POLYMERIZATION OF PURIFIED TUBULIN BY SYNTHETIC POLYCATIONS

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1. Introduction

Tubulin is the main component of microtubules. A preparation of brain tubulin capable of polymerization can be obtained by cyclic assembly–disassembly. Such a preparation contains, however, a number of minor components which can be resolved from tubulin by ion-exchange chromatography or gel filtration [1–3]. Chromatographically-purified tubulin fails to form microtubules in the standard conditions of polymerization unless minor proteins are returned to the preparation [4]. Tubulin polymerization can also be induced by polycations (e.g., histones, RNase, DEAE-dextran), although structures formed in this case usually differ from normal microtubules [5,6]. It was shown that some synthetic polycations induce formation of stable cooperative complexes of bovine serum albumin [7].

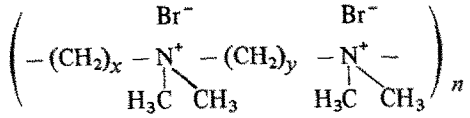
We have studied polymerization of chromatographically purified tubulin in the presence of different synthetic polymers of quaternary ammonium salts. It was found that these polycations were able to induce GTP-dependent and temperature-sensitive tubulin polymerization. The structure of the polymerization products was regular, although different from the normal microtubule structure, and was dependent on the polycation used.

2. Materials and methods

Tubulin was obtained from bovine brain by polymerization–depolymerization [8] as modified in [9] and further purified by column chromatography on phosphocellulose (Whatman P-11, batch 2111/582) according to [10]. A buffer solution containing 50 mM imidazole adjusted to pH 6.7 with HCl, 50 mM KCl, 0.5 mM $MgCl_2$ was used. During the last depolymerization and thereafter 2-mercaptoethanol and EDTA were added to the buffer to a final 1 mM and 0.1 mM, respectively.

Tubulin polymerization was induced by addition of GTP and EGTA up to 1 mM each and by raising to 37°C; it was monitored by turbidity at A_{330} [11]. After 15 min the samples were applied to grids coated with Formvar and carbon, and negatively stained with aqueous 2% uranyl acetate. The grids were examined in an Hitachi HU 11 electron microscope.

The synthetic polycations used to stimulate tubulin polymerization were regular polymers of quaternary ammonium salts [12] of the common formula:



These polycations are water soluble and their aqueous solutions have a neutral pH. The values of x and y and the mean molecular weights of the polycations are listed in table 1.

Table 1
Characteristics of the polycations

x	y	mol. wt	μg/ml used
3	3	5000	25
2	5	7000	62.5
5	6	25 000	25
6	10	20 000	25

3. Results

Solutions of the synthetic polycations and the phosphocellulose-purified tubulin were mixed at 0°C. High polycation concentrations caused strong precipitation of tubulin at this temperature. We used the maximal polycation concentrations that did not precipitate the protein at 0°C (table 1). These concentrations of polymers $x-3,y-3$ and $x-2,y-5$ did not induce tubulin precipitation at 37°C either. Polymers $x-5,y-6$ and $x-6,y-10$, however, caused a slight precipitation of tubulin when the solution was raised to 37°C.

In cold, all the 4 polycations induced formation of double rings both in the presence and absence of GTP (fig.1a). These rings had 430–500 Å o.d. and were morphologically identical with the rings observed in a depolymerized tubulin preparation before the ion-exchange chromatography [13]. The double rings were also found in the mixture of tubulin and the polycations after heating at 37°C in the absence of GTP. If, however, this mixture was heated at 37°C in the presence of GTP, tubulin formed regular structures described below.

The assembly of tubulin induced by polycation $x-3,y-3$ at 37°C was seen from the increase in the turbidity of the solution. Electron microscopy did not show any microtubules in the preparation. Instead of microtubules, tubulin formed strands of 130–150 Å diam. and of various length (fig.1b). The strands were composed of two tubulin protofilaments. Polycation $x-3,y-3$ did not stimulate tubulin polymerization in the absence of GTP. Incubation of the solution at 37°C without GTP did not increase its turbidity. The only regular structures found in this solution by electron microscopy were the double rings.

Similar results were obtained when polycation $x-2,y-5$ was used. This polycation also induced a GTP-dependent assembly of tubulin strands. The strands were formed by two parallel protofilaments and were much longer than those in the mixture of tubulin and polycation $x-3,y-3$. The strands formed

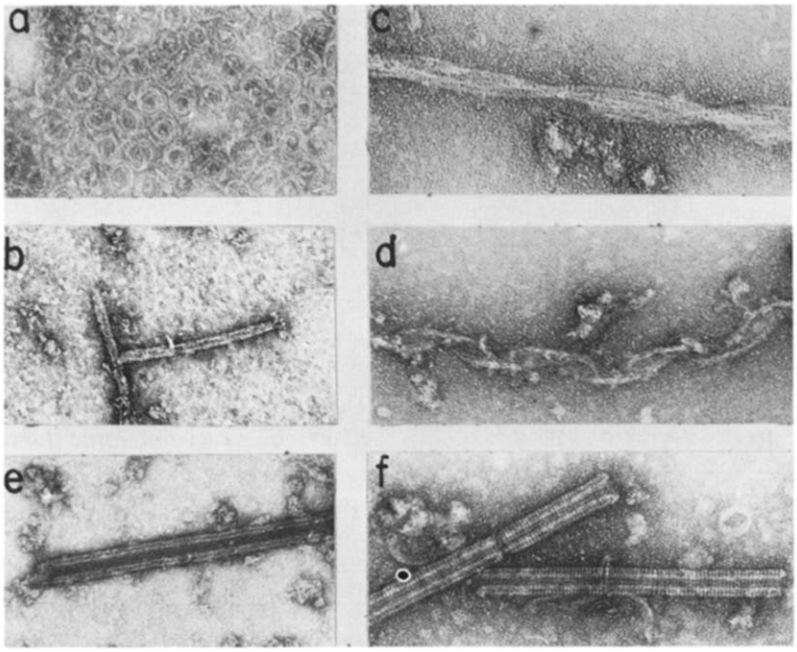


Fig.1. Structures formed by phosphocellulose-purified (a–e) and unchromatographed (f) tubulin in the presence of synthetic polycations. × 100 000. (a) Double rings formed in the presence of polycation $x-5,y-6$ at 0°C. (b) Strands formed in the presence of polycation $x-3,y-3$. (c) Strands and (d) twisted ribbons formed in the presence of polycation $x-2,y-5$. (e) Double-wall microtubules formed in the presence of polycation $x-5,y-6$. (f) Double-wall microtubules formed in the presence of polycation $x-2,y-5$.

in the presence of polycation $x-2,y-5$ twisted around one another (fig.1c). In addition to the strands which were the major component of the mixture, electron microscopy revealed, although not very frequently, twisted ribbons (fig.1d). Polycation $x-2,y-5$ did not stimulate tubulin assembly in the absence of GTP.

The turbidity of the mixture of tubulin and polycation $x-5,y-6$ increased during heating at 37°C both in the presence and in the absence of GTP. As shown by electron microscopy, in the presence of GTP, polycation $x-5,y-6$ induced assembly of a small number of regular structures, e.g., helices and double-wall microtubules (fig.1e). In the absence of GTP, only the rings were found in the preparation.

Polycation $x-6,y-10$ increased the optical density of the tubulin solution at 37°C both with and without GTP, but no regular polymers were formed in the mixture.

We also studied the effect of agents which depolymerize normal microtubules or prevent their assembly on the structures obtained in the presence of the synthetic polycations. Ca^{2+} at 2 mM (in the presence of 1 mM EGTA) did not depolymerize any of these structures. Cooling to 0°C resulted in depolymerization of the structures formed by tubulin in the presence of polycation $x-5,y-6$. Turbidity measurements showed that cooling reduced the optical density of the mixture of tubulin and polycation $x-2,y-5$ by 20–30% and did not depolymerize the structures formed in the presence of polycation $x-3,y-3$. Colchicine at 100 μM inhibited tubulin assembly in the presence of polycation $x-5,y-6$ almost completely. The assembly induced by polymers $x-2,y-5$ and $x-3,y-3$ was inhibited by 100 μM colchicine by 20–30%. Therefore the structures formed by tubulin in the presence of polycation $x-5,y-6$ were most sensitive to the inhibitors of tubulin assembly.

We also investigated the effects of the polycations on the assembly of tubulin purified solely by polymerization–depolymerization, without phosphocellulose chromatography. This tubulin formed both normal and double-wall (fig.1f) microtubules in the presence of each of the polycations.

4. Discussion

It is known that tubulin preparations purified from mammalian brain by polymerization–depolymerization contain tubulin oligomers (double rings) at 4°C and can form microtubules at 37°C in the presence of GTP. Phosphocellulose-purified tubulin cannot form either microtubules at 37°C or oligomers at 4°C, but addition of the components of the preparation adsorbed on phosphocellulose restored the polymerization ability [4]. We have shown that synthetic polycations, similarly to the proteins adsorbed on a phosphocellulose column, can induce the formation of double rings at 0°C and regular tubulin polymers at 37°C. The formation of regular structures in the presence of the polycations is GTP-dependent and partially temperature-sensitive. The structures obtained in the presence of the polycations are not, however, depolymerized by Ca^{2+}.

Our data partially confirm the hypothesis of Erickson [5,6] that native assembly factors act nonspecifically, due to their charge. Synthetic polycations do induce tubulin polymerization. Moreover, the formation of regular structures occurs only in the presence of GTP and at 37°C. However, the morphology of the structures depends on the polycation used. Polycations with different molecular weights and charge densities induce assembly of different structures: various strands, twisted ribbons and double-wall microtubules. This may indicate that the specificity of natural factor(s) is essential for the morphology of a resulting polymer rather than for the assembly per se.

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