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In Vitro Microtubule Assembly Regulation by Divalent Cations and Nucleotides†

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ABSTRACT: Mg^{2+} and guanosine 5′-triphosphate (GTP) are usually used to assemble microtubule protein (tubulin + microtubule-associated proteins) or tubulin into microtubules in vitro. Recently, it has been shown that Mn^{2+} will substitute for Mg^{2+} in inducing pure tubulin + GTP to assemble into microtubules. We find that Mn^{2+} also substitutes for Mg^{2+} in inducing two-cycle calf microtubule protein (MTP) + GTP to assemble into microtubules. Zn^{2+} or Co^{2+} induces MTP + GTP to form sheets with more than 13 protofilaments. We find that Co^{2+} also substitutes for Zn^{2+} in inducing tubulin to form 200-nm tubules similar to those reported with Zn^{2+}. To learn whether metal-induced assembly is due to direct binding and/or a metal–GTP complex, metal-induced assembly of MTP and tubulin was studied in the presence of Cr^{III}GTP or taxol, two probes which promote assembly in the absence of added GTP. With CrGTP, Mg^{2+} and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA) were required for optimal assembly into microtubules, and Mn^{2+} could substitute for Mg^{2+}. MTP incubated with Zn^{2+} and CrGTP assembled into sheets, shorter than but similar to those induced by Zn^{2+} + GTP. Mg^{2+}-induced microtubules and Zn^{2+}-induced sheets contained 0.45 mol of [8-^{3}H]-GDP/mol of tubulin if assembled from [8-^{3}H]GTP and a mixture of 0.25 mol of [8-^{3}H]CrGTP, 0.1 mol of [8-^{3}H]GDP, and 0.05 mol of [8-^{3}H]CrGDP/mol of tubulin if assembled from [8-^{3}H]CrGTP. Zn^{2+} induced taxol-treated MTP to form sheets. Sheets were also induced from tubulin + Zn^{2+} and either CrGTP or taxol. These studies suggest that the Zn^{2+}-induced structures are not due to a Zn–GTP complex and that Mg^{2+} does not promote assembly only through a Mg–GTP complex.

Mg^{2+} + guanosine 5′-triphosphate (GTP) promotes assembly of two-cycle microtubule protein (MTP)[1] into microtubules (22–24 nm in diameter and with 13–14 protofilaments) (Weisenberg, 1972; Pierson et al., 1978). Although it has been reported that Mn^{2+} does not affect MTP polymerization (Wallin et al., 1977), we find that it substitutes for Mg^{2+}. Our results agree with those of Buttlaire et al. (1980), who showed that Mn^{2+} will substitute for Mg^{2+} in promoting pure tubulin to assemble into microtubules. Zn^{2+} or Co^{2+} induces MTP + GTP to form sheets with considerably more than 13 protofilaments (Larsson et al., 1976; Gaskin et al., 1976; Gaskin & Kress, 1977). Zn^{2+} alters the alignment of protofilaments from a parallel to an antiparallel arrangement (Crepeau et al., 1978; Baker & Amos, 1978). We have previously shown that Zn^{2+} induces 6S tubulin + GTP to assemble into protofilamentous structures which are consistent with a tubular structure of 200–250 nm (Gaskin & Kress, 1977) and report that Co^{2+} will also substitute for Zn^{2+} in promoting these structures. GTP is usually used in MTP assembly experiments, and, thus, metal may be coordinated directly and/or through a metal–GTP complex. To test whether a Mg–GTP or a Zn–GTP complex promotes microtubules or sheets of protofilaments, respectively, we studied assembly of calf MTP in the presence of two probes which can substitute for GTP. One probe was CrGTP, a stable metal–nucleotide complex that has been shown to promote assembly of MTP in the presence of 1 mM Mg^{2+} and 1 mM EGTA (MacNeal & Purich, 1978). The other probe was taxol, a low molecular weight neutral plant product which has antitumor activity and which promotes assembly of MTP and tubulin in the presence of 0.5 mM Mg^{2+} and 1 mM EGTA (Schiff et al., 1979). The effects of Zn^{2+} on the assembly of tubulin in the absence of microtubule-associated proteins (MAPs) and in the presence of GTP, CrGTP, or taxol are also compared.

Experimental Procedures

Preparation of Protein. MTP was prepared from calf brain by the recyclization procedure of Shelanski et al. (1973). The final pellet of microtubules was resuspended in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid, pH 6.6 (Mes), and stored in liquid nitrogen. On the day of the experiment, 1–2 mL of protein was dialyzed for 3 h against 3 × 500 mL of 0.1 M Mes buffer (charged hourly) in a Crowe–Englander-type thin-film microdialyzer at 4 °C. The dialyzed sample was centrifuged at 120000*g* for 40 min at 4 °C, warmed to 37 °C for 30 min, and kept on ice for 10 min before the experiment was started. This MTP will not assemble into microtubules at 37 °C unless

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[1] Abbreviations used: C_c, critical concentration; CrGTP, chromium guanosine 5′-triphosphate; Me_2SO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; MAPs, microtubule-associated proteins; Mes, 2-(*N*-morpholino)ethanesulfonic acid; MTP, two-cycle microtubule protein (tubulin + microtubule-associated proteins).

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appropriate cofactors are added.

The P-cellulose chromatography procedure of Weingarten et al. (1975) was used to remove MAPs and purify tubulin from MTP preparations. Microtubule pellets were resuspended in 0.025 M Mes, 1 mM EDTA, and 1 mM mercaptoethanol. Elution of tubulin from the P-cellulose column was with the same buffer. Samples were diluted with 0.5 M Mes so that the final buffer was 0.12 M Mes, pH 6.6, 0.8 mM EDTA, and 0.8 mM mercaptoethanol.

Sodium dodecyl sulfate–polyacrylamide gradient slab gels (2.5–27%) (Margolis & Kenrick, 1968) were used to establish that MTP preparations contained several MAPs and P-cellulose chromatography removed most of the MAPs. Protein concentrations were determined by the method of Lowry et al. 1951.

Preparation of CrGTP. CrGTP was prepared as previously described (Cleland & Mildvan, 1979; MacNeal & Purich, 1978). Both GTP (Sigma type II) and [8-^{3}H]GTP (New England Nuclear) were used as starting materials. The purity of both cold and radioactive CrGTP was analyzed by column chromatography on 13 × 1 cm DEAE-cellulose Cl^- columns as previously described (MacNeal & Purich, 1978). This procedure gives an excellent separation of CrGTP, GDP, and GTP. The CrGTP preparations contained 95% CrGTP and less than 5% GTP. Treatment of the CrGTP preparations with standard buffer assembly conditions with Mg^{2+} or Zn^{2+} for 37 °C, 30 min, did not significantly change the elution profile.

In order to further purify CrGTP and selectively remove GTP, the CrGTP preparation was treated with glycerokinase, which binds GTP tighter than CrGTP (Janson & Cleland, 1974), in an assay system with glycerol as a substrate and Mg^{2+} as a cofactor. Conditions of the assay were 1.0 mM CrGTP preparation, 3 mM glycerol, 0.5 mM $MgCl_2$, and glycerokinase [from *Candida mycoderma*, Boehringer (50 μg/mL)] in 0.1 M Mes, pH 6.6. Analysis of enzyme-treated CrGTP on DEAE-cellulose columns showed a trace (<1%) of GTP. As a control, 0.5 mM GTP was treated by using the glycerokinase system. Addition of the enzyme-treated GTP (1 part) to 2.5 mg/mL MTP (4 parts) did not induce assembly although 0.1 mM GTP induces maximum assembly (A_{350} = 0.22) with no decay for at least 20 min.

Metal Ion Induction of Assembly. In some experiments, metal ions were added to protein at 4 °C. After 10 min, nucleotide or taxol (a gift from S. Horwitz) was added. In other experiments, nucleotide or taxol was added 10 min before metal ions. Assembly was initiated after 10 min, by warming to 37 °C. Since taxol is not very soluble in water, a small aliquot (5 μL or less) in Me_2SO was added to 1 mL of protein. The same volume of Me_2SO was added to protein samples before or after metal ions were added. Salts used were $MgSO_4$, $MnSO_4$, $ZnCl_2$, and $Co(C_2H_3O_2)_2$.

Turbidity Assay. A Cary 118 C recording spectrophotometer with a thermostatable sample changer was used for turbidimetric measurements—A_{350} as a function of time. Such measurements are proportional to the total concentration of microtubules (Gaskin et al., 1974). However, they are only qualitative for Zn^{2+}-induced sheet formation (Gaskin & Kress, 1977). All turbidimetric measurements were at 37 °C.

Electron Microscopy. Electron microscopy on samples was performed as previously described (Gaskin & Kress, 1977) by using a Siemens 101 electron microscope.

Results

Mg^{2+}- and Mn^{2+}-Induced Assembly of MTP + GTP. The assembly kinetics of MTP + 1 mM GTP + 0.2 mM Mg^{2+} are similar to those of MTP + 1 mM GTP + 0.2 mM Mn^{2+} (see Figure 1). Electron microscopy studies confirm the formation of microtubules. Addition of 1 mM GTP to MTP also promotes assembly into microtubules, but the lag time is longer, and the assembly rate is slower than MTP + GTP + Mg^{2+}. No significant assembly is found with 0.03 mM GTP unless 0.1 mM Mg^{2+} or Mn^{2+} is added (data not shown). These results are consistent with GTP and Mg^{2+} promoting assembly and show that Mn^{2+} can substitute for Mg^{2+} in MTP assembly.

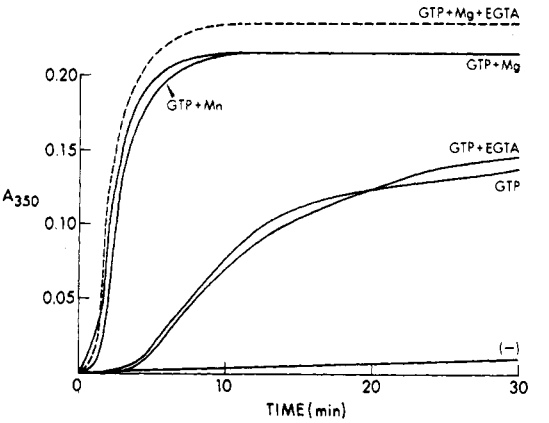

FIGURE 1: Effect of 0.2 mM Mg^{2+}, 0.2 mM Mn^{2+}, 1 mM EGTA, and 1 mM GTP on the rate of polymerization of MTP (2 mg/mL) at 37 °C. Curve is quantitatively the same for Mg^{2+} only, Mn^{2+} only, EGTA only, Mg^{2+} + EGTA, or no additions, and no microtubules were found in these treated samples. All other samples contained microtubules, quantitatively in proportion to A_{350}.

Assembly Studies at Low GTP Concentration. Studies were done to learn the minimum concentration of GTP required to promote assembly of MTP into microtubules (0.1–0.5 mM Mg^{2+}) or sheets of protofilaments (0.05–0.2 mM Zn^{2+}). Specifically, we were interested in this because our preparations of glycerokinase-treated CrGTP may contain a small percentage of GTP (<1%), and we wanted to be certain CrGTP itself was responsible for inducing assembly. Since most CrGTP experiments were done at 0.16–0.2 mM, the effective GTP concentration would be less than 0.002 mM. At 0.01 mM GTP, we found less than 25% assembly (based on A_{350}) with MTP (2.4 mg/mL) and 0.5 mM Mg^{2+}, plus or minus 1 mM EGTA. No significant assembly occurs with 0.002 mM GTP. With 0.1 mM Zn^{2+}, significant assembly of MTP into sheets did not occur below 0.005 mM GTP. However, structures similar to the 200-nm tubules previously described by Gaskin & Kress (1977) form (after a considerable lag) in the absence of added GTP, and these structures scatter more light at 350 nm than a comparable concentration of sheets.

Mg^{2+}-Induced Assembly of MTP + CrGTP. Assembly kinetics of MTP + 0.2 mM CrGTP + 0.2 mM Mg^{2+} and MTP + 0.2 mM CrGTP + 0.2 mM Mn^{2+} result in similar time curves (see Figure 2), and microtubules are confirmed by electron microscopy (Figure 3a). Although there is no assembly of MTP + CrGTP (Figure 2) into microtubules, short spirals are found (Figure 3b). Substantial assembly occurs with MTP + CrGTP + EGTA (Figure 2), and examination by electron microscopy shows sheets as well as microtubules (Figure 3c). Optimal assembly into microtubules occurs with MTP + CrGTP + Mg^{2+} + EGTA (Figures 2 and 3d). Presumably, EGTA binds an inhibitory metal (perhaps Ca^{2+}). The metal is probably in the MTP preparation, although CrGTP contamination by trace metal cannot be ruled out.

In order to eliminate the possibility that GTP contamination of CrGTP was responsible for nucleating assembly, we did

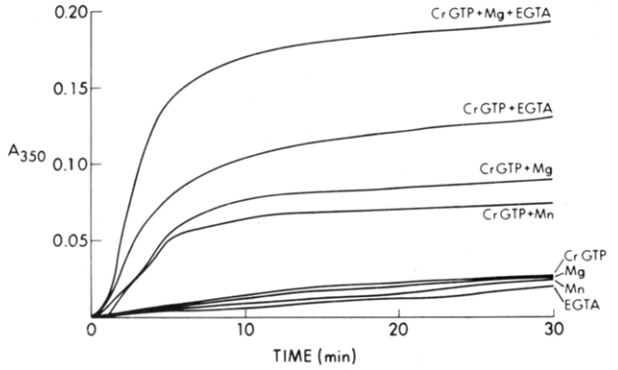

FIGURE 2: Effect of 0.2 mM Mg^{2+}, 0.2 mM Mn^{2+}, 1 mM EGTA, and 0.2 mM CrGTP on the rate of polymerization on MTP (2 mg/mL) at 37 °C. See Figure 3 for specific structures induced by the metal and nucleotide additions.

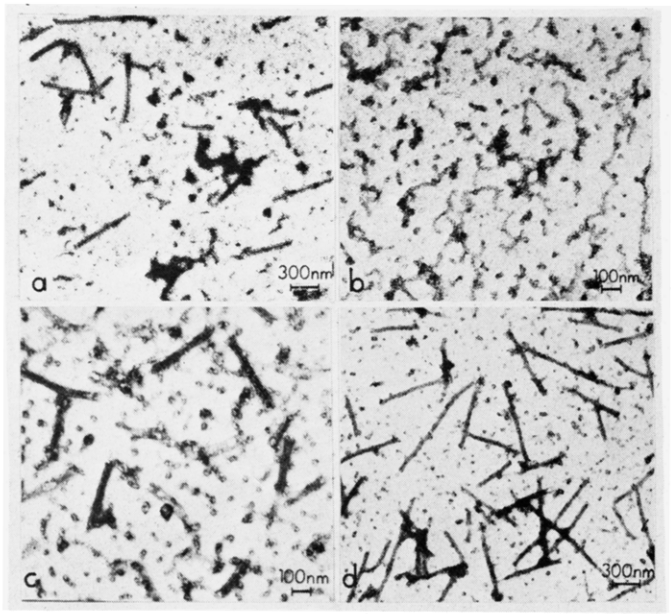

FIGURE 3: Structures induced by metal ions and CrGTP. See Figure 2 for polymerization kinetics. (a) CrGTP + Mn^{2+}. Slightly more microtubules were found than with CrGTP + Mg^{2+}. (b) CrGTP. (c) CrGTP + EGTA. (d) CrGTP + Mg^{2+} + EGTA.

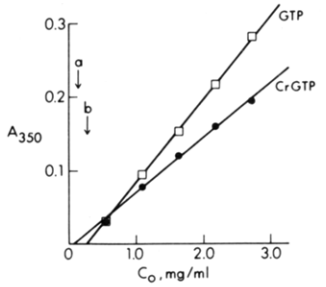

FIGURE 4: Comparison of critical concentrations (C_c) for MTP assembly induced by 0.2 mM GTP or CrGTP + 0.5 mM Mg^{2+} + 1 mM EGTA at 37 °C. C_0 is the total protein concentration. At point a, near the C_c for CrGTP, there are no microtubules in the CrGTP sample. At point b, near the C_c for GTP, there are a few long microtubules in the GTP sample and many short microtubules in the CrGTP sample.

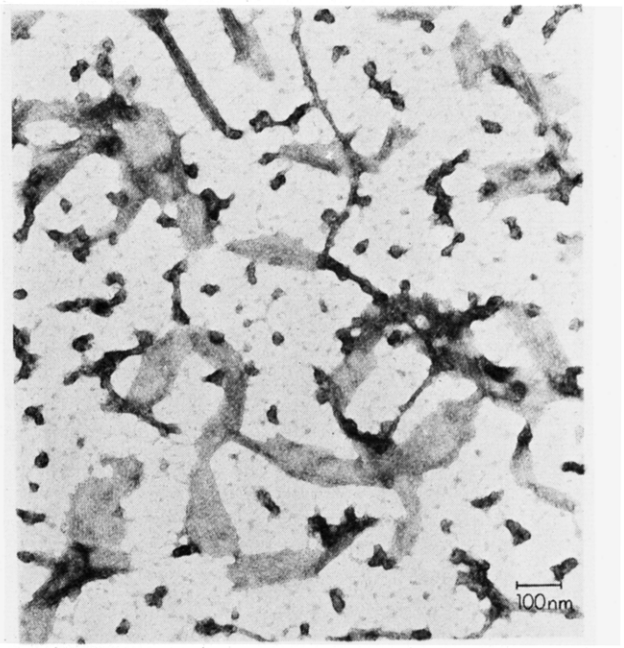

FIGURE 5: Typical zinc-induced sheets formed from MTP (2.0 mg/mL) + 0.2 mM CrGTP (glycerokinase treated) + 0.05 mM Zn^{2+}.

experiments with the glycerokinase-treated CrGTP. The treated CrGTP induced MTP to form microtubules. As expected, similarly treated 0.1 mM GTP did not induce assembly. When 3 mM EDTA was added to bind the Mg^{2+} cofactor used in the glycerokinase preincubation, there was no assembly of MTP into microtubules. Also, with 3 mM EDTA and 1 mM EGTA, CrGTP did not induce assembly of MTP into sheets or microtubules. These results are consistent with Mg^{2+} induction of CrGTP–MTP into microtubules.

Comparison of GTP- and CrGTP-Induced Assembly of MTP. As noted previously, optimal assembly occurs with Mg^{2+}, EGTA, and either GTP or CrGTP. However, we find that microtubules induced by CrGTP + Mg^{2+} + EGTA are considerably shorter than microtubules induced by GTP + Mg^{2+} + EGTA. Figure 4 compares the critical concentrations (C_c) for assembly with both nucleotides. In this study, the C_c is defined as the inverse equilibrium constant for the addition of a protein species (probably 6S tubulin) to a microtubule end. With 0.2 mM CrGTP, the C_c = 0.08 mg/mL, and with 0.2 mM GTP the C_c = 0.27 mg/mL. Lower A_{350} values are found for the CrGTP-induced microtubules and are probably due to the fact that the same total mass of MTP in short microtubules scattered somewhat less light than in long microtubules (Gaskin et al., 1974). Also, in agreement with MacNeal & Purich (1978), we find that the lag time for assembly is less and the rate faster for CrGTP-induced microtubules than GTP-induced microtubules. All of these results suggest that CrGTP promotes nucleation better than GTP.

Co^{2+}-Induced Assembly of Tubulin. Co^{2+} induces purified tubulin + GTP to form structures which are similar to the Zn^{2+}-induced 200-nm tubule structures [see Gaskin & Kress (1977) for negative staining and serial sections on pelleted Zn^{2+}-induced 200-nm structures]. However, higher concentrations of Co^{2+} are required. For example, with tubulin (1.5 mg/mL) and GTP (1 mM), optimal Zn^{2+} is 0.1–0.4 mM and optimal Co^{2+} concentration is 0.6–1.5 mM. Incubations were done at 37 °C for 1 h.

Zn^{2+}-Induced Assembly of MTP + CrGTP and Tubulin + CrGTP. A Zn^{2+} concentration of 0.05 mM induces MTP + enzyme-treated CrGTP to form flat sheets similar to but shorter than those induced by Zn^{2+} + GTP [see Figure 5 and Gaskin et al. (1976) and Gaskin & Kress (1977)]. Optimal conditions for MTP sheet formation are 0.05–0.15 mM Zn^{2+} at 0.2 mM CrGTP. The Zn^{2+} effect is reversed by adding 1 mM EGTA to the CrGTP or GTP sheets. If Zn^{2+} (up to 0.4 mM) is added to (CrGTP + Mg^{2+})-induced microtubules, most microtubules remain, and only a few sheets are found

Table I: Radioactive Nucleotide Binding to Microtubules or Sheets[a]

inducer	structures	N/D[b]	% GDP	% CrGTP	% CrGDP
Mg + GTP	MT[c]	0.47	95		
Zn + GTP	sheets	0.46	94		
Mg + CrGTP	MT	0.37	25	62	12
Zn + CrGTP	sheets	0.40	27	60	11

[a] MTP (2 mg/mL), 0.2 mM Mg^{2+} + 1 mM EGTA or 0.1 mM Zn^{2+}, 0.4 mM [8-^{3}H]GTP (specific activity 2.2 μCi/μmol), or 0.2 mM [8-^{3}H]CrGTP (specific activity 1.7 μCi/μmol). [b] Nucleotides/tubulin dimer. [c] MT, microtubules.

after 30 min. Higher concentrations of Zn^{2+} begin to induce precipitation and disrupt the preformed microtubules, but sheets are not found. However, addition of increasing concentrations of Zn^{2+} (up to 0.4 mM) to (GTP + Mg^{2+})-induced microtubules results in increasing numbers of sheets (many are wide and look like wrapped sheets) and decreasing numbers of microtubules [also see Gaskin et al. (1976)].

Incubation of Zn^{2+} with tubulin + CrGTP [using a wide range of Zn^{2+} concentrations (0.005–1 mM)] failed to produce the characteristic 200-nm tubules that formed from tubulin + Zn^{2+} + GTP. Conditions such as tubulin (0.03 mM), $ZnCl_2$ (0.4 mM), and CrGTP (0.2 mM) resulted in sheets.

Comparison of Nucleotide Binding in Structures Induced by MTP + GTP or CrGTP in the Presence of Mg^{2+} + EGTA or Zn^{2+}. After being assembled with 0.4 mM [8-^{3}H]GTP and divalent metal, and centrifuged through a 2-mL 30% sucrose cushion for 2 h at 200000*g*, 0.2 mM Mg^{2+}-induced microtubules and 0.1 mM Zn^{2+}-induced sheets contain approximately 0.45 mol of [8-^{3}H]GDP/mol of tubulin (see Table I). Using radioactive 0.2 mM CrGTP and divalent metal to promote assembly results in 0.4 mol of nucleotides/tubulin dimer, and the distributions of CrGTP, GDP, and CrGDP are similar in both the Mg^{2+}- and Zn^{2+}-induced structures (Table I). These results show that 0.2 mM Mg^{2+} and 0.1 mM Zn^{2+} do not significantly change the nucleotides bound in the assembled structures. The microtubules assembled with CrGTP + Mg^{2+} + EGTA showed a similar distribution of label as those described by MacNeal & Purich (1978).

Assembly Studies with Taxol and Zn^{2+}. Schiff et al. (1979) reported that taxol induced MTP or tubulin to assemble into microtubules in the absence of GTP at the exchangeable site. We found that Mg^{2+} does not need to be added to promote assembly of taxol–MTP into microtubules. A comparison of MTP (2 mg/mL) assembly with taxol (0.02 mM) in the absence and presence of 0.05 mM Zn^{2+} resulted in mainly microtubules and sheets of 8–20 protofilaments, respectively. Addition of 1 mM EGTA to the Zn^{2+}–taxol-induced sheets results in microtubules. The taxol-induced microtubules are stable to Zn^{2+} (up to 0.5 mM), and higher concentrations of Zn^{2+} result in precipitation.

With tubulin + taxol + Zn^{2+}, sheets similar to, but wider than, those induced by MTP + taxol + Zn^{2+} are found.

Discussion

Table II summarizes the effects of (1) the metals Mg^{2+}, Mn^{2+}, Zn^{2+}, and Co^{2+}, (2) the nucleotides GTP and CrGTP, and (3) taxol on MTP and tubulin assembly. This table demonstrates the following features: (1) Mn^{2+} can substitute for Mg^{2+} in MTP assembly into microtubules. (2) Co^{2+} can substitute for Zn^{2+} in MTP and tubulin assembly into sheets and 200-nm tubules, respectively. (3) CrGTP can substitute for MgGTP in MTP assembly into microtubules. (4) Mg^{2+} and MgGTP are probably required for optimal assembly into microtubules, since Mg^{2+} + CrGTP + EGTA results in more assembly into microtubules than CrGTP + EGTA. (5) CrGTP shifts ring $\rightleftharpoons$ spiral equilibrium to spirals. Kirschner et al. (1974) previously showed that GTP shifts the equilibrium to spirals. (6) Zn^{2+} promotes sheets of MTP in the presence of GTP, CrGTP, or taxol. Zn^{2+} + MTP results in the formation of a few 200-nm tubules. Three-dimensional reconstruction of tubulin in the CrGTP–Zn sheets, taxol–Zn sheets, and 200-nm tubules is required to learn if the alignment of protofilaments is identical with that in the GTP–Zn sheets. (7) Many more 200-nm tubules are promoted from 6S tubulin + GTP + Zn^{2+} than by Zn^{2+}. Sheets are induced from 6S tubulin + taxol + Zn^{2+} or CrGTP + Zn^{2+}.

Addition of Zn^{2+} to (GTP + Mg^{2+} + EGTA)-induced microtubules results in loss of microtubules and the formation of wide sheets. Zn^{2+} induces few sheets from (CrGTP + Mg^{2+} + EGTA)-induced microtubules and essentially no sheets from taxol-induced microtubules, which are more stable ($C_c \leq 0.01$ mg/mL; Schiff et al., 1978) than CrGTP-induced microtubules ($C_c = 0.08$ mg/mL; this work), which are more stable than GTP-induced microtubules ($C_c = 0.27$ mg/mL; this work). Thus, addition of Zn^{2+} to a microtubule–tubulin mixture results in sheets only if there is sufficient tubulin.

With experimental conditions which result in 200-nm tubules, there is a long lag time (~15 min with 1.2 mg/mL 6S tubulin, 0.2 mM Zn^{2+}, and 1 mM GTP at 30 °C) (Gaskin & Kress, 1977). Substitution of 1×10^{-5} M taxol for GTP results in sheets after a short lag (<5 min). The 200-nm structures are probably the result of wide sheets which wrap due to the

Table II: Effect of Metals, Nucleotides, and Taxol on MTP and Tubulin Assembly

sample	no additions	0.4 mM GTP	0.2 mM CrGTP	0.02 mM taxol
MTP[a] + Mg (0.2 mM)	rings	MT[b]	some MT, long spirals	MT
MTP + Mn (0.2 mM)	rings	MT	some MT, long spirals	nd
MTP (no added metals)	*c*	MT, but slow assembly	long spirals	MT
MTP + EGTA (1 mM)		MT, but slow assembly	some MT, some sheets	MT
MTP + EGTA (1 mM) + Mg (0.2 mM)	rings	MT	MT	MT
MTP + EDTA (7 mM)				
MTP + Zn (0.05 mM)	rings, few 200-nm tubules	sheets	sheets	sheets
MTP + Co (0.15 mM)	rings	sheets	nd[d]	nd
tubulin[e] + Mg (0.5 mM)				MT[f]
tubulin + Zn (0.2 mM)	few 200-nm tubules	200-nm tubules	sheets	sheets[f]
tubulin + Co (0.6 mM)		200-nm tubules	nd	nd

[a] 2 mg/mL. [b] Microtubules. [c] No specific aggregates. [d] Not determined. [e] 3 mg/mL. [f] Incubation mixture contained 0.03 mM taxol.

orientation and alignment of protofilaments. It is interesting that structures similar to the Zn^{2+}-induced 200-nm tubules of 6S tubulin + GTP are found in zinc-treated cultures of mouse central and peripheral nerve tissue (Gaskin et al., 1978; Kress et al., 1980). A recent study on long-term effects of excess zinc in the central nervous system of rats shows the slow appearance (after 16 weeks) of the 200-nm structures in the central nervous system of adult Lewis rats in which zinc wires were implanted (Kress et al., 1980). Thus, microtubule assembly in cells is susceptible to the concentration of zinc ions. Such studies strongly suggest the the divalent cationic environment can influence the structure and function of cells containing neurotubules.

Cleland & Mildvan (1979) have recently reviewed the advantages of substituting Cr nucleotides for Mg nucleotides. The CrGTP isomers have been separated into stereochemically distinct coordination isomers whose structures are known, and several reports on the stereospecificity of CrGTP with enzymes are in the literature. Thus, the exact coordination geometry of MgGTP on tubulin could be determined when the isomer of CrGTP which binds to tubulin is known, and such a study should give useful information about the role of MgGTP during assembly. Since chromium is paramagnetic, it is possible to determine the distance between CrGTP and a paramagnetic ion such as Mg^{2+} bound at the tight binding site of tubulin by using electron-spin resonance. Also, distances between CrGTP and other reactants can be determined from nuclear magnetic resonance of key atoms.

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