Polymorphism in the self-assembly of tubulin dimer and microtubule protein (tubulin plus the microtubule-associated proteins) has been investigated as a function of systematic variation of solution composition (i.e. buffer ion, [glycerol] and [Mg$^{2+}$]). The nature of the assembly product was examined using negative staining and thin sectioning electron microscopy.

The morphology of the product of assembly of tubulin dimer was found to be strongly influenced by the concentration of glycerol and Mg$^{2+}$ in Pipes and Mes buffers; the effects are less marked in phosphate buffer. Formation of bona fide microtubules in 0.1 M-Pipes occurs for a limited range of solution conditions (e.g. with [glycerol] < 2 M and [Mg$^{2+}$] < 1 mM). Conditions of elevated [glycerol] and [Mg$^{2+}$], which enhance the rate and extent of assembly, have the adverse effect of strongly promoting the formation of polymorphic forms in addition to, and in place of, the normal microtubule morphology.

In both Pipes and Mes buffers, increasing [glycerol] from 1 to 3 M favours the formation of extended multiply curved sheets, apparently made up from a basic structure with an S-like cross-section. By contrast, increasing [Mg$^{2+}$] promotes the formation of junctions between microtubule walls, giving products whose cross-section shows multiple hook-like appendages, attached to closed microtubules. The assembly of tubulin dimer in a typical ‘dimer assembly buffer’ (e.g. 0.05–0.1 M-Mes, with 1–3.4 M-glycerol and 2–7 mM-Mg$^{2+}$), invariably produces substantial proportions of non-microtubule structures such as open sheets, ribbons, and hooked structures.

We conclude that the self-assembly of tubulin dimer exclusively into bona fide microtubules occurs over a very restricted range of solution conditions in the normally used Pipes- and Mes-based buffers. Deviation from these conditions readily promotes the formation of mixtures of polymorphic forms. Many buffer systems used for the assembly and disassembly of microtubules composed of tubulin dimer appear likely to promote the formation of structures related to, but significantly different from, normal microtubules. This represents a cautionary factor in the interpretation of in vitro assembly and disassembly properties of microtubules.

Key words: tubulin, microtubules, assembly, morphology, negative staining, thin section, electron microscopy.

Introduction

Much interest attaches to the properties of microtubules assembled in vitro as models of the mammalian cytoplasmic and mitotic microtubule systems in vivo. For measurements on the model systems to be fully relevant, it is necessary to ensure that the product assembled in such systems reproduces the known morphology of genuine microtubules in order to relate to in vitro and in vivo results.

The assembly of tubulin exhibits a wide range of polymorphism. Although most microtubules observed in vitro appear to be composed of 13 protofilaments (Tilney et al. 1973; Pierson et al. 1978; Scheele et al. 1982), microtubules consisting of other than 13 protofilaments have also been observed in certain cells (Pierson et al. 1978; Scheele et al. 1982; Nagano and Suzaki, 1975; Burton et al. 1975). Structural variation of microtubules is more widespread amongst in vitro rather than in vivo assemblies (Burton, 1981; Dustin, 1984) and is, generally, more common in tubulin assemblies performed in the absence of MAPs (microtubule-associated proteins) (Pierson et al. 1978; Scheele et al. 1982; Bohm et al. 1984; Fakhari et al. 1984). In addition to variation in the number of protofilaments, polymorphism of tubulin assemblies can also involve the formation of various atypical structures such as open sheets, ribbons, incomplete microtubules and macrotubules (Erickson, 1974; Amos, 1979; Dustin, 1984). The formation of these polymorphic assemblies is strongly dependent on sol-
ution conditions (e.g. the presence of reagents like DEAE-dextran, polycations, Vinca alkaloids, glycerol, dimethyl sulfoxide, Zn\(^{2+}\), Ca\(^{2+}\)), as well as by alterations in the pH and temperature (Dustin, 1984).

The purpose of this work is therefore to investigate systematically the influence of solution composition on the morphology of the product obtained from the assembly of a consistent preparation of tubulin dimer, with parallel studies performed on MT-protein (microtubule protein comprising tubulin and microtubule-associated proteins) as control. In the commonly used systems based on Pipes and Mes zwitterionic buffers, both the rate and extent of tubulin assembly are increased by the inclusion of glycerol and Mg\(^{2+}\). The effects of increasing concentrations of these components have therefore been specifically studied. Specific and contrasting effects of both glycerol and magnesium are found, which contribute to the extensive polymorphism in the products of the in vitro assembly of tubulin dimer in commonly used buffer systems.

**Materials and methods**

**Materials**

Mes, Pipes, MgCl\(_2\) and GTP were obtained from Sigma. Reagents for electron microscopy were obtained from Agar Scientific Ltd, and those for electrophoresis were obtained from Sigma and BDH.

**Protein preparation**

MT-protein was prepared by two cycles of assembly and disassembly in the presence of glycerol, using a modification of the method of Shelanski et al. (1973). (See Clark et al. 1981.)

Pure tubulin dimer was prepared by cation exchange chromatography on phosphocellulose (Whatman II) presaturated with Mg\(^{2+}\) according to the method of Williams and Detrich (1979). The protein obtained by this procedure was examined by SDS-polyacrylamide gel electrophoresis followed by Coomassie Blue R staining of overloaded gels to ensure the absence of HMW-MAPs (high molecular weight microtubule-associated proteins).

**Negative staining**

Carbon-coated grids were successively floated on: (1) the protein solution for 10—15 s; (2) buffer solution for 5 s; (3) a 1% solution of uranyl acetate in assembly buffer for 10 s. The grids were blotted on filter paper after each step. The dried grids were stored for subsequent examination in a Philips 300 electron microscope.

**Thin sectioning of assembled microtubules**

Samples of assembled microtubules were fixed for 30 min at 37° C in a 0.25% glutaraldehyde solution in the appropriate assembly buffer. The fixed protein was pelleted by centrifugation in an MSE Microcentaur bench centrifuge and was fixed overnight at room temperature in a 1% glutaraldehyde solution in assembly buffer. The pellets were post-fixed in 1% osmium tetroxide solution in assembly buffer, stained 'en-bloc' with 1% aqueous uranyl acetate solution, dehydrated in a graded ethanol series and embedded in Araldite resin. Thin sections (60 nm) were typically prepared and collected on 200 mesh copper grid, and stained with uranyl acetate (70% in ethanol solution) followed by Reynold's lead citrate solution. Grids were extensively examined to ensure representative sampling, and predominantly longitudinal or cross-sectional orientations were chosen for photography.

**Assembly buffers for tubulin and MT-protein assembly**

Assembly of protein was performed in Pipes (P), Mes- (M) or phosphate-based buffers in the presence of 0—3.4 M-glycerol and 0.5—16 mM-Mg\(^{2+}\). The exact nature of the assembly buffer used in individual experiments is described in Results. All assemblies were performed in the presence of 0.5 mM-GTP at 37° C. Protein concentrations were chosen to be approximately 1 mg ml\(^{-1}\) over the critical concentration for a given buffer, and were in the range 1.5 to 4.5 mg ml\(^{-1}\), the lower range being used for buffers containing higher glycerol (G) and Mg\(^{2+}\) (Mg) concentrations.

**Results**

The assembly product of tubulin dimer in aqueous Pipes buffer

Aqueous Pipes buffer was used by Mitchison and Kirschner (1984) in studies involving nucleated tubulin assembly. Fig. 1A shows the negatively stained assembly product obtained from tubulin dimer in PEM100 buffer (100 mM-Pipes, 0.1 mM-EGTA, 0.5 mM-MgCl\(_2\), pH 6.5). The protofilament structure is characteristic of the morphology of normal microtubules. Thin sectioning of this
Table 1. Influence of solution conditions on the morphology of tubulin assemblies

<table>
<thead>
<tr>
<th>Protein preparation</th>
<th>Solution conditions</th>
<th>[G] (M)</th>
<th>[Mg$^{2+}$] (mM)</th>
<th>Normal MTs (O-shaped)</th>
<th>S-sheets</th>
<th>Complexes of S-sheets</th>
<th>6 and 8 shapes</th>
<th>Complexes of 6 and 8 shapes</th>
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<tr>
<td>MT-protein</td>
<td>MEM100</td>
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<td>0.5</td>
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<td></td>
<td>MEM100</td>
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<tr>
<td>MgPC-tubulin</td>
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<td></td>
<td>PEM100</td>
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<td></td>
<td>PEM100</td>
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<td>0.5</td>
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<tr>
<td></td>
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<td>MEM50</td>
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<td></td>
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<td>16.0</td>
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Crosses indicate the approximate proportions of different morphologies observed in transverse thin sections from tubulin dimer (1–2.5 mg ml$^{-1}$) assembled under varying solution conditions.

G, glycerol.

The assembly product of tubulin dimer in Pipes buffer in the presence of glycerol

Tubulin dimer assembled in PEM100 buffer in the presence of 1 M-glycerol (PEM100/G) showed a similar morphology to the material obtained from the assembly of tubulin dimer in PEM100 with a slightly increased proportion of assemblies with the profile of normal microtubules. Similar results were obtained in the presence of 2 M-glycerol (see Table 1). However, in 3 M-glycerol, there was a significant change in the morphology of the assembly product. Negatively stained samples (Fig. 2A) and longitudinal views of thin-sectioned material (Fig. 2B) indicate the presence of open curved sheets of varying width. Cross-sectional views (Fig. 2C) clearly show that the proportion of normal 'O'-shaped microtubules is very low and the majority of the structures have an elongated 'S'-shaped profile. This structure, which we term an S-sheet, comprises an extended curved sheet with a single point of inflection linking two surfaces of opposite curvature. The profile occurs so frequently as to suggest that this is in some way a basic structure that can be formed by a specific lateral association of groups of protofilaments. In some cases a number of these S-sheets associate laterally at a point to form a cluster of S-sheets, or to form a relatively regular single continuous surface of alternating curvature.

Fig. 2. Tubulin assemblies from MgPC-tubulin (2.2 mg ml$^{-1}$) in PEM100/3G buffer: negatively stained sample (A); longitudinal view of thin-sectioned sample (B); cross-sectional view of thin-sectioned sample (C). Bar, 135 nm.
The assembly product of tubulin dimer in Pipes buffer with increasing [Mg\textsuperscript{2+}]

Mg\textsuperscript{2+} is believed to be important for the in vitro assembly into microtubules (Olmsted and Borisy, 1975; Lee and Timasheff, 1977). Increasing [Mg\textsuperscript{2+}] up to 20 mM in the assembly buffers has been shown to stimulate the rate of tubulin assembly (Lee and Timasheff, 1977).

Tubulin dimer was assembled in 0.1M-Pipes in the presence of 5.0mM-Mg\textsuperscript{2+} (PEM100/5Mg; 5Mg denotes 5 mM) and sampled as before. Negatively stained samples (Fig. 3A) indicate the presence of normal microtubules although some lateral association is obvious. In longitudinal views of thin-sectioned material (Fig. 3B), structures significantly wider than normal microtubules are frequently seen. A clearer picture of the structures involved is obtained from the cross-sectional views (Fig. 3C). Evidently under these solution conditions, there is only a small proportion of closed O-shaped microtubules while the majority of the structures show 'hooks'. Some of these structures have a simple 6-shaped profile, whereas others have a more complex cross-section. The assembly products of tubulin dimer in 0.1M-Pipes in the presence of 1M-glycerol and 5.0mM-Mg\textsuperscript{2+} (PEM100/G/5Mg buffer) showed a diversity similar to that for assembly in PEM100/5Mg.

The assembly of tubulin dimer in Mes buffer in the presence of glycerol and Mg\textsuperscript{2+}

Assembly of tubulin dimer in aqueous Mes buffers does not normally occur at protein concentrations below 8mg ml\textsuperscript{-1} (Himes et al. 1977). However, assembly can be induced in Mes buffer at protein concentrations lower than 8mg ml\textsuperscript{-1} in the presence of glycerol and excess Mg\textsuperscript{2+} (Carlier and Pantaloni, 1978). The nature of the assembly product of tubulin dimer in 0.05M-Mes, 3.4M-glycerol, 0.1mM-EGTA, 2.0mM-Mg\textsuperscript{2+}, pH 6.5 (MEM50/3.4G/2Mg buffer; MEM is PEM with Mes substituted for Pipes) at a low (1.0mg ml\textsuperscript{-1}) and a high (4.5mg ml\textsuperscript{-1}) protein concentration are shown in Figs 4 and 5, respectively. The negatively stained samples in both cases (Figs 4A and 5A) clearly indicate that although some normal microtubules are present, a high proportion of the polymers are open sheets, ribbons, or even composite structures that are part normal and part open sheets. Longitudinal views show extended open structures (Figs 4B and 5B). The cross-sectional views (Figs 4C and 5C) show the presence of S-sheets as a major component of the population. At the higher protein concentration there appears to be a higher extent of clustering amongst the S-sheets.

The morphology of the assembly product of tubulin dimer in MEM50/3.4G was examined as a function of

Fig. 3. Tubulin assemblies from MgPC-tubulin (2.8 mg ml\textsuperscript{-1}) assembled in PEM100/5Mg buffer: negatively stained sample (A); longitudinal view of thin-sectioned sample (B); cross-sectional view of thin-sectioned sample (C). Bar, 105 nm.

Fig. 4. Tubulin assemblies from MgPC-tubulin (1.0 mg ml\textsuperscript{-1}) in MEM50/3.4G/2Mg; negatively stained sample (A); longitudinal view of thin-sectioned sample (B); cross-sectional view of thin-sectioned sample (C). Bar, 105 nm.
[Mg$^{2+}$]. Fig. 6 shows several examples of the product of assembly of tubulin dimer in MEM50/3.4G/6mM-Mg$^{2+}$. The aberrant nature of the structures is clearly indicated. The population consists of a variety of open sheets and ribbons, as well as composite structures that are part normal microtubules and part open sheets. In cross-sectional view (Fig. 6C) a small proportion of normal O-shaped or simple S-shaped profiles is evident, with the majority of the assemblies being complexes of hooked structures.

It was established that the minimum conditions for self-assembly of tubulin dimer at 2.3 mg ml$^{-1}$ in 100 mM-Mes are 1 M-glycerol and 3 mM-Mg$^{2+}$ (MEM100/1G/3Mg). Negative staining and longitudinal sections of the assembly product (Fig. 7A and B) indicate the presence of open sheets and of lateral association, while cross-sections (Fig. 7C) clearly show that the assembly product comprises only a small proportion of normal microtubules and a large proportion of hooked structures of various degrees of complexity.

The assembly product of MT-protein in Mes buffer: effects of increasing [Mg$^{2+}$]

Assembly of MT-protein is a simple control for comparison with assembly of tubulin dimer. Further, increasing [Mg$^{2+}$] in the assembly solutions of MT-protein has been reported to influence various aspects of the assembly/disassembly reactions (Huang et al. 1985; Martin et al. 1987; Barton et al. 1987; Bal et al. 1988). A solution of MT-protein was assembled to steady state in MEM100 buffer and samples for negative staining and thin sectioning were prepared as before. On negative staining (Fig. 8A) the material thus obtained has the appearance of normal microtubules, approximately 30 nm in diameter and showing clear protofilament structure. Longitudinal views of thin sections of these microtubules (Fig. 8B) show the absence of lateral association. Fine projections are evident on the surface of these microtubules, which are due to decoration by MAPs. In cross-section (Fig. 8C), these microtubules have the appearance of O-shaped structures consistent with the end-on view of a closed cylindrical structure, and fine projections can be detected (cf. Dentler et al. 1975; Murphy and Borisy, 1975; Amos, 1977; Zingsheim et al. 1979).

Thin sections of the product of assembly of MT-protein in MEM100 in the presence of 5.0 mM-Mg$^{2+}$ are shown in Fig. 9. There are few normal microtubules, and the predominant cross-section is of hooked structures (6- and 8-shaped profiles) and their complexes. Very few S-sheet profiles are seen. It therefore appears that the morphology of the assembly product of MT-protein, like that of tubulin dimer, can be significantly influenced by the concentration of Mg$^{2+}$ in the assembly buffer.
Fig. 7. Tubulin assemblies from MgPC-tubulin (2.3 mg ml\(^{-1}\)) in MEM100/G/3Mg: negatively stained sample (A); longitudinal view of thin-sectioned sample (B); cross-sectional view of thin-sectioned sample (C). Bar, 165 nm.

Fig. 8. Microtubules assembled from MT-protein (1.8 mg ml\(^{-1}\)) in MEM100 buffer: negatively stained sample (A); longitudinal view of thin-sectioned sample (B); cross-sectional view of thin-sectioned sample (C). Bar, 85 nm.

Fig. 9. Tubulin assemblies from MT-protein (1.8 mg ml\(^{-1}\)) in MEM100/5Mg buffer: longitudinal view of thin-sectioned sample (A); cross-sectional view of thin-sectioned sample (B). Bar, 170 nm.

Assembly of tubulin dimer in phosphate-glycerol buffer

Lee and Timasheff (1977) and Lee et al. (1978) reported assembly of tubulin dimer (prepared by an ammonium sulphate fractionation procedure) in PhMG buffer (10 mM-sodium phosphate, 3.4 M-glycerol, 16 mM-Mg\(^{2+}\), 1.0 mM-EGTA, pH 7.0). Normal microtubules were reported. Since we have found that elevated glycerol and Mg\(^{2+}\) concentrations in Pipes and Mes buffer cause the formation of aberrant structures, it is interesting to examine the product of assembly in this PhMG buffer with the standard MgPC-tubulin preparation (pure tubulin dimer prepared by cation exchange chromatography on phosphocellulose). The assembly product of tubulin dimer (2.4 mg ml\(^{-1}\)) in PhMG buffer comprised a somewhat heterogeneous population. However, the majority of the profiles were apparently normal microtubules, as found by Lee et al. (1978), whereas a small proportion were S-sheets and simple hooked structures (Fig. 10). Similar results were obtained in PhMG buffer at pH 6.5, suggesting that this is not a pH effect as reported by Burton and Hymes (1978), for tubulin assembled in the presence of dimethyl sulphoxide (DMSO) or glycerol at pH 6.5 to 6.9 with other buffer ions but at [Mg\(^{2+}\)]=0.5 mM. Therefore, the assembly product of tubulin dimer in PhMG buffer had a very similar morphology to the assembly product obtained in
**Polymorphism of tubulin assembly**

The stability of the various aberrant tubulin assemblies obtained under certain solution conditions was investigated to determine whether such assemblies can revert to normal microtubules or to other types of aberrant structures, as recently reported by Bohm et al. (1987). To this end, tubulin dimer was assembled in PEM100/3G (to obtain S-sheets) and in PEM100/G/5Mg (to obtain hooked structures). The solutions were then either subjected to agitation with a Pasteur pipette to disrupt any metastable structures or were left undisturbed for about 2 h to allow re-equilibration of the assembled structures.

With both S-sheets, and with the hooked structures, neither agitation nor time had any significant effect on the morphology of the assembly products nor on the relative proportions of the various structures. These polymorphic structures thus appear to be relatively stable end-products of assembly under these conditions.

**Discussion**

*Comments on the ultrastructural techniques used*

In this study electron-microscopic techniques were used for the direct investigation of the morphology of the assembly product of tubulin dimer or MT-protein under different solution conditions. The use of negative staining electron microscopy provided detailed longitudinal views of the assembled polymer as well as evidence for the presence of polymers other than normal microtubules. However, this method could not always distinguish between open sheets, laterally associated normal microtubules and hooked structures, since their appearance in negative staining was often similar. Longitudinal and especially transverse views of thin-sectioned material were extremely useful in providing clear distinction between the various types of structures involved. Thus, a combination of negative staining and thin-sectioning techniques was essential to obtain a fuller picture of the nature of the assembly product.

The limitation of the ultrastructural approach is that it is often difficult to analyse the data in a fully objective and quantitative manner. Thus in the assembly products of tubulin dimer comprising various types of structure (sheets, hooked structures, and normal microtubules), only their relative proportions could reasonably be estimated by visual inspection of representative fields; small changes induced by varying the solution conditions were therefore not readily resolvable.

*The polymorphism of tubulin assemblies is dependent on solution conditions*

The findings of the present study are summarised in Table 1, which illustrates the type of structures seen in cross-sections of the assembled material under various solution conditions, together with their approximate relative proportions. Table 1 shows that the assembly product of tubulin dimer is extremely prone to polymorphism and that the structures formed depend strongly on solution conditions. This is in agreement with similar conclusions reached by a number of workers (Matsumura and Hayashi, 1976; Amos, 1979; Fakhari et al. 1984).

The most striking finding is that it is extremely difficult to produce normal microtubules as the exclusive product of assembly of tubulin dimer. The conditions under which they are the predominant product is also surprisingly limited. This result has important implications when considering the wide range of solution conditions used in studies of the mechanism of tubulin dimer assembly and disassembly, reported in the literature. It is clear that inconsistencies must exist, due to the variety of morphologies of the assembly products formed under these different conditions.

Table 1 shows that normal microtubules, appearing as O-shaped structures in cross-sections, are the predominant assembly product of tubulin dimer in PEM100 at low glycerol concentration (0–2 M) and low [Mg$^{2+}$] (0.5 mM). However, a small proportion of aberrant structures (S-sheets and microtubules with hooks) are also present. At glycerol concentrations higher than 2 M, the formation of normal microtubules is suppressed, with
concomitant formation of S-sheets and complex lateral associations including extended sheets of alternating curvature. Increasing the Mg\(^{2+}\) concentration from 0.5 mM to 5.0 mM, causes a decrease in the proportion of normal microtubules and an increase in the proportion of 6- and 8-shaped structures and complex hooked structures, which, in longitudinal views, may resemble laterally associated normal microtubules.

The assembly of tubulin dimer in Mes buffer (0.05M or 0.1M) and in the presence of low (1.0M) or high (3.4M) glycerol and Mg\(^{2+}\) in the range 2.0–7.0 mM invariably promotes the formation of aberrant structures, such as S-sheets, ribbons, hooked structures, and more complex clusters. The concentration of Mg\(^{2+}\) influences the nature of the aberrant structures formed in the presence of elevated glycerol concentrations. At [Mg\(^{2+}\)] = 2.0 mM, the assembly product is predominantly S-sheets; as [Mg\(^{2+}\)] increases hooked structures of increasing complexity are seen. At [Mg\(^{2+}\)] > 5.0 mM, clusters of these 6- and 8-shaped structures predominate. Increased protein concentration also enhances the degree of clustering amongst the various assemblies.

The heterogeneous nature of the assembly product of tubulin in MEM50 with 3.4M-glycerol was previously observed by Carlier and Pantaloni (1978), who reported that increasing the [Mg\(^{2+}\)] to 10 mM gave a reduced proportion of sheets and an increased proportion of normal microtubules. At [Mg\(^{2+}\)] = 15 mM, amorphous aggregates predominated. They observed S-sheets, and doublet and triplet structures at [Mg\(^{2+}\)] = 10 mM. The buffer MEM50/3.4G/7Mg has subsequently been extensively used as ‘dimer assembly buffer’. However, the evidence presented in Figs 4, 5 and 6 shows that aberrant structures persist at all Mg\(^{2+}\) concentrations in this buffer system. As summarised in Table 1, the effect of increased [glycerol] in both Pipes and Mes buffers is an increase in S-sheets; increasing [Mg\(^{2+}\)] causes increased proportions of complex branched and hooked structures. Thus in our experience, normal microtubules do not appear as the predominant assembled structure in MEM50/3.4G/7Mg buffer. Our finding that the characteristics of the assembly products are a function of glycerol, Mg\(^{2+}\) and protein concentration indicates that it is most probable that assemblies based in this type of buffer system will contain a range of assembly morphologies.

This finding may help to resolve certain differences in interpretation of measurements of kinetics of assembly of tubulin dimer, microtubule disassembly induced by different solution conditions, and the relationship between microtubule assembly and GTP hydrolysis. Such data come from measurements in a range of buffer systems that support tubulin assembly. These differences may be due, at least in part, to the possibility that the variations in experimental conditions may have resulted in the formation of assembly products of significantly different morphologies, which could exhibit substantially different assembly properties.

*The influence of buffer ion*

The detailed ultrastructural investigation performed on the assembly product of tubulin dimer in PEM100 and PEM100/G has confirmed that under these conditions normal microtubules predominate amongst the assembled structures. Consequently, these buffers can be considered suitable for performing studies on the assembly of tubulin dimer.

The reason for the differences between Pipes- and Mes-based systems remains unresolved. Waxman *et al.* (1981) performed a comparative study on the morphology of the assembled product of tubulin dimer in Pipes and in Mes buffers. Their study was performed in the absence of glycerol and added Mg\(^{2+}\) and in the presence of high concentrations (0.8–1.7M) of Pipes and Mes. Under these conditions, Pipes buffer showed a greater ability than Mes in support assembly, but the products were extended sheet and ribbon structures. In our studies, at lower buffer ion concentrations and in the absence of glycerol and high Mg\(^{2+}\), Pipes appears considerably more effective than Mes in supporting assembly into normal microtubules at appropriate protein concentrations.

In view of the increased polymorphism observed when tubulin dimer is assembled in Mes or Pipes buffer in the presence of high glycerol/Mg\(^{2+}\), it was interesting to note that tubulin assembly in 10 mM-phosphate/3.4M glycerol/16 mM-Mg\(^{2+}\) (PhMG buffer) resulted in the formation of predominantly normal microtubules. The reason for the difference is not clear. It is possible that the presence of the phosphate ion somehow alleviates the otherwise aberrant effects of Mg\(^{2+}\)/glycerol on tubulin–tubulin interactions. Alternatively, the effects of glycerol and Mg\(^{2+}\) on the morphology of tubulin assemblies may be established only in the presence of certain ions (e.g. Pipes, Mes) but not others (e.g. phosphate). This would imply that the nature of the assembly product of tubulin is influenced not solely by the interactions between the protein and individual components of the assembly mixture but also by interactions occurring amongst the various solution components.

*The origins of polymorphic behaviour of tubulin dimer*

We now examine how the aberrant structures that appear in cross-sectional view as S-sheets, 6- and 8-shaped structures, as well as more complex forms, can be accommodated within the known features of microtubule polymorphism.

In a variety of reported polymorphic tubulin assemblies, such as sheets and hoops, it appears that the longitudinal interactions (i.e. the interactions occurring between adjacent tubulin subunits within a protofilament) are generally similar to those occurring in normal microtubules. Variation occurs in the relative orientation of adjacent protofilaments (parallel or anti-parallel). A slight variation in the lateral binding angle between adjacent protofilaments can result in variation in the number of protofilaments that can be accommodated in the completed microtubule structure (Amos, 1979; Burton, 1981). It thus appears that significant variation can be tolerated in the specification of lateral interactions between tubulin protofilaments. Furthermore, Mandelkow and Mandelkow (1979) distinguished two different
types of lateral binding that can occur in assembled microtubules: (1) lateral binding between adjacent protofilaments within a microtubule (intramicrotubular lateral binding); and (2) lateral binding between protofilaments belonging to different microtubular walls (intermicrotubular lateral binding). The latter give rise to junctions between walls of different microtubules.

The major polymorphic forms observed in this study appear to be derived from different spatial relationships of protofilaments similar to those observed in normal microtubules. Therefore, the observed polymorphism amongst these aberrant tubulin assemblies must be the result of altered lateral interactions between adjacent protofilaments. Elevated concentrations of either glycerol or Mg$^{2+}$ stimulate the formation of polymorphic tubulin assemblies. However, the nature of the assemblies promoted by these two reagents is apparently distinct. Increasing [glycerol] favours the formation of S-sheets and related complexes, whereas increasing [Mg$^{2+}$] promotes formation of products composed of various hooked 6- or 8-shaped structures, and clusters of these assemblies. The formation of hooked structures appears to involve a change in the balance between intermicrotubular and intramicrotubular lateral binding. We therefore conclude that increased Mg$^{2+}$ enhances the tendency to formation of junctions between microtubule walls.

It is interesting that, whereas elevated Mg$^{2+}$ leads to junction formation, elevated [Zn$^{2+}$] (with MT-protein) produces extended flat sheets of antiparallel protofilaments (Crepeau et al. 1977; Baker and Amos, 1978). Sheet structures are also formed from tubulin dimer with dimethyl sulphoxide (Himes et al. 1977), DEAE-dextran or poly(ethylene glycol) (Hertzog and Weber, 1978). These conditions appear related to the high-glycerol buffer systems in that they involve a significant reduction in the molar fraction of water in the solvent. However, the glycerol-induced S-sheets have the distinctive feature of a dual curvature in cross-section, which appears surprisingly uniform. This could be rationalised by a combination of 'intramicrotubular' lateral association of parallel protofilaments, producing normal curvature, together with a defined and periodic change in polarity, i.e. an antiparallel interaction, and further normal interaction, which would now give the opposite curvature in cross-section. Alternatively the change in curvature could reflect the presence of a junction interaction via an 'intramicrotubular' lateral association, which could then preserve the relative polarity of the two oppositely curved regions. Repetition of the interaction giving the point of inflection would produce extended structures, as the result of the further repetition of the basic S-sheet motif.

The presence of 1–2 M-glycerol and increasing [Mg$^{2+}$] in Pipes buffer stimulates the rate and extent of assembly of tubulin (Karecla and Bayley, unpublished observations). The presence of these reagents in the assembly mixture promotes the formation of polymorphic assemblies. Therefore, there appears to be a correlation between conditions that greatly enhance the assembly of tubulin dimer and the formation of polymorphic assemblies. This may suggest that under assembly-enhancing conditions the mechanisms that normally impose structural specificity in the assembly process are inadequate and, as a result, alternative interactions other than those leading to the formation of bona fide microtubules become effective and may predominate. One interesting exception appears to be the effect of phosphate ion, which appears to maintain the integrity of the normal microtubule structure, even in the presence of elevated [glycerol] and [Mg$^{2+}$]. Phosphate ion is, of course, a product of the GTP hydrolysis reaction; the possible link between this ion and control of microtubule morphology is the subject of continuing investigation.

References


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