Stability of Tubulin Polymers Formed with Dideoxyguanosine Nucleotides in the Presence and Absence of Microtubule-associated Proteins*

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We have examined the effects of dilution, Ca²⁺, reduced temperature, and triphosphate depletion on microtubules formed from purified tubulin, heat-treated microtubule-associated proteins (MAPs), and either GTP, 2',3'-dideoxyguanosine 5'-diphosphate (ddGDP), or 2',3'-dideoxyguanosine 5'-triphosphate (ddGTP). The stability of the polymer formed with tubulin plus ddGTP without MAPs was also examined. In all cases dilution resulted in rapid depolymerization of polymer until a new turbidity plateau was established. These experiments yielded estimates of the critical concentration of tubulin of 0.09 mg/ml with GTP plus MAPs, 0.04 mg/ml with either ddGDP or ddGTP plus MAPs, and 0.07 mg/ml with ddGTP minus MAPs. Addition of CaCl₂ to polymer resulted in depolymerization of microtubules formed with either GTP or ddGTP plus MAPs; but both with and without MAPs the polymer formed with ddGTP was stable to Ca²⁺. The polymer formed with ddGTP minus MAPs was the most cold-labile, major depolymerization occurring at 25 °C. With MAPs, microtubules were progressively less cold-labile when formed with GTP, ddGDP, or ddGTP. Depolymerization with GTP was virtually complete at 15 °C, with ddGDP at 5 °C, and with ddGTP at 0 °C. Rapid triphosphate depletion was achieved with phosphofructokinase. GTP-formed tubules were rapidly and completely depolymerized at all GTP concentrations after the enzyme was added to the reaction mixture. Both with and without MAPs polymer formed with ddGTP was progressively more stable upon enzyme addition, the higher the initial ddGTP concentration. At specific ddGTP concentrations, however, less depolymerization was observed following enzyme addition if MAPs were present. Microtubules formed with ddGDP plus MAPs were unaffected by phosphofructokinase addition. This comparison of the properties of microtubules formed with MAPs and either ddGDP or ddGTP demonstrates that their stability is enhanced rather than reduced following nucleotide hydrolysis. The greater stability of microtubules formed with ddGTP plus MAPs than of the polymer formed with ddGTP minus MAPs similarly implies substantial enhancement of microtubule stability by the MAPs.

Tubulin binds 2 molar eq of guanosine nucleotide (1–4). These have been termed the nonexchangeable GTP and the exchangeable GTP, since the former can only be removed by denaturing the protein while the latter can be displaced by exogenous nucleotide. Microtubule assembly is generally accompanied by the hydrolysis of exchangeably bound GTP to GDP (5–10), but this reaction is not obligatory in the polymerization of tubulin.

The initial evidence for this was the assembly of microtubules with the nonhydrolyzable GTP analogs p(CH₂)ppG¹ and p(NH)ppG (6, 7, 11, 12). It was then found that GDP would permit the elongation of microtubule seeds, but not de novo polymerization (13, 14), and that the nonhydrolyzable GDP analog p(CH₂)ppG supported a weak polymerization reaction (15). Although no turbidity developed with p(CH₂)ppG, a pellet consisting in part of short microtubules was obtained by centrifuging an incubated mixture of the analog and either tubulin or microtubule protein. It was next found that the drug taxol could promote efficient microtubule formation in the absence of GTP, with GDP in the exchangeable site (16–18).

Most recently we have found that a potentially hydrolyzable GTP analog, ddGDP, can promote efficient microtubule assembly in a MAP-dependent reaction (19). The analog is incorporated unaltered in the polymer in near stoichiometric amounts. In addition we have observed that ddGTP supports vigorous microtubule assembly with MAPs and the formation of a mixture of microtubules and sheets of parallel protofilaments without MAPs (20). The triphosphate is hydrolyzed during these polymerization reactions (19) and is incorporated in the polymer as ddGDP.²

Since GTP hydrolysis is not essential for microtubule assembly, but nevertheless occurs if GTP is present during polymerization, there has been much speculation about the role of GTP hydrolysis in the reaction. One suggestion, based on studies with p(NH)ppG, is that hydrolysis in effect destabilizes the microtubule and is required for its rapid disassembly. Microtubules formed with p(NH)ppG were found to be more resistant to depolymerization by both calcium and dilution than those formed with GTP (6, 11, 21). One limitation to this conclusion, however, is that a nucleotide analog may

¹ The abbreviations used are: p(CH₂)ppG, guanosine 5'-β,γ-imido-5'-(β,γ-methylene)triphosphate; p(NH)ppG, guanosine 5'-β,γ-imido-5'-(β,γ-methylene)triphosphate; p(CH₂)ppG and p(NH)ppG, guanosine 5'-β,γ-imido-5'-(β,γ-methylene)triphosphate; ddGDP and ddGTP, 2',3'-dideoxyguanosine 5'-diphosphate; MAPs, microtubule-associated proteins; Mes, 2-(N-morpholino)ethanesulfonate.
² E. Hamel, A. A. del Campo, and C. M. Lin, unpublished observations.

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Received for publication, June 16, 1983

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produce unanticipated secondary effects on the conformation and properties of the tubulin-nucleotide complex. The enhanced stability of p(NH)ppG tubules may derive from such effects rather than from the lack of nucleotide hydrolysis.

The effects of the dideoxyguanosine nucleotides on tubulin polymerization allow a direct approach to this hypothesis, despite potential secondary effects, since microtubules containing ddGDP can be formed from ddGTP (with nucleotide hydrolysis) or directly from ddGDP (without nucleotide hydrolysis). In this study we have compared the stability of microtubules formed with these analogs to each other, to microtubules formed with GTP, and to the ddGTP-dependent polymer formed without MAPs.

EXPERIMENTAL PROCEDURES

Materials—The preparation of GTP, ddGTP, ddGDP, and purified tubulin and heat-treated MAPs from calf brain have been described elsewhere (4, 18-20). The nucleotides were homogeneous on thin layer chromatography on polyethyleneimine cellulose in 1 m KH2PO4. The heat-treated MAPs and tubulin were both free of nucleoside diphosphate kinase and ATPase activities. In 0.1 N Mes, 0.5 mM MgCl2, these two protein components were also free of nucleoside diphosphate kinase activity, measured by Weisenberg and Deery (21) initially observed that microtubules formed with p(NH)ppG resisted depolymerization upon dilution compared to those formed with GTP. They in fact reported that with p(NH)ppG the critical concentration for tubulin polymerization was a negative value, implying indefinite stability. Karr et al. (14), however, found no difference between the critical concentrations of tubulin with GTP and p(NH)ppG. These workers did observe a much slower depolymerization of microtubules formed with p(NH)ppG as compared to those formed with GTP.

Methods—All reaction mixtures contained 0.1 M Mes, 0.5 mM MgCl2, and other components as indicated. Tubulin polymerization was followed turbidimetrically (23) by change in absorbance at 350 nm of a Gifford model 250 or model 2400-S spectrophotometer. Both instruments were equipped with Gifford "Thermoset" units for electronic temperature control, rather than the thermostatically controlled circulating water bath we had used in our previous studies (19, 26). All reaction components were mixed at 0 °C, with tubulin added last, and added to cuvettes at 0 °C. Base-lines were established at 0 °C, and then the temperature was set at 37 °C (indicated on the abscissa of the figures by an arrow). About 70 s later the instrument readout reached 37 °C (indicated on the abscissa of the figures by a second arrow), and this point is arbitrarily defined as zero time. When the temperature reached 37 °C, the temperature of the contents of the cuvettes was 34 °C. The cuvettes' contents were at 36.5 °C at 15 s, at 36.5 °C at 30 s, and had equilibrated at 37 °C by 60 s. We have used this method, rather than adding a nucleotide to reaction mixtures at 37 °C, because we have found that polymerization with more active nucleotides, including GTP, began while the cuvette contents were being mixed.

Dilutional disassembly of microtubules was performed as described by Weisenberg and Deery (21) and Karr et al. (14). Polymer was formed, with the reaction followed spectrophotometrically, during a 45-min incubation at 37 °C, well into the plateau phase. The desired amount of reaction mixture was then rapidly diluted into an appropriate volume of buffer containing Mes, MgCl2, and nucleotide at the same concentrations as originally present in the polymerization reaction. The drop in turbidity was followed continuously for at least 5 min and then intermittently for another 30 min. Seven dilutional points were obtained in each experiment, 3 followed successively in one spectrophotometer and 4 followed successively in the second instrument.

Polymer treated with Ca2+ was studied in the electron microscope. Polymer was formed, with the reaction followed spectrophotometrically, during a 45-min incubation at 37 °C, well into the plateau phase. CaCl2 to the desired final concentration was added, and incubation continued 45 min at 37 °C. Pellets were obtained by centrifugation of the reaction mixtures for 40 min at 35,000 rpm in a Beckman Ti 50 rotor which had been warmed to 30°-35 °C. The pellets were fixed, and thin sections were prepared and examined as described previously (18).

The polymer formed with 1 mM ddGTP minus MAPs (in the presence of fructose 6-phosphate) and persisting after addition of phosphofructokinase to the reaction mixture was also examined in the electron microscope. The initial incubation was for 10 min at 37 °C, at which point phosphofructokinase was added. After another 20 min at 37 °C the reaction mixture was centrifuged and the pellet processed as described above. Nucleotide degradation was followed by thin layer chromatography and autoradiography as described previously (4, 19).

RESULTS

Stability of Polymer to Dilution—Weisenberg and Deery (21) initially observed that microtubules formed with p(NH)ppG resisted depolymerization upon dilution compared to those formed with GTP. They in fact reported that with p(NH)ppG the critical concentration for tubulin polymerization was a negative value, implying indefinite stability. Karr et al. (14), however, found no difference between the critical concentrations of tubulin with GTP and p(NH)ppG. These workers did observe a much slower depolymerization of microtubules formed with p(NH)ppG as compared to those formed with GTP.

Fig. 1 presents our observations on the dilutional stability of microtubules formed in the presence of MAPs with GTP (Fig. 1A), ddGTP (Fig. 1C), and ddGDP (Fig. 1D) and of the polymer formed with ddGTP without MAPs (Fig. 1B). These experiments indicate critical concentrations of about 0.09 mg/ml with GTP, about 0.04 mg/ml with both ddGDP and ddGTP with MAPs, and about 0.07 mg/ml with ddGTP without MAPs. There is no significant difference between the critical concentrations and the dilutional stabilities of the microtubules formed with ddGTP and ddGDP in the presence of MAPs, but the MAPs significantly lower the critical concentration of the ddGTP-supported reaction.

Fig. 2 demonstrates that depolymerization upon dilution was rapid under all four conditions, with the turbidity read-

![Fig. 1. Dilutional stability of tubulin polymers. A, microtubules formed with GTP plus MAPs. B, polymer formed with ddGTP without MAPs. C, microtubules formed with ddGTP plus MAPs. D, microtubules formed with ddGDP plus MAPs. For the polymerization reactions 0.45 ml reaction mixtures were prepared containing 1.0 mg/ml of tubulin, 0.25 mg/ml of heat-treated MAPs as indicated, and the appropriate nucleotide at 0.4 mM. The reactions were followed spectrophotometrically for 45 min at 37 °C, well into the plateau phase in all cases. Aliquots of the polymerization reaction mixtures were then diluted to the final tubulin concentrations shown in the figure with the appropriate amounts of solutions at 37 °C containing 0.1 M Mes, 0.5 mM MgCl2, and the appropriate nucleotide at 0.4 mM. The diluting solutions were in cuvettes, and the drop in turbidity was monitored as soon as the reaction mixture and the diluting solution were mixed. Samples were monitored continuously for about 5 min following dilution and then intermittently for an additional 30 min. Each set of symbols represents a different experiment.](http://www.jbc.org/Downloaded_from)
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FIG. 2. Time course of dilutional depolymerization. Typical depolymerization time course curves obtained in the experiments described in Fig. 1, all at a final tubulin concentration of 0.17 mg/ml. A, GTP plus MAPs. B, ddGTP without MAPs. C, ddGTP plus MAPs. D, ddGDP plus MAPs.

ings at 10 min differing little from those obtained a half hour later. In no experiment was a slow depolymerization, similar to that reported by Karr et al. (14) with p(NH)ppG tubules, observed.

Stability of Polymer to Ca2+-Both Weisenberg et al. (6) and Arai and Kaziro (11) have reported that p(NH)ppG tubules, unlike those formed with GTP, are stable in Ca2+. We, therefore, next examined the effect of Ca2+ on microtubules formed with GTP, ddGTP, and ddGTP with MAPs and on the polymer formed with ddGTP without MAPs. Effects on both turbidity readings and on polymer morphology were studied.

When 1 mM CaCl2 was added to microtubules formed with GTP, the turbidity reading fell rapidly to less than half the initial value (Fig. 3A). Increasing the CaCl2 to 2 mM led to a further decrease in turbidity, the total drop being about 85% of the initial reading. No significant change occurred when still more CaCl2 was added, first to 5 mM, then to 10 mM. When the reaction mixture was centrifuged, a small pellet was obtained. Electron microscopy demonstrated that this pellet consisted of amorphous aggregate (Fig. 4A).

With ddGTP plus MAPs successive additions of CaCl2 to 1, 2, 5, and 10 mM had only transient and minimal effects on the turbidity reading (Fig. 3C). Pellets were obtained by centrifugation of reaction mixtures after the addition of CaCl2 to both 5 and 10 mM. Electron microscopic examination demonstrated that the microtubules originally present had persisted with no obvious morphological change (Fig. 4C) in 5 mM CaCl2. At the higher CaCl2 concentration, although most of the polymer was still in the form of microtubules, a number of enlarged tubules was also observed (Fig. 4D). The average diameter of these structures was 70 nm. They have not been observed in the absence of Ca2+.

A third pattern was observed with ddGDP plus MAPs. Successive additions of CaCl2 to 1, 2, 5, and 10 mM caused large volume decreases to about 50% of the initial value. No further decrease in turbidity could be observed with additional CaCl2. In fact, addition of CaCl2 to 20 mM caused a rapid rise in turbidity due to aggregation (data not presented). Centrifugation of the reaction mixture with 10 mM CaCl2 and examination of the pellet in the electron microscope indicated that the residual turbidity was due to aggregate rather than to persistent polymer (Fig. 4B). No trace of the microtubules formed with ddGDP remained after the addition of CaCl2.

With ddGTP minus MAPs’ addition of 1 mM CaCl2 led to an initial small drop, followed by a steady rise in the turbidity reading (Fig. 3B). If 5 mM CaCl2 was added, a rapid rise in turbidity occurred (Fig. 3B). Pellets obtained by centrifugation of these reaction mixtures differed little in their morphological appearance. In the absence of Ca2+ (Fig. 4E), addition of 1 mM Ca2+ (data not presented) and after addition of 5 mM Ca2+ (Fig. 4F) both open sheets and microtubules were observed. With Ca2+, however, there appeared to be significantly more amorphous aggregate in the pellets (Fig. 4, E and F). This probably explains the rise in turbidity observed after the addition of CaCl2 (Fig. 3B).

Stability of Polymer to Low Temperatures. In addition to their instability to Ca2+ and upon dilution, microtubules also disassemble at low temperatures. Since we had previously observed that microtubules formed with ddGTP plus MAPs were still more CaCl2 was added, first to 5 mM, then to 10 mM. This probably explains the rise in turbidity due to aggregation (data not presented). Centrifugation of the reaction mixture with 10 mM CaCl2 and examination of the pellet in the electron microscope indicated that the residual turbidity was due to aggregation rather than to persistent polymer (Fig. 4B). No trace of the microtubules formed with ddGDP remained after the addition of CaCl2.

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FIG. 4. Effects of Ca$^{2+}$ on the morphology of tubulin polymers. Each 0.4-ml reaction mixture contained 1.0 mg/ml of tubulin, 0.25 mg/ml of heat-treated MAPs as indicated, and the appropriate nucleotide at 0.4 mM during the polymerization reaction. CaCl$_2$ to the indicated concentration was then added in stages as in Fig. 3. Further details are given in the text. All magnifications are ×27,000. A, pellet obtained after 5 mM CaCl$_2$ was added to the microtubules formed with GTP plus MAPs. B, pellet obtained after 10 mM CaCl$_2$ was added to the microtubules formed with ddGDP plus MAPs. C, pellet obtained after 5 mM CaCl$_2$ was added to the microtubules
had enhanced temperature stability (20), a detailed examination of the effects of temperature on polymerization and depolymerization with these analogs was undertaken. Fig. 5 presents a study in which the reaction temperature was first raised and then lowered stepwise. As before, the reactions with GTP, ddGDP and ddGTP with MAPs, and with ddGTP without MAPs were compared.

With GTP (Fig. 5, curve 1) there was no change in turbidity at temperatures below 20°C. At 20°C a sluggish rise in turbidity occurred, but at 25°C there was a brisk reaction. The rise in turbidity at 25°C represented about 50% of the total reaction with GTP. A smaller additional rise occurred when the temperature was raised to 30°C, and a minimal further reaction occurred at 37°C. When the temperature was decreased, a small drop in turbidity occurred at 25°C. The largest changes occurred at 20 and 15°C. There was little additional change thereafter.

The polymerization reaction with ddGTP plus MAPs (Fig. 5, curve 3) was very different. The major rise in turbidity occurred at 10°C with progressively smaller increases at 15, 20, and 25°C. At 37°C there was actually a downwar drift in the turbidity plateau. (Cf. Fig. 3C; this downward drift occurs about half the time in the reaction with ddGTP plus MAPs and is of uncertain significance.) The first clearcut depolymerization was a small drop in turbidity at 10°C, followed by a larger decline at 5°C. Full depolymerization of ddGTP microtubules, however, only occurred at 0°C, with over half the depolymerization reaction occurring at this low temperature.

The polymerization reaction with ddGDP plus MAPs (Fig. 5, curve 2) was more temperature-dependent than either the ddGTP- or GTP-supported reaction. Although there was minimal turbidity development at 20 and 25°C, a substantial polymerization reaction did not occur until the temperature was raised to 30°C. Another large rise in turbidity occurred at 37°C. Once formed, however, these ddGDP microtubules were much more cold-stable than GTP microtubules. No depolymerization occurred until the temperature was lowered to 10°C. The depolymerization occurring at this temperature was relatively slow, but more extensive than the depolymerization of ddGTP microtubules (curve 3). The major depolymerization of the microtubules formed with ddGDP occurred at 5°C. The formation of microtubules with ddGDP plus MAPs thus requires a substantially higher temperature than the formation of microtubules with ddGTP plus MAPs. Once formed, ddGDP-microtubules are relatively cold-stable, but nevertheless more labile than those formed with ddGTP plus MAPs.

The polymerization reaction with ddGTP without MAPs (Fig. 5, curve 4) was also more temperature-dependent than the reactions with GTP or ddGTP plus MAPs. A minimal rise in turbidity at 25°C was followed by large reactions at both 30 and 37°C. Unlike the microtubules formed with ddGDP plus MAPs, however, this MAP-independent polymer was quite cold-labile. Although relatively slow, extensive depolymerization occurred at 25°C, with about 70% of the total drop in turbidity occurring at this relatively high temperature. Successively smaller drops in turbidity occurred at 20 and 15°C. The MAP-independent polymer thus is relatively temperature-dependent, both for its formation and for its maintenance.

**Stability of Polymer to Triphosphate Depletion**—To examine the stability of microtubules formed with ddGTP or GTP plus MAPs and the MAP-independent polymer formed with ddGTP to rapid triphosphate depletion we used the phosphofructokinase system described by Margolis (24) (Fig. 6). In these experiments, to assess the extent of depolymerization obtained with phosphofructokinase, the reaction mixtures were chilled to 0°C after the new turbidity plateaus were established.

The GTP-formed microtubules disappeared rapidly upon addition of the enzyme (Fig. 6, A–C), with essentially identical patterns observed at all nucleotide concentrations. A range from 10 to 1000 μM GTP was examined in our system, disassembly of GTP microtubules was almost complete following the addition of phosphofructokinase, as there was little further drop in turbidity when the temperature was reduced to 0°C.

Not surprisingly, since ddGDP supports microtubule formation with MAPs, the microtubules formed with ddGTP plus MAPs were much less affected by the addition of phosphofructokinase (Fig. 6, D–F). At ddGTP concentrations below the nucleotide threshold for polymerization with ddGDP (about 200 μM, see Ref. 19) there was partial depolymerization following enzyme addition (Fig. 6, D and E). The relative amount of enzyme-induced depolymerization, as compared to the subsequent cold depolymerization, was greater at 10 μM ddGTP (Fig. 6D) than at 100 μM ddGTP.
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Fig. 6. Effects of rapid triphosphate depletion on polymer stability. Each 0.4-ml reaction mixture initially contained 1.0 mg/ml of tubulin, 20 mM fructose 6-phosphate, 0.4 mg/ml of heat-treated MAPs as indicated, and nucleotide as indicated. At the times indicated by the breaks in the curves (and, in addition, by an arrow in I), a mixture of 7 μl of 0.1 M Mes and 1 μl containing 2.1 units of phosphofructokinase in 1.4 M (NH₄)₂SO₄ was added to each reaction. The first arrow on the abscissa indicates the point at which the temperature control unit reached 37°C (see text for further details). The second arrow on the abscissa indicates the point at which the temperature control unit was set at 0°C. A, 10 μM GTP plus MAPs. B, 0.1 mM GTP plus MAPs. C, 1.0 mM GTP plus MAPs. D, 10 μM ddGTP plus MAPs. E, 0.1 mM ddGTP plus MAPs. F, 1.0 mM ddGTP plus MAPs. G, 0.1 mM ddGTP without MAPs. H, 1.0 mM ddGTP without MAPs. I, 1.0 mM ddGDP plus MAPs. Experiments with [γ-³²P]GTP and [γ-³²P]ddGTP were performed under comparable conditions to assess the extent of nucleotide degradation with phosphofructokinase. These data are presented in the figure as per cent of original triphosphate remaining, indicated by the open circles. The first aliquot was taken immediately prior to the addition of phosphofructokinase, and additional aliquots were taken at the indicated times.

With 1 mM ddGDP plus MAPs extensive microtubule assembly occurs (19) and with 1 mM ddGTP plus MAPs addition of phosphofructokinase resulted in only an abrupt, but small, drop in turbidity with no subsequent depolymerization (Fig. 6F) until the temperature was reduced to 0°C.

Without MAPs the polymer formed with ddGTP was significantly more labile when the triphosphate was degraded (Fig. 6, G and H). As with MAPs the extent of depolymerization following enzyme addition varied with the initial ddGTP concentration. With 100 μM ddGTP complete depolymerization occurred following phosphofructokinase addition (Fig. 6G), with no cold-labile polymer surviving. At 1 mM ddGTP (Fig. 6H) only about 60% depolymerization occurred following enzyme addition, with a substantial further drop in turbidity following reduction of the reaction temperature to 0°C.

The variability, as a function of ddGTP concentration, in the extent of depolymerization observed both with and without MAPs following phosphofructokinase treatment does not appear to be caused by a variable extent in the amount of nucleotide degradation. As shown in Fig. 6, D–H, the remaining ddGTP was rapidly degraded following enzyme addition at all concentrations. Similarly, the remaining GTP was rapidly degraded following phosphofructokinase addition (Fig. 6, A–C).

Fig. 6I demonstrates that addition of phosphofructokinase to the polymer formed with ddGDP plus MAPs is without significant effect on the extent of polymerization.

The polymer formed with 1 mM ddGTP minus MAPs which persisted after ddGTP degradation was examined in the electron microscope (Fig. 7A), and it was found to consist entirely of open sheets. We, therefore, also examined the polymer prior to the addition of phosphofructokinase (Fig. 7B), and it, too, was found to consist only of sheets. Additional studies have confirmed that polymer morphology without MAPs varies with the ddGTP concentration. At lower nucleotide concentrations variable numbers of microtubules are observed (20), but at 1 mM ddGTP only sheets appear to be formed.

Discussion

Comparing microtubules formed with ddGTP plus MAPs (with nucleotide hydrolysis) to those formed directly from ddGDP plus MAPs (without hydrolysis), we have studied their depolymerization upon dilution, the addition of Ca²⁺, and the reduction of reaction temperature. Table I presents a summary of the observations presented here. The dilutional stability of microtubules formed with the two nucleotides was essentially identical, and comparable estimates of the critical
The microtubules in the form of ddGDP, it is possible that the ddGTP-formed microtubules derive their greater stability, relative to ddGDP-formed microtubules, not from the hydrolysis of ddGTP associated with polymerization but from the persistence of minor amounts of ddGTP at microtubule ends. If this were the case, addition of ddGTP to preformed ddGDP-microtubules should result in enhanced stability of these microtubules to reduced temperatures and Ca²⁺ addition. When such experiments were performed, the temperature stability properties of ddGDP-formed microtubules were unaltered. They still depolymerized more extensively at 10 and 5 °C than did ddGTP-formed microtubules despite the addition of ddGTP at the turbidity plateau. With Ca²⁺, however, prior addition of ddGTP to ddGDP-formed microtubules appeared to stabilize the polymer. There was no significant drop in turbidity, and both microtubules and 70-nm tubules were found in thin sections of a glutaraldehyde-fixed pellet when ddGTP was added to ddGDP-formed microtubules 15 min before Ca²⁺ was added.

Moreover, these studies on their stability demonstrate that ddGTP- and ddGDP-formed microtubules are not identical structures. Despite their similar morphology (19, 20), with both types of microtubule containing near stoichiometric amounts of ddGDP (19), their properties clearly differ. This
would seem to exclude simple reversible reactions (e.g. tubulin-\(ddGTP\) \(\rightleftharpoons\) microtubule-\(ddGD\) \(\rightleftharpoons\) tubulin-ddGDP) as an adequate description of microtubule assembly with dideoxyguanosine nucleotides.

At present we do not know the basis for the different stability properties of the \(ddGDP\)- and \(ddGTP\)-formed microtubules. One intriguing possibility lies in differing properties of \(ddGTP\)- and \(ddGDP\)-formed microtubules could be a manifestation of microtubule growth in different directions. Assuming this is the case, tubulin with bound \(ddGTP\) might add preferentially at one end of a microtubule, while tubulin with bound \(ddGD\) would tend to add at the other.

The studies presented here also emphasize the importance of the MAPs in stabilizing tubulin polymers, as exemplified here in the generally greater stability of microtubules formed with \(ddGTP\) plus MAPs than of the polymer formed with \(ddGD\) minus MAPs. The most striking effect in this comparison was observed in the temperature studies. Formation of the MAP-independent polymer in significant amounts required a temperature \(20^\circ C\) higher than with MAPs (30 versus \(10^\circ C\)), and this \(20^\circ C\) difference was also observed in the depolymerization reactions. Significant depolymerization was observed at \(25^\circ C\) without MAPs, but only at \(5^\circ C\) with MAPs. In addition, the dilutional studies demonstrated that the critical concentration for tubulin with \(ddGTP\) was approximately halved by adding MAPs to the reaction; and with phosphofructokinase the MAP-independent polymer was more labile to rapid triphosphate depletion at all nucleotide concentrations. Only with \(Ca^{++}\) were similar stabilities observed with the MAP-dependent and -independent polymers. \(Ca^{++}\) addition had minimal effects on \(ddGTP\)-supported polymers both with and without MAPs.

There is a remarkable similarity in the effects on tubulin polymerization of the dideoxyguanosine nucleotides and of the \(\alpha\beta\) methylene GDP and GTP analogs studied by San doval et al. (15, 29). Although we have found that MAP-independent polymerization is much more marked with \(ddGTP\) than with \(pp(CH_2)pG\) (20) and \(ddGD\) is substantially more potent than \(p(CH_2)pG\) in supporting polymerization (19), in polymerization with MAPs both \(ddGTP\) and \(pp(CH_2)pG\) produce structures with enhanced stability to dilution, \(Ca^{++}\), and cold as compared to GTP-formed microtubules. These qualitative similarities despite major differences in the nucleotide modifications probably derive from comparable effects on the conformation of the tubulin-nucleotide complex.

Finally, we would like to point out that microtubules formed with tubulin plus heat-treated MAPs were completely labile to rapid GTP depletion with phosphofructokinase plus fructose 6-phosphate at all GTP concentrations, unlike the partial stability reported by Margolis for microtubules formed with microtubule protein (24). Lee et al. (30) have recently reported that the extent of disassembly of microtubules with phosphofructokinase varies with the method of preparation of microtubule protein. It is, therefore, possible that either heat treatment or another step in our MAPs preparation results in the loss of a MAP which maintains microtubules in the presence of GDP.

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J. Biol. Chem. 1984, 259:2501-2508.

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