyme activity (0.015–0.02 unit/mg) and the time. The maximum GTP concentration after 6 min is only 0.2 μM , assuming that the CTP concentration is non-limiting and without correcting for the back-formation of CTP from the generated GTP and CDP. No assembly is observed at such low GTP concentrations for unseeded preparations (Fig. 1).

Nucleation, requiring the presence of approx. 4 μM -GTP (Fig. 1), provides a further test of the role of NDP kinase. The lag phase for GTP assembly was 2 min, whereas that for CTP-induced assembly was 6 min. Consequently, 4 μM -GTP must be generated within 4 min if the NDP kinase is obligatory. First, this is inconsistent

with the constant duration of the lag phase, which was independent of the CTP concentration above $50\text{ }\mu\text{M}$, since NDP kinase functions via a 'Ping-Pong' mechanism (Islam & Burns, 1985b; Parks & Agarwal, 1973). Furthermore, the lag phase was shorter with CTP than with ATP (9 min; K. Islam & R. G. Burns, unpublished work), even though ATP is the preferred substrate.

Secondly, the threshold CTP concentration for nucleation was $12\text{ }\mu\text{M}$. The concentration of GTP generated by the NDP kinase during the lag period from the CTP ($12\text{ }\mu\text{M}$) and GDP ($8\text{ }\mu\text{M}$) concentrations is insufficient to promote nucleation. Even non-limiting CTP concentrations would only yield approx. 130 nm-GTP during the increase in the lag period.

The analysis of both the maximum rate of elongation and the lag period for assembly with CTP strongly suggests that NDP kinase is not required. However, the maximum rate of elongation depends on the product of the number concentration of microtubule ends, determined by the efficiency of nucleation, and the subunit concentration. Therefore, although the initial subunit concentration is constant, a maximum rate of elongation which is higher than that observed with GTP could be achieved if there is a higher number of nucleation events. Clearly, though, if CTP promotes assembly by the NDP kinase-mediated formation of GTP, the number of nucleation events would be the same as that on addition of exogenous GTP. In the following experiment, the possible effects on nucleation events have been eliminated by comparing the seeded assembly with GTP and CTP.

Kinetics of seeded microtubule assembly with GTP and CTP

Assembly was initiated by the addition of $10\text{ }\mu\text{M}$ -GTP or $500\text{ }\mu\text{M}$ -CTP in the presence and absence of seeds. Both the GTP- and CTP-induced assembly showed the characteristic lag phase when no seeds were added, but this was totally abolished by inclusion of seeds (Fig. 4).

Fig. 4. Microtubule elongation induced by either GTP or CTP in the presence or absence of microtubule seeds

Microtubule protein (1.0 mg/ml) was incubated at 30°C with either $10\text{ }\mu\text{M}$ -GTP (●, ○) or $500\text{ }\mu\text{M}$ -CTP (■, □) in the presence (●, ■) or absence (○, □) of microtubule seeds. The assembly was initiated by the addition of the nucleoside triphosphate alone or the simultaneous addition of the seeds, and was monitored at 350 nm .

The observed rate of elongation with CTP, during the lag phase of the unseeded preparation, was only half that with GTP, but was 9-fold faster than that observed in the absence of added nucleotide (i.e. $8\text{ }\mu\text{M}$ -GDP; Fig. 2).

In this experiment, both the number of nucleation events and the initial subunit concentration were controlled, yet there was no detectable lag phase with CTP. Consequently, there cannot have been any significant generation of GTP by the NDP kinase before the onset of elongation.

DISCUSSION

The assembly kinetics of MAP2-tubulin microtubule protein are complex and must include terms representing the addition and loss of tubulin dimers and MAP2-tubulin oligomers (Burns & Islam, 1985; Islam & Burns, 1985a). Furthermore, the association and dissociation rate constants for oligomer addition and loss are affected by the GTP occupancy of the oligomers, which in turn is affected by the exogenous GTP concentration. This is illustrated by the marked increase in the apparent elongation rate constant between $100\text{ }\mu\text{M}$ - and 1 mM -GTP (Fig. 1), even though the GTP occupancy of the tubulin dimers is approximately constant over this concentration range.

In particular, the existence of MAP2-tubulin oligomers permits, in contrast with the assembly of pure tubulin, the addition of tubulin:E-site GDP subunits, as indicated by the assembly of the unfractionated protein at sub-stoichiometric GTP concentrations (Fig. 1). However, it must be noted that no assembly is observed below a threshold of about $4\text{ }\mu\text{M}$ -GTP (Fig. 1), and that this must represent a nucleation requirement as seeded preparations exhibit elongation (Fig. 2).

The role of NDP kinase in the promotion of assembly by non-GTP nucleoside triphosphates can be evaluated with respect to:

(a) the direct formation of GTP from tubulin:E-site GDP;

(b) the release of GDP from the tubulin:E-site, charging to GTP in solution, and subsequent rebinding to the tubulin:E-site.

As NDP kinase functions by a 'Ping-Pong' mechanism, the rate of formation of the phosphoenzyme intermediate will depend on the concentrations of the donor nucleoside triphosphate and the enzyme and on the K_m for donor, whereas the rate of phosphoryl transfer will depend on the concentrations of the acceptor nucleoside diphosphate and the phosphoenzyme and the K_m for acceptor. CTP is an inefficient phosphoryl donor (Islam & Burns, 1985b), yet induces microtubule assembly (Fig. 3).

By characterizing the minimal GTP requirements for assembly (Fig. 1), the properties of the chick brain NDP kinase (Islam & Burns, 1985b), and by using highly purified microtubule protein, which contains a low NDP kinase activity, we find that CTP-induced assembly cannot be accounted for by the charging of GDP released from the tubulin:E-site and the subsequent rebinding of GTP. In particular:

(a) insufficient GTP can be generated within the required time from the maximum GDP content to account for either the observed rate of elongation or the duration of the lag phase of unseeded assembly;

(b) the observed constant lag phase with increasing CTP concentrations ($50\text{--}500\text{ }\mu\text{M}$) is inconsistent with the

formation of a phosphoenzyme intermediate, since, even for ATP, the preferred phosphoryl donor, the corrected K_m for ATP is 5×10^{-4} M (Islam & Burns, 1985b);

(c) the CTP-induced assembly of seeded protein fails to exhibit any lag phase (Fig. 4).

Consequently, there is strong evidence that the CTP-induced assembly of microtubules does not result from the NDP kinase-mediated charging of GDP released from the tubulin:E-site and then subsequently rebound.

However, GDP bound to the tubulin:E-site might be charged up *in situ*. The analysis of the rate of assembly as a function of the GTP concentration (Fig. 1) demonstrates that possible interactions with both tubulin dimers and tubulin-MAP2 oligomers must be considered.

Specific charging of tubulin dimer:E-site GDP could alter the rate of GTP generation, but this would not lead to the preferential accumulation of tubulin:E-site GTP dimers, as both bound GDP and GTP are readily exchanged with the nucleotides in solution (Arai *et al.*, 1975). As the maximum rate of assembly at 500 μ M-CTP is 2.8 times that with 10 μ M-GTP, the assembly cannot be due to the preferential accumulation of tubulin:E-site GTP dimers. Furthermore, although the NDP kinase and tubulin tend to co-purify, there is no evidence for a specific interaction between the NDP kinase and the tubulin dimer (Islam & Burns, 1985b).

Consideration of the possible preferential charging of tubulin oligomer:E-site GDP is more complex. As the GTP occupancy determines the association rate constant for oligomer incorporation, any direct charging of the oligomer:E-site GDP by NDP kinase and CTP would also alter the GTP/GDP occupancy. The oligomer:E-site only exchanges slowly (Zackroff *et al.*, 1980; Weisenberg *et al.*, 1976), so that charged oligomers could preferentially accumulate and hence enhance the rate of oligomer utilization.

Such charging would require an interaction between the NDP kinase and the MAP2-tubulin oligomers. Although attempts to demonstrate such an interaction have been unsuccessful (Islam & Burns, 1985b), transient binding of a phosphoenzyme intermediate might not be observed in the steady-state studies.

Comparison of the kinetics of seeded and unseeded assembly with CTP and GTP demonstrate that such a model is invalid. No assembly is initiated until the tubulin:E-site GTP concentration is equivalent to that found with an exogenous GTP concentration of 4 μ M (Fig. 1). The actual concentration is unknown, but will depend on the dimer/oligomer equilibrium, the dimer/oligomer exchange rate, and the rates of binding of GTP to dimers and oligomers. This state is not achieved with 500 μ M-CTP until 6 min (Fig. 3), so that the rate of E-site GTP formation must be comparatively slow. By contrast, there is no lag phase before the onset of CTP-induced elongation on to seeds (Fig. 4), and the immediate elongation rate is 50% of that achieved with 10 μ M-GTP and 9 times that with GDP (Fig. 2). Again, the constant lag phase observed for unseeded assembly by all CTP concentrations above 50 μ M, and the shorter lag phase with CTP than with ATP, are inconsistent with the formation of a phosphoenzyme intermediate.

Consequently, no model for the NDP kinase-mediated charging of tubulin:E-site GDP, of either tubulin dimers or MAP2-tubulin oligomers, either *in situ* or in solution, can be supported.

The role of NDP kinase has been previously examined by using both phosphoryl donors, such as ATP and UTP, and the non-hydrolysable ATP analogues adenosine 5'-[β,γ -methylene]triphosphate and adenosine 5'-[β,γ -imidio]triphosphate. The assembly of unfractionated microtubule protein with ATP and UTP was deduced to result from the direct charging of E-site GDP (Penningroth & Kirschner, 1977), but this was subsequently challenged, as the conditions for NDP kinase charging and for exogenous GTP binding were not equivalent (Jacobs, 1979; Terry & Purich, 1979). Similarly, our studies with CTP are inconsistent with the assembly resulting from direct charging. By contrast, the assembly induced by the non-hydrolysable ATP analogues was argued to result from the direct binding to the tubulin subunit (Penningroth & Kirschner, 1978). Our current results support this second interpretation. Indeed, the kinetics observed with ATP are also inconsistent with the generation of GTP by NDP kinase (Islam & Burns, 1984b). Such promotion of assembly by non-GTP nucleoside triphosphates may be effected by direct binding to the low-affinity (2×10^{-4} M) ATP-binding site located on α -tubulin (Zabrecky & Cole, 1982, 1983), i.e. at a site distinct from the GTP-binding E-site located on β -tubulin (Geahlen & Haley, 1979; Nath *et al.*, 1985).

However, such a conclusion, based on the assembly of unfractionated microtubule protein, is in direct conflict with the findings on the assembly of pure tubulin by ATP in the presence of added pure NDP kinase (Huitorel *et al.*, 1984). These authors failed, however, to consider the inhibition of microtubule assembly by high GTP concentrations (Murphy & Hiebsch, 1979; Jameson & Caplow, 1980), the possible presence of tubulin oligomers (Kravitz *et al.*, 1984), which may contain unexchangeable tubulin:E-site GDP, and provide no evidence for the required protein-protein interactions between NDP kinase and tubulin. However, the possibility cannot be discounted that the assembly characteristics of microtubule protein containing MAPs are significantly different from those with pure tubulin.

We conclude that there is no role for NDP kinase in mediating the assembly of unfractionated microtubule protein by non-GTP nucleoside triphosphates, and that its role in the assembly of pure tubulin remains ambiguous.

This work was supported by the Cancer Research Campaign. We also thank Mr. A. Luttriano of Ross Breeders Ltd. for generously donating the chicks.

REFERENCES

- Arai, T., Ihara, Y., Arai, K. I. & Kaziro, Y. (1975) *J. Biochem. (Tokyo)* **77**, 647-658
- Burns, R. G. & Islam, K. (1981) *Eur. J. Biochem.* **117**, 515-519
- Burns, R. G. & Islam, K. (1984a) *FEBS Lett.* **173**, 67-74
- Burns, R. G. & Islam, K. (1984b) *Biochem. J.* **224**, 623-627
- Burns, R. G. & Islam, K. (1984c) *Eur. J. Biochem.* **141**, 599-608
- Burns, R. G. & Islam, K. (1985) *Ann. N.Y. Acad. Sci.*, in the press
- Burns, R. G., Islam, K. & Chapman, R. (1984) *Eur. J. Biochem.* **141**, 609-615
- Carlier, M.-F. & Pantaloni, D. (1978) *Biochemistry* **17**, 1908-1915
- Carlier, M.-F., Hill, T. L. & Chen, Y. D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 771-775
- Geahlen, R. L. & Haley, B. E. (1979) *J. Biol. Chem.* **254**, 11982-11987

- Hamel, E., del Campo, A. A., Lowe, M. C. & Lin, C. M. (1981) *J. Biol. Chem.* **256**, 11887–11894
- Hartree, E. F. (1972) *Anal. Biochem.* **48**, 422–427
- Herzog, W. & Weber, K. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1860–1864
- Huitorel, P., Simon, C. & Pantaloni, D. (1984) *Eur. J. Biochem.* **144**, 233–241
- Islam, K. & Burns, R. G. (1984a) *Anal. Biochem.* **137**, 8–14
- Islam, K. & Burns, R. G. (1984b) *FEBS Lett.* **178**, 264–270
- Islam, K. & Burns, R. G. (1985a) *Ann. N.Y. Acad. Sci.*, in the press
- Islam, K. & Burns, R. G. (1985b) *Biochem. J.* **232**, 651–656
- Jacobs, M. (1979) in *Microtubules* (Roberts, K. & Hyams, J. S., eds.), pp. 255–278, Academic Press, New York
- Jameson, L. & Caplow, M. (1980) *J. Biol. Chem.* **255**, 2284–2292
- Johnson, K. A. & Borisy, G. G. (1977) *J. Mol. Biol.* **117**, 1–31
- Kravitz, N. G., Regula, C. S. & Berlin, R. D. (1984) *J. Cell Biol.* **99**, 188–198
- Kuriyama, R. & Sakai, H. (1974) *J. Biochem. (Tokyo)* **75**, 463–471
- Lee, S-H., Kristofferson, D. & Purich, D. L. (1982) *Biochem. Biophys. Res. Commun.* **105**, 1605–1610
- MacNeal, R. K. & Purich, D. L. (1978) *J. Biol. Chem.* **253**, 4683–4687
- Mitchison, T. & Kirschner, M. W. (1984) *Nature (London)* **312**, 237–242
- Murphy, D. B. & Hiebsch, R. R. (1979) *Anal. Biochem.* **96**, 225–235
- Nath, J. P., Eagles, G. R. & Himes, R. H. (1985) *Ann. N.Y. Acad. Sci.*, in the press
- Parks, R. E. & Agarwal, R. P. (1973) *Enzymes* 3rd Ed. **8**, 307–333
- Penningroth, S. M. & Kirschner, (1977) *J. Mol. Biol.* **115**, 643–673
- Penningroth, S. M. & Kirschner, M. W. (1978) *Biochemistry* **17**, 734–740
- Terry, B. J. & Purich, D. L. (1979) *J. Biol. Chem.* **254**, 9469–9476
- Weisenberg, R. C., Deery, W. J. & Dickinson, P. (1976) in *Cell Motility*, vol. C (Goldman, R., Pollard, T. D. & Rosenbaum, J. L., eds.), pp. 1123–1132, Cold Spring Harbor Press, New York
- Zabrecky, J. R. & Cole, R. D. (1982) *Nature (London)* **296**, 757–759
- Zabrecky, J. R. & Cole, R. D. (1983) *Arch. Biochem. Biophys.* **225**, 475–480
- Zackroff, R. V., Deery, W. J. & Weisenberg, R. C. (1980) *J. Mol. Biol.* **139**, 641–659

Received 9 May 1985/29 July 1985; accepted 14 August 1985