Separation of Tubulin from Microtubule-Associated Proteins on Phosphocellulose. Accompanying Alterations in Concentrations of Buffer Components†

Robley C. Williams, Jr.,* and H. William Detrich, III‡

ABSTRACT: Tubulin can be conveniently separated from the microtubule-associated proteins by chromatography on phosphocellulose [Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y., & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1858]. Concentrations of Mg^{2+}, GTP, GDP, and glycerol were measured in the various fractions collected during several such separations. The phosphocellulose was found to sequester Mg^{2+} under the conditions employed for the separation and to retard GTP, GDP, and glycerol relative to tubulin. Polymerization of the resulting phosphocellulose–tubulin was found to be inhibited by this Mg^{2+} depletion. The composition of the buffer in which tubulin emerges from the chromatographic column was found to depend in a sensitive way upon the retardation of the other components of the buffer and to be a sensitive function of the width of the pooled tubulin peak and of the ratio of the volume of the chromatographic column to that of the column load. The bearing of these findings on interpretation of existing literature is briefly discussed. Efficient separation of tubulin from microtubule-associated proteins can also be obtained on phosphocellulose columns that have been saturated with Mg^{2+}. Such saturation of the column, or addition of Mg^{2+} to the collected fractions, followed by equilibration of the tubulin with known buffer, is suggested as an improvement to the preparative scheme.

When it is isolated by means of alternate cycles of assembly and disassembly (Shelanski et al., 1973), microtubule protein is found to consist of the major protein tubulin and several microtubule-associated proteins (MAPs)[1] (Murphy & Borisy, 1975; Weingarten et al., 1975; Sloboda et al., 1976; Murphy et al., 1977; Berkowitz et al., 1977). Weingarten et al. (1975) first demonstrated that tubulin could be rapidly and conveniently separated from the MAPs by means of chromatography on a column of phosphocellulose (PC). Chromatography on PC has since become an important last step in a large number of reported preparative schemes. Several reports have noted that PC-purified tubulin (PC–tubulin) does not polymerize into microtubules under conditions that allow unchromatographed microtubule protein[2] to do so (Weingarten et al., 1975; Bryan, 1976; Sloboda et al., 1976; Witman et al., 1976; Cleveland et al., 1977). Others (Himes et al., 1976, 1977) have reported polymerization of PC–tubulin under solvent conditions (glycerol or dimethyl sulfoxide concentrations of more than 1 M) not necessary for the polymerization of unchromatographed microtubule protein. Recently, Herzog & Weber (1977) and Detrich & Williams (1978) have obtained microtubule assembly from PC–tubulin in buffers that contain no special additives. It is thus clear that tubulin prepared by this method can form microtubules.

In using PC–tubulin, we have observed variable polymerization behavior from one preparation to another. Cleveland et al. (1977) have noted both variability in polymerizability (attributed by them to differences between different batches of phosphocellulose) and rapid loss of polymerizability on storage at 4 °C. Instability of polymerizability has also been observed by us and reported by Himes et al. (1977). Both the conflicting reports regarding the capacity of PC–tubulin to polymerize and the observations of instability of polymerizability suggest that the PC column may act to alter some essential component or components of the buffer.

Himes et al. (1977) noted that phosphocellulose binds Mg^{2+} very well and showed that addition of Mg^{2+} to PC–tubulin stimulates its polymerization in the presence of 10% dimethyl sulfoxide. Herzog & Weber (1977) have also shown that addition of Mg^{2+} to PC–tubulin stimulates its polymerization. Detrich & Williams (1978) found that batch-to-batch uniformity and long-term stability of the protein could be obtained by equilibrating it after chromatography with the same buffer used to load and elute the PC column. These findings suggested that Mg^{2+} might be removed from the buffer and that the concentrations of other components might be perturbed in an uncontrolled way by the PC column.

Materials and Methods

Materials. Phosphocellulose (Whatman P-11, control no. 2111 62) was precycled in 0.5 N HCl and in 0.5 N NaOH according to the manufacturer's instructions. Pipes, GTP (grade II-S), EGTA, and DTE were obtained from Sigma Biochemicals, St. Louis, MO. Eriochrome black T (Mordant Black 11, CI 14645) was purchased from Matheson Coleman and Bell Chemical Co. and was washed and recrystallized as described by Diehl & Lindstrom (1959). All other chemicals were reagent grade.

Microtubule Protein and Tubulin. Microtubule protein was prepared by three cycles of assembly and disassembly according to the method of Shelanski et al. (1973) as modified by Berkowitz et al. (1977). Chromatography on PC was carried out at 4 °C in column buffer (0.1 M Pipes, 1 mM $MgSO_4$, 2 mM EGTA, 0.1 mM GTP, and 2 mM DTE, pH 6.90). Approximately 150-mL wet-settled volume of precycled PC was washed in two 250-mL changes of column buffer. The slurry was then titrated to pH 6.90 with NaOH, and the PC

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‡ Formal affiliation: Yale University, New Haven, CT.

[1] Abbreviations used: DTE, dithioerythritol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; MAPs, microtubule-associated proteins; $NaDodSO_4$, sodium dodecyl sulfate; PC, phosphocellulose; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

[2] In this paper microtubule protein refers to tubulin plus all of the associated proteins (MAPs) which copurify with tubulin through cycles of in vitro assembly and disassembly. Tubulin refers specifically to the $\alpha\beta$-dimer of the main microtubule protein.

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was further washed with two 500-mL changes of column buffer, poured to make a 2.5 × 25 cm chromatographic column, and washed with another 500 mL of column buffer before application of the sample. The pH and conductivity of the eluted buffer were not measurably different from their values in the buffer going into the column.

Mg^{2+}-Saturated Phosphocellulose Column. In a separate experiment, Mg^{2+}-saturated PC in column buffer was prepared by washing a 100-mL wet-settled volume of precycled PC with two changes (500 mL each) of 0.1 M $MgSO_4$ in H_2O and then with ~2 L of column buffer. The physical appearance of the PC is altered by treatment with $MgSO_4$: it contracts and becomes less easily dispersed in the buffer. A 0.9 × 15 cm column was poured from part of this slurry and washed with ~500 mL of column buffer before application of the sample. The pH, conductivity, and Mg^{2+} concentration of the eluted buffer were not measurably different from their values in the buffer going into the column.

Mg^{2+} Assay. Concentrations of Mg^{2+} in buffers and protein solutions were measured by a modification of the method employed by Cantor & Hearst (1970). Recrystallized eriochrome black T (0.03 g) was dissolved in 100 mL of 95% ethanol and filtered through Whatman No. 1 paper to remove traces of insoluble material. The resulting solution was stable for 4 weeks at 4 °C. A 12-mL amount of this dye solution was diluted with 8 mL of an alkaline buffer (285 mL of NH_4OH of specific gravity 0.88 and 35 g of NH_4Cl brought to 500 mL with water) to make an operating dye solution that was stable for ~12 h. To 0.07 mL of the sample in column buffer were added 0.10 mL of 1.40 mM $CaCl_2$, 0.63 mL of H_2O, and 0.20 mL of operating dye solution. (The $CaCl_2$ is added to release Mg^{2+} from its complex with the EGTA present in the column buffer. The specified amount is $2Ca^{2+}$ per EGTA, which produces optimal sensitivity of the assay to Mg^{2+}.) Within 5 min the absorbance of the mixture was read at 626 nm and at 580.5 nm. (The dye shows an isosbestic point at 580.5 nm with an observed absorbance of 1.17 ± 0.01 for the assay as described.) The observed value of A_{626} was corrected for any small pipetting error by multiplying it by the ratio $1.17/A_{580.5}$. The Mg^{2+} concentration was estimated by comparison of the corrected A_{626} with a standard curve which is linear up to 0.5 mM Mg^{2+}, with a slope of $-1.49A$/mM. The assay was insensitive to the presence of 1 M NaCl or 10^{-4} M protein. The experimental uncertainty of the assay was about ±8 μM.

Guanosine Phosphate Analysis. GTP, GDP, and GMP were separated from each other and from the other ultraviolet-absorbing materials in the column buffer by chromatography at 24 °C on a 0.9 × 25 cm column of DEAE-Sephadex A-25-120 (40–120-μm beads) in 0.22 M ammonia buffer, pH 8.60 (made by adding 3.72 mL of NH_4OH of specific gravity 0.88 to 245 mL of H_2O and using this solution to titrate 1 L of 0.22 M NH_4Cl to pH 8.60). Samples of 1 mL in column buffer were applied to the column, eluted at a pumped flow rate of 1.0 mL/min, and detected in the eluant by a Pharmacia UV monitor. DTE elutes first, followed by GMP, GDP, and GTP. Amounts of each nucleotide were estimated by integrating the appropriate peaks and comparing the areas to those produced by solutions of the known nucleotides at known concentrations.

Miscellaneous Methods. Glycerol concentration in column buffer was calculated from the measured refractive index of the sample. A refractive index increment of 1.058×10^{-2} M^{-1} was employed. Protein concentrations were measured by the method of Bradford (1976). The assay was calibrated as

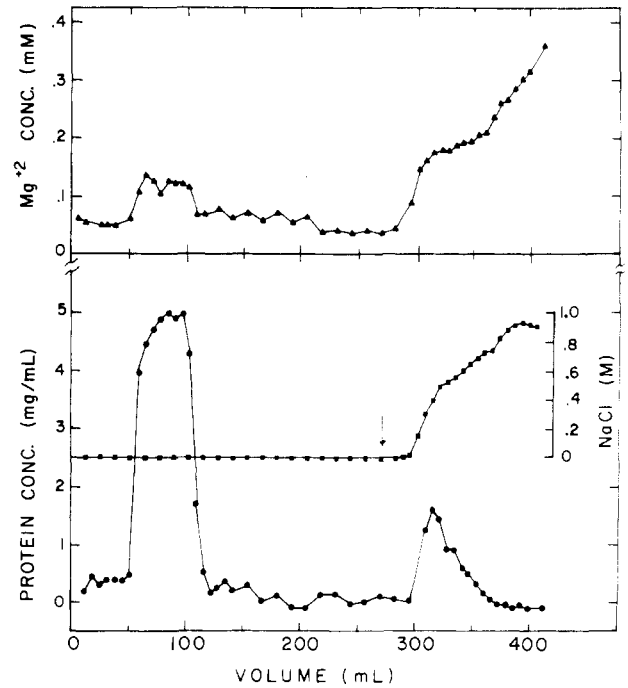

FIGURE 1: Mg^{2+} content of fractions from the PC column. The first protein peak is tubulin, and the second is primarily MAPs. Microtubule protein, prepared as described in the text, was allowed to assemble at 34 °C for 30 min in a buffer consisting of 0.1 M Pipes, 1 mM $MgSO_4$, 2 mM EGTA, 2 mM DTE, 0.5 mM GTP, and 4 M glycerol, pH 6.9 The tubules were pelleted by centrifugation at 28 °C (45 min at 35 000 rpm in a Beckman T35 rotor), and the pellet was resuspended at 0 °C in about 4 pellet volumes of a buffer identical with the above but lacking glycerol. After incubation for 30 min at 0 °C to disassemble tubules, the solution was centrifuged at 0 °C to remove undissociated aggregates (60 min at 35 000 rpm in the T35 rotor). The supernatant was equilibrated with column buffer by passage through a 2 × 60 cm column of Sephadex G-25. The sample (55 mL at 5.4 mg/mL) was then applied to the PC column. Elution with column buffer at ~0.5 mL/min was continued until the volume indicated by the arrow, where a change to column buffer + 0.8 M NaCl was carried out. Concentrations of protein (●), of NaCl (■), and of Mg^{2+} (▲) were measured in the collected fractions as described in the text.

described by Detrich & Williams (1978). Concentrations of NaCl in column buffer were estimated from conductivity measurements made with a Radiometer CDM 2 conductivity meter. Microtubule assembly was assessed by measurement of turbidity at 34 °C as apparent absorbance at 340 or 350 nm in a Cary 118c recording spectrophotometer. The zero level of apparent absorbance was adjusted before the start of a measurement with buffer in both cuvettes. Specimens were prepared for electron microscopy as described by Detrich & Williams (1978). $NaDodSO_4$–polyacrylamide electrophoresis was carried out on 7.5% slab gels by the method of Laemmli (1970).

Results

Mg^{2+} Sequestration by Phosphocellulose. Separation of tubulin from its asociated proteins was carried out by elution of tubulin in column buffer, followed by elution of the associated proteins with column buffer to which had been added 0.8 M NaCl. Figure 1 shows such a separation, together with the Mg^{2+} concentration observed in each fraction. Several points can be made about this experiment. First, the Mg^{2+} concentration in the eluant is initially only about 5% of the Mg^{2+} concentration in the column buffer, and even by the end of the chromatographic process it has risen only to about 40% of that concentration. Second, the rise in Mg^{2+} concentration

coincides with the rise in NaCl concentration that results from the buffer change. Third, a small increase in Mg^{2+} concentration is seen in the tubulin-containing fractions (60–110 mL).

To assess the Mg^{2+}-sequestering capacity of phosphocellulose under the conditions of chromatography, a titration of buffer with PC was carried out at 4 °C. To 15 mL of column buffer were added aliquots of 50 μL each of a particular slurry of PC. The PC had been precycled as described under Materials and Methods and then equilibrated with column buffer lacking Mg^{2+}. The suspension was mixed, the PC was pelleted by centrifugation, and the Mg^{2+} content of the supernatant buffer was measured. Successive aliquots of PC were added in the same way until the Mg^{2+} content of the buffer dropped below 0.1 mM. Aliquots of the slurry were then extensively washed with water and dried to obtain the mass of PC per unit volume of slurry (0.049 g/mL). From the slope of a linear plot of moles of Mg^{2+} removed vs. grams of PC added, a Mg^{2+} capacity was found of 1.0 ± 0.1 (mequiv/dry g) in column buffer. This number corresponds to approximately 0.12 mequiv/mL of PC column volume. In a confirmatory experiment, a 1.5 × 8 cm column of Mg^{2+}-free PC was poured and then washed at 4 °C with column buffer to which had been added 10 mM $MgSO_4$. The Mg^{2+} concentration of the eluant buffer reached 0.1 mM, and rose precipitously thereafter, when 160 mL of this buffer had flowed through. In this way an effective Mg^{2+} capacity of 1.0 mequiv/dry g or 0.11 mequiv/mL of column volume was obtained. These capacity figures can be used to predict the fact that the 2.5 × 25 cm column of Figure 1 would have a Mg^{2+} capacity of about 14 mequiv (14 L) in column buffer. In the "equilibration" process, it was exposed to only ~2 L of 1 mM Mg^{2+}, or 2 mequiv. Thus, the low Mg^{2+} concentrations observed in Figure 1 would be expected from the capacity of the column. The elution of Mg^{2+} in response to elevated NaCl concentration, while not measured above, can be reproduced in a chromatographic column to which no protein has been applied, but which is otherwise prepared and eluted as in Figure 1.

The elevated Mg^{2+} level in the tubulin-containing fractions (Figure 1) can be related to the tubulin concentration. It is assumed that there is a base line concentration of Mg^{2+}, represented by the mean of the contents of the fractions lying between 0 and 50 mL and between 130 and 280 mL, of 0.061 ± 0.010 mM. When this base line is subtracted from the Mg^{2+} contents of each of the tubulin-containing fractions and the difference is divided by the molar concentration of tubulin in the fraction, a mean ratio of 1.15 ± 0.38 Mg^{2+}/tubulin dimer is obtained. This level probably represents the tightly bound Mg^{2+} reported previously (Olmsted & Borisy, 1975) and known to be present in PC–tubulin (Himes et al., 1977).

Retardation of Small Molecules on the PC Column. In our usual preparation of tubulin, the microtubule protein was assembled in a buffer that contained 4 M glycerol and 0.5 mM GTP. It was then pelleted and resuspended at 4 °C in a buffer that contained no glycerol but which did contain 0.5 mM GTP. This resuspended material was ordinarily subjected to a centrifugation to remove undissociated aggregates and then loaded directly onto the PC column without the intervening desalting step described in the caption to Figure 1. In Figure 2 is shown a chromatogram of such a preparative separation. Refractometric estimation of the concentration of glycerol in the loading solution gave 0.92 M. This much glycerol was evidently carried over from the pellet of the last warm centrifugation. Measurement of the ratio of GTP to total guanosine nucleotide in this loading solution gave a value of 0.7 ± 0.2. Evidently, substantial hydrolysis of GTP must have occurred in the preceding polymerization and depolymerization steps. In Figure 2 are shown measurements of the concentration of glycerol, the summed concentrations of GTP and GDP, and the fraction of GTP in the eluant in the vicinity of the tubulin peak. It can be seen that all three are retarded by the column relative to the tubulin peak. Since glycerol is electrostatically uncharged and since GTP and GDP bear the same charge as the column's phosphate groups, it seems unlikely that an ion-exchange process is responsible for the observed retardation. Rather, it seems likely that an anomalous adsorption or gel filtration process is taking place. Regardless of the origin of the retardation, it is clear from examination of Figure 2 that the composition of the buffer in the pooled tubulin fractions will depend critically upon such factors as the width of the pooled zone, the ratio of the volume of the loading solution to the volume of the column, and any subsequent dilution of the fractions, even with column buffer.

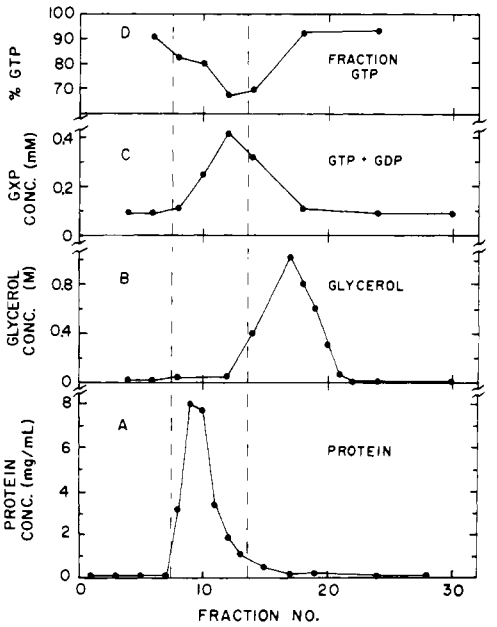

FIGURE 2: Preparative chromatography of microtubule protein on phosphocellulose. A 2.5 × 25 cm PC column was prepared as described in the text. Microtubule protein (30 mL at 8.4 mg/mL) was prepared, applied, and eluted with column buffer as described in the caption to Figure 1, except that the desalting step on G-25 was omitted. The dashed lines indicate the width of the pooled zone in this preparation. (A) Protein concentration; (B) glycerol concentration inferred from the refractive index; (C) total guanosine nucleotide concentration, measured chromatographically as described in the text; (D) the fraction of total guanosine nucleotide present as GTP.

Polymerization of PC–Tubulin. Polymerization of PC–tubulin from the pooled fractions shown in Figure 2 (between the dashed lines) is shown in Figure 3A as curves 1 and 2. At a concentration of 4 mg/mL, the protein will polymerize, but the rate of polymerization is greatly enhanced by the addition of 1 mM $MgSO_4$. Because of the residual amounts of Mg^{2+} and glycerol present in the PC–tubulin preparations and because of the variable ratios of GTP/GDP in these preparations, it is not possible to specify with certainty which of these factors limits polymerization in a given case. The preparation shown in Figure 2 had residual glycerol in it at a concentration of about 0.15 M. Desalting an aliquot of this preparation on a column of Sephadex G-25 previously equilibrated with a buffer that contained all of the elements of the column buffer but Mg^{2+} produced the results shown as curve 3 in Figure 3B. Addition of 1 mM $MgSO_4$ produced polymerization (curve

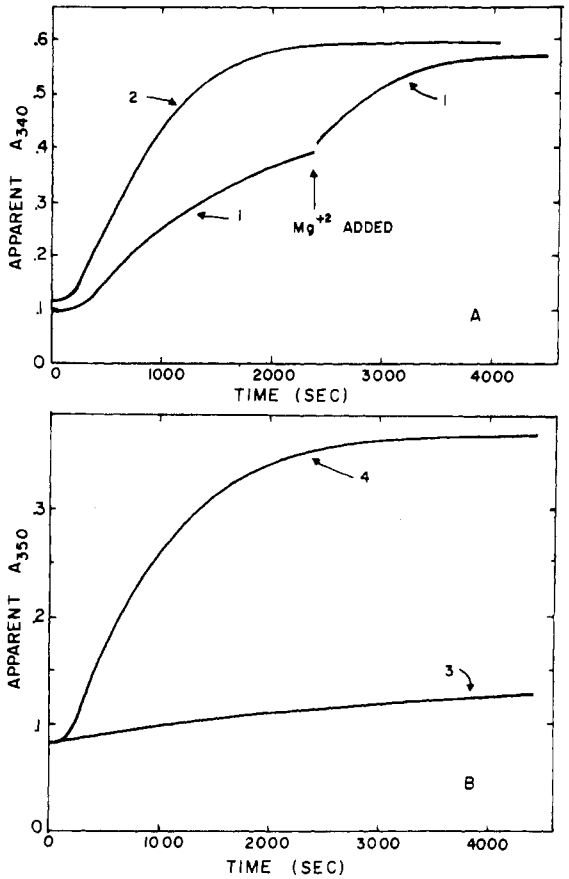

FIGURE 3: Tubulin polymerization. (A) Curve 1 represents pooled PC–tubulin as it comes from the column in Figure 2. At zero time the temperature was raised from 4 to 34 °C. At 2400 s sufficient $MgSO_4$ (20 μL of 50 mM $MgSO_4$ to 1 mL of protein solution) was added to bring the solution to 1 mM Mg^{2+}. Curve 2 represents another aliquot of the same PC–tubulin to which the same amount of cold $MgSO_4$ was added just before zero time. The experiment was otherwise identical. Tubulin concentrations were 3.92 mg/mL after additions of $MgSO_4$. (B) An aliquot (2 mL) of the PC–tubulin from Figure 2 was subjected to buffer exchange on a 0.9 × 25 cm column of Sephadex G-25 into 0.1 M Pipes, 2 mM EGTA, 0.1 mM GTP, and 2 mM DTE, pH 6.9 (i.e., column buffer without Mg^{2+}). Curve 3 represents a 1-mL aliquot of this material to which 20 μL of water was added. Curve 4 represents a 1-mL aliquot of this material to which 20 μL of 50 mM $MgSO_4$ was added. Tubulin concentrations were 2.75 mg/mL. Other conditions were as in (A).

4) as shown. This indicates that Mg^{2+} has a plausible role as the missing factor whose absence inhibits polymerization.

It might be supposed that the stimulation of assembly of PC–tubulin observed upon addition of the MAPs could be an artifact of the elevation of Mg^{2+} levels in the nonequilibrated (and therefore Mg^{2+}-depleted) solutions by dialyzed (and therefore Mg^{2+}-containing) solutions of MAPs. The assembly of buffer-equilibrated PC–tubulin at low protein concentration in the presence and absence of buffer-equilibrated MAPs is shown in Figure 4. It is readily apparent that stimulation of assembly is observed and that qualitatively this stimulation is *not* solely the result of a change in buffer conditions.

Chromatography on Mg^{2+}-Saturated Phosphocellulose. To determine whether the separation of tubulin from MAPs is affected by the binding of Mg^{2+} by the PC, chromatography of 9 mg of microtubule protein was carried out on the Mg^{2+}-saturated column described under Materials and Methods. The prior treatment of the microtubule protein and conditions of elution were as described in the caption to Figure 1, except that the experiment was scaled down. (Centrifugation was carried out in a Beckman Type 40 rotor, desalting

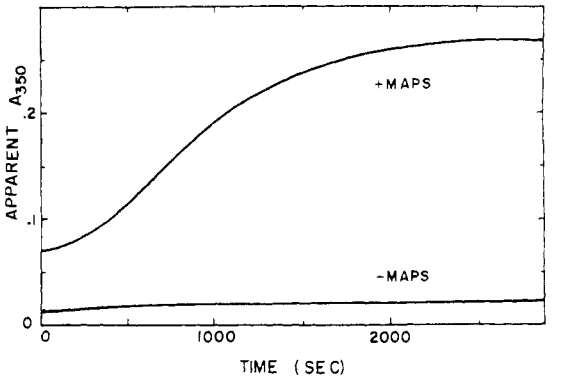

FIGURE 4: Effect of MAPs on polymerization of buffer-equilibrated PC–tubulin. PC–tubulin was equilibrated with column buffer by Sephadex G-25 gel filtration. An aliquot was diluted to a protein concentration of 0.98 mg/mL with buffer (curve labeled –MAPs) and warmed to 34 °C at time zero. To a second aliquot (labeled +MAPs) was added column buffer and a sufficient amount of the pooled, dialyzed second peak of Figure 1 to produce a tubulin concentration of 0.98 mg/mL and a MAP concentration of 0.50 mg/mL. The MAP peak was exhaustively dialyzed against column buffer before the addition.

was accomplished with a 1.5 × 30 cm column of Sephadex G-25, and the flow rate in the PC column was about 0.07 mL/min.) The resulting graph of protein concentration vs. elution volume was essentially identical with that in Figure 1, scaled down. $NaDodSO_4$ gel electrophoresis of the two pooled peaks showed patterns of proteins indistinguishable in each case from those obtained from the two pooled peaks of Figure 1 (data not shown). It thus appears that an equally good separation of MAPs from tubulin can be obtained on Mg^{2+}-saturated columns as on columns that are not saturated. Concentrations of Mg^{2+} were 1.0 mM in the collected fractions until buffer containing 0.8 M NaCl was applied, whereupon they rose rapidly.

Discussion

The results of the experiments demonstrate two main facts: first, PC columns operated under the conditions employed for tubulin preparation sequester the Mg^{2+} from the tubulin solutions; second, the small molecules accompanying the microtubule protein as it is loaded onto the column are partially, but not completely, separated from the tubulin. A demonstrated consequence of these facts is that PC–tubulin emerges from the column in a variable and unpredictable, but always Mg^{2+}-depleted, solvent medium. Because of this Mg^{2+} depletion, the stability and polymerizability of PC–tubulin are impaired. Because of the variability in the tubulin's small molecular environment, the degree of impairment varies from one preparation to another.

These findings are not unanticipated. The solubility products of magnesium phosphates (Linke, 1965) are small, PC is well-known to sequester divalent cations (Peterson, 1970; Cooper, 1977), and the Mg^{2+} affinity (although not the capacity) of PC has been noted in the microtubule literature (Himes et al., 1977). That Mg^{2+} is required for microtubule asembly in vitro has been known for some time (Weisenberg, 1972; Olmsted & Borisy, 1975; Lee & Timasheff, 1977). A paper of Herzog & Weber (1977) has noted that Mg^{2+} must be added to PC–tubulin to cause it to polymerize, although this paper did not point out the sequestration of Mg^{2+} by PC.

The results, relatively unimportant unless viewed in the context of the widespread use of PC chromatography in the preparation of tubulin, may help to resolve some unexplained differences in results reported in the literature. Although it

may have been carried out in some instances and not been reported, exchange (as, for example, by gel filtration or dialysis) of the buffer in which the tubulin fraction is recovered during PC chromatography has not been noted in the literature (e.g., Weingarten et al., 1975; Bryan, 1976; Erickson & Voter, 1976; Himes et al., 1976, 1977; Witman et al., 1976; Cleveland et al., 1977; David-Pfeuty et al., 1977, 1978; Fellous et al., 1977; Herzog & Weber, 1977; Nagle et al., 1977; Wehland et al., 1977; Burton & Himes, 1978; Lockwood, 1978; Sandoval et al., 1978). (The MAP-containing fraction, however, is desalted in most reported investigations.) Thus, a given observation of nonassembly could be the simple result of absence of Mg^{2+}. A dilution series carried out to establish a critical concentration or some other result should also be considered to see if some important buffer component (GTP, GDP, Mg^{2+}, glycerol) might be changing its concentration together with the concentration of the protein.

Although the qualitative observation (Weingarten et al., 1975) that the polymerization of PC–tubulin is stimulated by the addition of associated proteins is confirmed by the present results, they do serve to call into question the magnitude of the effect that may be observed in a given case. If Mg^{2+} is added, along with a known factor, to a Mg^{2+}-depleted solution of PC–tubulin, the effects of the Mg^{2+} and of the factor will be confounded. In order to obtain reproducible results in experiments with PC–tubulin, it is clear that one must equilibrate the protein with the buffer of interest by dialysis or by gel filtration salt exchange. Manipulations such as the dilution with column buffer of PC–tubulin fractions (such as those shown in Figure 2) or the addition of buffer components in an attempt to make the buffer up to a new set of conditions will not produce known or reproducible concentrations of small molecules. The existing literature must be evaluated with these facts in mind.

The problem of sequestration of Mg^{2+} by the PC column can evidently be circumvented by performing chromatography on a Mg^{2+}-saturated column. Alternatively, the addition of a small amount (enough to produce 1–2 mM) of $MgSO_4$ or $MgCl_2$ to the fractions as they emerge from the column increases the stability of the tubulin until equilibration with a known buffer can be carried out. As reported by Detrich & Williams (1978), PC–tubulin that has been equilibrated with column buffer by exchange on Sephadex G-25 within a few hours of its elution from the PC column has been found to be relatively stable upon storage and to polymerize readily to microtubules at concentrations above ~2 mg/mL, essentially in the manner previously described by Herzog & Weber (1977) for PC–tubulin in the presence of added Mg^{2+}. The time course and extent of assembly of tubulin treated this way were found to be quite reproducible from one preparation to the next.

Acknowledgments

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References

Berkowitz, S. A., Katagiri, J., Binder, H. K., & Williams, R. C., Jr. (1977) *Biochemistry 16*, 5610–5617.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Bryan, J. (1976) *J. Cell Biol. 71*, 149–167.

Burton, P. R., & Himes, R. H. (1978) *J. Cell Biol. 77*, 120–133.

Cantor, K. P., & Hearst, J. E. (1970) *J. Mol. Biol. 49*, 213–229.

Cleveland, D. W., Hwo, S.-Y., & Kirschner, M. W. (1977) *J. Mol. Biol. 116*, 207–225.

Cooper, T. G. (1977) *The Tools of Biochemistry*, p 387, Wiley, New York.

David-Pfeuty, T., Erickson, H. P., & Pantaloni, D. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5372–5376.

David-Pfeuty, T., Laporte, J., & Pantaloni, D. (1978) *Nature (London) 272*, 282–284.

Detrich, H. W., III, & Williams, R. C., Jr. (1978) *Biochemistry 17*, 3900–3907.

Diehl, H., & Lindstrom, F. (1959) *Anal. Chem. 31*, 414–418.

Erickson, H. P., & Voter, W. A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2813–2817.

Fellous, A., Francon, J., Lennon, A.-M., & Nunez, J. (1977) *Eur. J. Biochem. 78*, 167–174.

Herzog, W., & Weber, K. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1860–1864.

Himes, R. H., Burton, P. R., Kersey, R. N., & Pierson, G. B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4397–4399.

Himes, R. H., Burton, P. R., & Gaito, J. M. (1977) *J. Biol. Chem. 252*, 6222–6228.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lee, J. C., & Timasheff, S. N. (1977) *Biochemistry 16*, 1754–1764.

Linke, W. F. (1965) *Solubilities*, 4th ed., p 520, American Chemical Society, Washington, D.C.

Lockwood, A. H. (1978) *Cell 13*, 613–627.

Murphy, D. B., & Borisy, G. G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2696–2700.

Murphy, D. B., Vallee, R. B., & Borisy, G. G. (1977) *Biochemistry 16*, 2598–2605.

Nagle, B. W., Doenges, K. H., & Bryan, J. (1977) *Cell 12*, 573–586.

Olmsted, J. B., & Borisy, G. G. (1975) *Biochemistry 14*, 2996–3005.

Peterson, E. A. (1970) in *Laboratory Techniques in Biochemistry and Molecular Biology* (Work, T. S., & Work, E., Eds.) Vol. 2, pp 289–290, North-Holland Publishing Co., New York.

Sandoval, I. V., Jameson, J. L., Niedel, J., MacDonald, E., & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3178–3182.

Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.

Sloboda, R. D., Dentler, W. L., & Rosenbaum, J. L. (1976) *Biochemistry 15*, 4497–4505.

Wehland, J., Herzog, W., & Weber, K. (1977) *J. Mol. Biol. 111*, 329–342.

Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y., & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1858–1862.

Weisenberg, R. C. (1972) *Science 177*, 1104–1105.

Witman, G. B., Cleveland, D. W., Weingarten, M. D., & Kirschner, M. W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4070–4074.