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Purification of assembly-competent tubulin from *Saccharomyces cerevisiae*

Christine BELLOCQ[1], , Isabelle ANDREY-TORNARE[1], Anne-Marie PAUNIER DORET[1], Bernard MAEDER[1], Lawrence PATURLE[2], Didier JOB[2], Jacques HAIECH[3] and Stuart J. EDELSTEIN[1]

[1] Department of Biochemistry, University of Geneva, Switzerland
[2] INSERM Unité 244, Centre D'Etudes Nucléaires de Grenoble, France
[3] CNRS Chimie Bactérienne, Marseille, France

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We have developed a straightforward, two-step procedure to isolate highly purified yeast tubulin that reproducibly assembles into microtubules. The starting extracts are obtained from cells genetically engineered to overproduce both the α and β subunits of tubulin, under control of the galactose promoter, to approximately 10-times wild-type levels. The first step of purification is carried out with the high-speed supernatant of lysed cells loaded onto a DEAE-Sephadex column; after this step the tubulin preparation is approximately 30% pure. In the second step, the tubulin fractions are loaded onto an immunoaffinity column prepared by coupling the anti-(α-tubulin) monoclonal antibody YL 1/2 to Sepharose-4B. Following elution with 0.8 M KCl, the tubulin present in the peak is 90% pure. Upon addition of porcine brain microtubule-associated proteins or DEAE-dextran, this tubulin preparation is functionally active for assembly into microtubules, as visualized by electron microscopy on negatively stained samples. Virtually identical microtubule structures are produced in parallel experiments on the assembly of yeast or porcine brain tubulin, with differences observed only at acidic pH values. Overall, this relatively simple procedure provides a useful tool for the production of functional tubulin suitable both for structural studies and for investigations of the assembly process.

Microtubules are dynamic organelles of eucaryotic cells involved in mitosis and many other cytoskeletal functions (for reviews, see Dustin, 1984; Vallee and Sheptner, 1990). In nearly all species they occur as hollow cylinders of about 25 nm diameter composed of 13 parallel protofilaments, each formed by the alignment of alternating α and β subunits of tubulin. This apparent uniformity has obscured an enormous complexity of structure and function for microtubules in higher eucaryotes. Both α-tubulin and β-tubulin in vertebrates are encoded by families of up to six or seven functional genes (for reviews, see Cleveland, 1987; Sullivan, 1988). However, the number of isogenes is lower than the number of identified polypeptides (Field et al., 1984). The high degree of polypeptide diversity is in part explained by the characterization of post-translational events such as tyrosination–detyrosination, acetylation and glutamylation of α-tubulin (Raybin and Flavin, 1977; L'Hernault and Rosenbaum, 1985; Edde et al., 1990), as well as phosphorylation of β-tubulin (Gard and Kirschner, 1985). Very recently, a non-tyrosinatable variant of brain α-tubulin was characterized (Paturle-Lafanechère et al., 1991). A third level of complication arises from the different interactions of tubulin with microtubule-associated proteins (MAPs; Olmsted, 1986) and with other interacting proteins, such as the product of the *mipA* gene of *Aspergillus nidulans*, designated γ-tubulin (Oakley and Oakley, 1989).

Previous attempts to characterize microtubules more fully have focused on microtubule structure studied by electron microscopy or X-rays at relatively low resolution (Crepeau et al., 1978; McEwen and Edelstein, 1980; Amos, 1982; Amos et al., 1984; Mandelkow and Mandelkow, 1985; Rozycki et al., 1988), or on *in vitro* assembly in numerous biochemical studies (see, for example, Weisenberg, 1972; Shelanski et al., 1973; Timasheff, 1979; Mitchison and Kirschner, 1984; Serano et al., 1984; Evans et al., 1985; Karecla et al., 1989; Schilstra et al., 1991). For both approaches, large amounts of native purified tubulin are essential, and brain tubulin, in spite of its complexity, has most commonly been employed. However, yeast tubulin possesses two major advantages for such studies. First, β-tubulin is encoded by only one gene and α-tubulin by two genes, only one of which is essential (Neff et al., 1983; Schatz et al., 1986); second, yeasts are genetically very tractable. In addition, the intrinsically low abundance of tubulin has been partially overcome in strains of *Saccharomyces cerevisiae* that overproduce tubulin at about 10-times the basal level (Burke et al., 1989; Bollag et al., 1990; Katz et al., 1990; Weinstein and Solomon, 1990).

This paper reports a simple and rapid method of yeast tubulin purification by enrichment on DEAE-Sephadex chromatography (Weisenberg et al., 1968), followed by isolation using an immunoaffinity column (Paturle et al., 1989). We demonstrate that, beginning with strains possessing overexpression plasmids, it is possible to obtain significant

Correspondence to S. J. Edelstein, Dept. of Biochemistry, 30 Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland
Fax: +41 22 702 6476.

Abbreviations. MAPs, microtubule-associated proteins; PC-tubulin, phosphocellulose-purified tubulin.

quantities of highly purified, assembly-competent tubulin. This method, applicable for larger scale purification, is based on principles different from those used by Kilmartin (1981) or Barnes et al. (1992). The procedure is suitable for structural studies on active tubulin, as well as for routine functional assays that may be employed to identify yeast MAPs.

MATERIALS AND METHODS

Yeast strains

Saccharomyces cerevisiae strain F808, also called F0, (*MATa, Leu2-3, Leu2-112, His4-519, Ade1-100, ura3-52, GAL+*) which contains the PDB68 plasmid (Burke et al., 1989), is designated FLT12. The plasmid PDB68 contains α and β genes of yeast tubulin, under the control of the *Gal10* promoter. In the presence of galactose, the FLT12 strain overexpresses tubulin at approximately 10-times the concentration in F0 (Bollag et al., 1990).

Cell induction and lysis

Yeast cultures were induced with galactose in yeast extract/peptone/glucose medium; 30–40 g wet cells were lysed as previously described (Bollag et al., 1990) by vortexing with glass beads (425–600 μm, Sigma) in 1 vol buffer A (100 mM Pipes/KOH pH 6.9, 1 mM $MgCl_2$, 2 mM EGTA). In addition, the extraction buffer contained 0.2 M KCl. Following centrifugation, the pellet was immediately re-extracted with 1 vol buffer A. After high-speed centrifugation, crude extracts (about 100 ml) were immediately loaded onto chromatography columns for purification. Protein concentrations were determined after each step of purification by the Coomassie blue binding assay (Bradford, 1976) using bovine serum albumin for the standard curve.

Isolation of yeast tubulin

The high-speed supernatant from lysed yeast cells was loaded at 4 °C on a 7-ml DEAE-Sephadex A-50 (Sigma) column (Weisenberg et al., 1968) previously equilibrated in buffer A containing 0.2 M KCl. The column was washed with 10 bed volumes of buffer A containing 0.2 M KCl and tubulin fractions were eluted with buffer A containing 0.5 M KCl. The peak fractions (approximately 15 ml) were desalted immediately by the centrifugation/filtration method on Bio-Gel P-6DG from Bio-Rad laboratories (Penefsky, 1977) and loaded on a 3-ml immunoaffinity column. The immunoaffinity column, was prepared according to the procedure of Paturle et al. (1989) by coupling the rat monoclonal anti-(α-tubulin) antibody YL 1/2 (Wehland et al., 1983) to cyanogen-bromide-activated Sepharose 4B at an antibody concentration of 5 mg/ml activated gel. The column, previously equilibrated in buffer A, was loaded with tubulin-containing fractions from the preceding DEAE-Sephadex column, and washed with 30 bed volumes of buffer A containing 0.1 M KCl. Tubulin fractions (about 8 ml) were eluted by raising the concentration of KCl in buffer A to 0.8 M and then immediately desalted on Bio-Gel P-6DG. The resulting sample was concentrated to about 1.0 mg/ml with a Centricon 30 microconcentrator (Amicon). The concentrated tubulin was divided into aliquots, frozen in liquid nitrogen, and stored at −70 °C. There was no loss of competence in assembly for up to 2 months.

An alternative approach was to eliminate the first step on the DEAE-Sephadex column. In this case, the immunoaffinity column was loaded directly with 100 ml high-speed supernatant following lysis. The tubulin fractions were eluted and concentrated exactly as described in the preceding paragraph.

Isolation of porcine brain tubulin and MAPs

Porcine brains were obtained from freshly slaughtered animals. Microtubule proteins were isolated by two cycles of polymerization and depolymerization as described by Shelansky et al. (1973), with the addition of 1 mM phenylmethylsulfonyl fluoride to inhibit endogenous proteases. The microtubule pellets were rapidly frozen and stored at −70 °C. Immediately before use, a frozen sample was dissolved in buffer B (10 mM Mes/KOH pH 6.4, 1 mM $MgCl_2$, 2 mM EGTA) at 4 °C, centrifuged, and the supernatant used to separate tubulin from MAPs by chromatography on a phosphocellulose column (Whatman P11) equilibrated in buffer B (Sloboda and Rosenbaum, 1982). Purified tubulin (PC-tubulin) was eluted in the void volume, whereas MAPs were eluted in the buffer B containing 0.8 M KCl. The fractions containing MAPs were pooled, desalted, and concentrated to 3 mg/ml with a Centriprep 30 (Amicon). PC-tubulin and MAPs were frozen in liquid nitrogen and stored in aliquots at −70 °C. When the MAP fractions were examined for trace amounts of contaminating brain tubulin, small quantities were observed by Coomassie-blue-stained SDS/PAGE. However, these trace amounts were found to be present in a partially insoluble form and were eliminated by a centrifugation step at 165000 *g* for 10 min at room temperature. As a result, no tubulin bands were detectable by immunoblotting of SDS/PAGE, under overloading conditions, with the rat monoclonal anti-(α-tubulin) antibody YOL 1/34 (Kilmartin et al., 1982).

Assembly of yeast and porcine brain tubulin

Assembly experiments were performed in a 'standard' buffer: 50 mM buffer B pH 6.4, DNase (100 μg/ml), RNase (50 μg/ml), leupeptin (50 μg/ml), 10% glycerol, and 0.25 mg/ml of phosphocellulose-purified porcine brain MAPs. Immunoaffinity-purified yeast tubulin or brain PC-tubulin were added to a final concentration of 0.7 mg/ml or 0.5 mg/ml, respectively. In order to minimize the quantities used, assembly reactions were carried out in a final volume of 25 μl. Assembly was initiated by the addition of 1 mM GTP with incubation for 45 min at 30 °C for yeast tubulin or at 37 °C for brain tubulin. In certain experiments MAPs were replaced by DEAE-dextran from Pharmacia at a final concentration of one seventh the tubulin concentration.

Following assembly, 3 μl of each sample was placed on a copper grid previously coated with 1.5% collodion and carbon. Samples were stained with 0.5% uranyl acetate as described by McEwen and Edelstein (1980). The grids were examined on a Zeiss 109 electron microscope operating at 80 kV.

Immunoblotting

SDS/PAGE (8%) was performed using a Bio-Rad Mini-Protean II apparatus. After electrophoresis, the protein bands were stained with Coomassie brilliant blue R (Sigma) and scanned with a Beckman DU-65 spectrophotometer. Immunoblots were processed according to the method of Towbin et al. (1979) with the omission of methanol in the transfer buffer and with a second antibody linked to alkaline phospha-

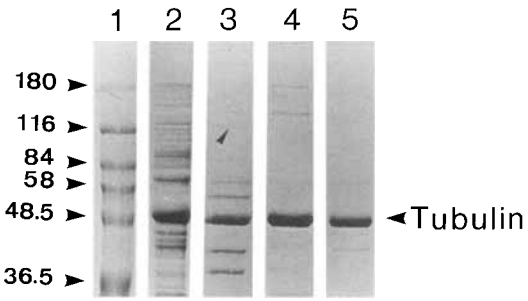

Fig. 1. Analysis of the purification of tubulin from the overproducing strain FLT12. For each sample, 5 µg was examined by Coomassie-blue-stained 8% SDS/PAGE. Lane 1, prestained molecular mass markers (Sigma; values in kDa on left); lane 2, tubulin peak fractions from DEAE-Sephadex chromatography; lane 3, tubulin peak fractions after purification by immunoaffinity chromatography alone; lane 4, samples prepared as in lane 3, but preceded by a DEAE-Sephadex chromatography; lane 5, porcine brain tubulin isolated by phosphocellulose chromatography. The arrow in lane 3 indicates the position of the 110-kDa band present in the peak eluted from the immunoaffinity chromatography alone.

tase. Rat monoclonal anti-(α-tubulin) antibodies YOL 1/34 and YL 1/2 (Kilmartin et al., 1982; Wehland et al., 1983) were purchased from Sera-Lab (Sussex, UK) and rabbit polyclonal antisera raised against the yeast γ-tubulin C-terminal peptide were generously provided by F. Solomon (MIT, Cambridge MA).

RESULTS

Purification of yeast tubulin

Yeast tubulin was isolated from the high-speed supernatant of the lysate of FLT12 cells grown under conditions for overexpression, followed by two chromatographic steps. The starting crude extract contained 8–10 mg/ml of soluble protein. After the first step, a DEAE-Sephadex column, tubulin-containing fractions examined on Coomassie-blue-stained SDS/PAGE were found to contain numerous bands other than tubulin (Fig. 1, lane 2). The results of this purification step were somewhat variable, but generally the tubulin band represents 30% of the total peak fraction, as judged by densitometry. The protein concentration in the pooled tubulin fraction was consistently in the range 0.8–1.0 mg/ml.

This partially purified tubulin was rapidly desalted and loaded for the second step onto an immunoaffinity column coupled to the monoclonal antibody YL 1/2, which recognizes the C-terminus of the α subunit of tubulin (Wehland et al., 1983). Tubulin-containing fractions were eluted by increasing the KCl concentration to 0.8 M. Tubulin was then identified both by its molecular mass on Coomassie-stained gels and by its recognition on Western blots with the monoclonal anti-(α-tubulin) antibodies (YOL 1/34 or YL 1/2) and rabbit anti-(β-tubulin) serum (data not shown).

Densitometry of SDS/PAGE revealed that the yeast tubulin was 90% pure (Fig. 1, lane 4). At the end of the purification, the eluted peak was desalted and concentrated to a volume of approximately 200 µl, in order to provide solutions at sufficiently high concentrations for assembly studies. Final concentrations of 1.0–1.5 mg/ml were routinely obtained. The total yield of the purified protein was at least 0.03% of the total protein present in the crude extract (see Table 1).

Table 1. Flow chart of yeast tubulin purification.

Step	Protein concentration	Purity
	mg/ml	%
Yeast cells (40 g)		
↓ vortex with glass beads, 100000 × *g* centrifugation		
High-speed supernatant (≈80 ml)	8	–[a]
↓ DEAE-Sephadex column, tubulin eluted with 0.5 M KCl		
Tubulin fractions (≈10 ml)	1	30
↓ immunoaffinity column, tubulin eluted with 0.8 M KCl, Centricon 30		
Concentrated tubulin (≈200 µl)	1	90

[a] Not determined.

Upon examination of overloaded gels, it was possible to detect seven minor bands present in the peak fractions of the immunoaffinity column (Fig. 1, lane 4), with apparent molecular masses of 38, 45, 65, 75, 140, 160 and 175 kDa. These bands could be tubulin-binding proteins (such as MAPs) or proteins sharing a common epitope with tubulin recognized by the YL 1/2 antibodies used for the immunoaffinity chromatography. However, only the 38-kDa and 45-kDa bands, which may be proteolysis products of tubulin, were specifically recognized by the YL 1/2 antibody on Western blots.

When the purification of tubulin was carried out with the crude extract loaded directly on the immunoaffinity column, without the prior DEAE-Sephadex step, a peak profile was obtained (Fig. 1, lane 3) that was similar to the highly purified tubulin (lane 4), but with a larger proportion of the contaminating proteins, as well as an additional 110-kDa band (indicated by the arrow), strongly recognized by YL 1/2 on Western blots. Since the purification was clearly superior when the DEAE-Sephadex step was included, we generally carried out assembly experiments with tubulin purified through the two columns.

Assembly of yeast tubulin into microtubules

In order to achieve reproducible and straightforward assembly assays, we established conditions for MAP-dependent formation of microtubules at moderate tubulin concentrations. These experiments were routinely carried out in the presence of porcine brain MAPs, purified by phosphocellulose chromatography, at a concentration of 0.25 mg/ml. Tubulin concentrations in the range of 0.025–2.0 mg/ml were employed, with 0.7 mg/ml generally used for studying the effects of various assembly conditions. The concentration of MAPs of 0.25 mg/ml was selected to be within the optimal range for yeast tubulin at 0.7 mg/ml, on the basis of studies of tubulin–MAP stoichiometry of Kim et al. (1979). Although brain tubulin assembly can occur in the absence of MAPs,

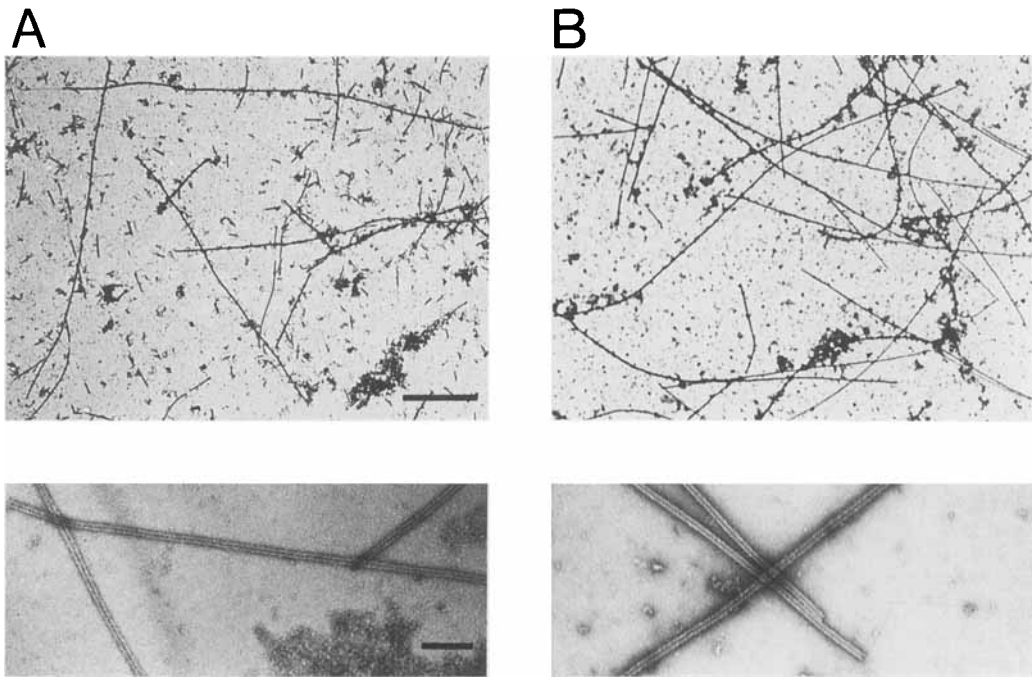

Fig. 2. Electron micrographs of negatively stained microtubules assembled *in vitro* with porcine brain MAPs. (A) Microtubules assembled 45 min at 30 °C with 0.7 mg/ml of yeast tubulin purified by DEAE-Sephadex and immunoaffinity chromatography, in the presence of 0.25 mg/ml of brain MAPs. (B) Microtubules assembled for 45 min at 37 °C with 0.5 mg/ml of porcine brain tubulin purified by phosphocellulose column chromatography, in the presence of 0.25 mg/ml of MAPs. For each experiment results are presented for micrographs recorded at magnifications of 4300 (top) and 32000 (bottom). The bars represent 2 μm (top) and 200 nm (bottom).

the critical concentration is lowered when MAPs are present (Timasheff, 1979). Under the conditions we investigated, MAPs were essential for the formation of microtubules at all of the tubulin concentrations examined (up to 2 mg/ml).

Each assembly experiment was performed in parallel with yeast tubulin, immuno-purified from galactose-induced FLT12 cells, and with porcine PC-tubulin. We observed, following centrifugation of the assembly mixture, that about 50% of the tubulin sediments as microtubules, when determined by measurements on the pellet and supernatant fractions using SDS/PAGE. Similar values have been reported by other investigators (Barnes et al., 1992). The products of the assembly reactions were examined by electron microscopy. Long, intact microtubules, either isolated or in bundles, with individual protofilaments readily apparent, were routinely observed by electron microscopy for both yeast and porcine brain tubulin, with identical diameters of 24 nm (Fig. 2). When decreasing concentrations of immunoaffinity-purified yeast tubulin or PC-tubulin were assayed for assembly in the standard buffer with a fixed concentration of MAPs (0.25 mg/ml), a reduction in the numbers of microtubules proportional to the dilution was observed, down to concentrations of 50 μg/ml with either yeast or brain tubulin. This result is in agreement with the critical concentration for assembly reported by Kim et al. (1979). Below this concentration, no microtubules were observed with tubulin from either source.

Having established the standard conditions of assembly, we proceeded to characterize the general features of yeast microtubule assembly compared to brain microtubules. Assembly experiments may also be successfully carried out with non-microtubule-associated proteins. Histones and RNases, both basic proteins, or polycationic macromolecules such as poly-(L-lysine) or DEAE-dextran, are known to replace MAPs by facilitating microtubule assembly (Erickson, 1975; Erickson and Voter, 1976). When we replaced MAPs by DEAE-dextran (at a concentration of 1/7th of the concentration of either immunoaffinity-purified yeast tubulin or PC-tubulin from brain), the assembly of microtubules was obtained. The microtubules consisted of a normal structure surrounded by a spiral wrapping made of sheets apparently also composed of protofilaments (Fig. 3) but with fewer outer spirals in the case of yeast tubulin. In spite of the special morphology induced by DEAE-dextran, other features of the assembly (number and length of microtubules) were relatively unchanged compared to assembly with MAPs. Overall, these structures are very similar to those observed previously with brain tubulin, designated 'double wall microtubules' by Erickson and Voter (1976).

We also investigated the effect of pH. Preliminary results at pH values above that of the standard assembly buffer (pH 6.4) indicated a loss of stability of the yeast tubulin. When the pH was lowered, bringing it closer to the theoretical isoelectric points of yeast α-tubulin (pI = 4.71) and β-tubulin (pI = 4.36), assembly was observed, but with alterations of the structures formed (Fig. 4). As the pH was made more acidic, the microtubules produced were significantly shorter, with the observation of closely spaced breaks in microtubules on electron microscope grids, indicative of relatively fragile structures. The effect, already visible at pH 5.9, was more pronounced at pH 5.2, with virtually all microtubules present as contiguous fragments (Fig. 4 A). Finally, only a small number of very short microtubule fragments were present at pH 4.5 (data not shown). Under similar conditions, the microtubules produced from porcine brain tubulin remained intact at pH 5.9, but were partially opened into sheets of protofilaments at pH 5.2 or pH 4.5 (Fig. 4 B).

The effect of temperature on the assembly of microtubules was also investigated. The standard brain tubulin purification

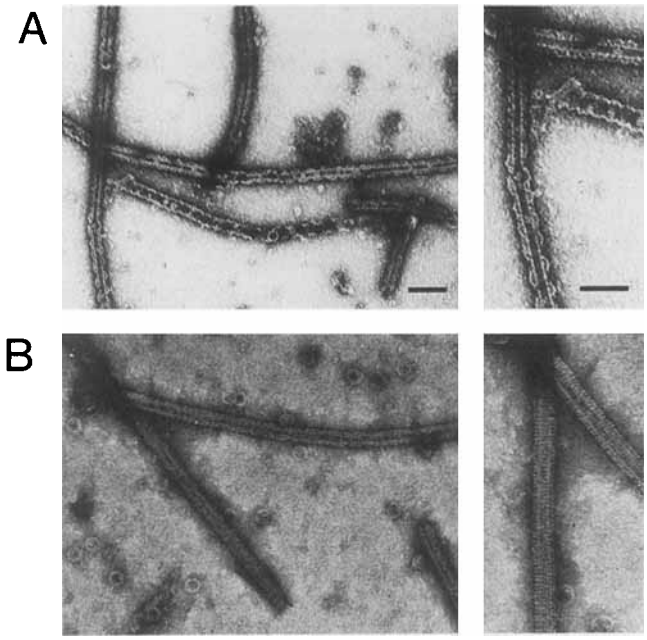

Fig. 3. Electron micrographs of negatively stained microtubules assembled *in vitro* with DEAE-dextran. (A) Microtubules assembled with 0.7 mg/ml of yeast tubulin as described in Fig. 2A, except that MAPs were replaced by 0.1 mg/ml of DEAE-dextran. (B) Microtubules assembled with 0.5 mg/ml of brain tubulin as described in Fig. 2B, except that MAPs were replaced by 0.07 mg/ml of DEAE-dextran. For each experiment results are presented for micrographs recorded at magnifications of 32000 (left) and 48000 (right). The bars represent 100 nm (left) and 84 nm (right).

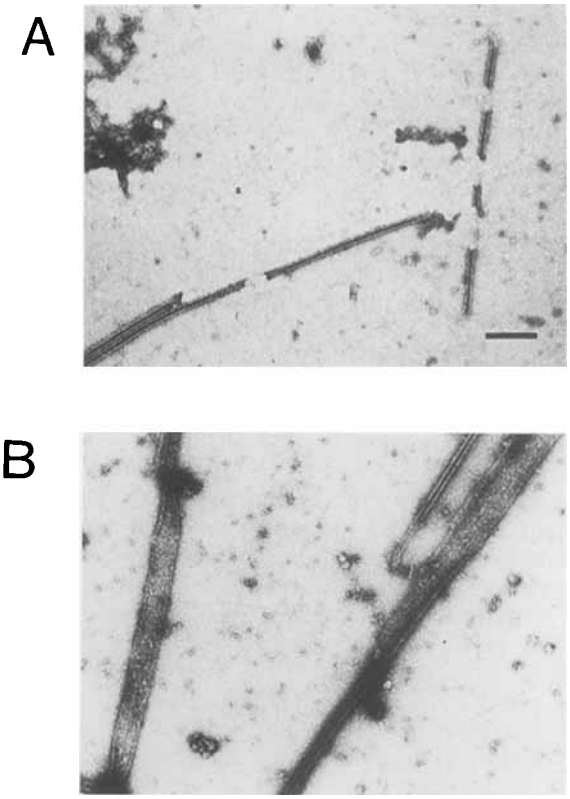

Fig. 4. The effect of pH on *in vitro* assembly of microtubules. Assembly was performed with yeast tubulin (A) or porcine brain tubulin (B) under the conditions described in Fig. 2, with the exception that the pH was lowered to pH 5.2. The bar represents 200 nm.

makes use of cycles of assembly at 37 °C and disassembly in the cold (Weisenberg, 1972; Shelansky et al., 1973), which were also used by Kilmartin (1981) for yeast tubulin from *Saccharomyces uvarum*. In order to compare the cold stability of microtubules produced from yeast tubulin purified by immunoaffinity chromatography with microtubules produced from brain PC-tubulin, samples were polymerized *in vitro* and then incubated for various periods of time on ice (0, 5, 15, 30, or 45 min). Observations by electron microscopy indicated that both types of microtubules were similar in their behavior in the cold. They became significantly shorter after 30 min of incubation in the cold and disappeared completely after 45 min (data not shown).

DISCUSSION

The construction of yeast strains overproducing tubulin under the control of an inducible promoter was an essential element in our studies of the purification and assembly of yeast tubulin. Indeed, the non-overproducing wild-type yeast strain FO provided a level of endogenous tubulin insufficient for routine purification and biochemical characterization. The purification based on an immunoaffinity column preceded by an initial DEAE-Sephadex chromatography yielded a tubulin preparation of high purity, particularly well suited for structural studies. For the immunoaffinity column, the antibody YL 1/2, which recognizes the C-terminal Tyr of the α subunit of brain tubulin, has a higher affinity when Phe is present at this position (Wehland et al., 1984), as is the case for *S. cerevisiae*. Furthermore, since detyrosination may not occur in *Schizosaccharomyces pombe* (Alfa and Hyams, 1991), it is plausible that for *S. cerevisiae* the C-terminal Phe is also maintained and consequently recognized by YL 1/2. In support of these conclusions, very little tubulin passed directly through the immunoaffinity column, as visualized on overloaded Western blots. Hence, the YL 1/2 antibody is suitable for purification of tubulin from *S. cerevisiae*. We noted that each immunoaffinity column may be used at 4 °C up to 20–30 times over a period of six months without loss of efficiency.

The routine purification of functional yeast tubulin has permitted investigations of various aspects of microtubule assembly. At the level of purity achieved, assembly was obtained in the presence of MAPs with relatively low tubulin concentrations (as low as 50 µg/ml), although concentrations in the range of 0.5–0.7 mg/ml are necessary to observe a field of microtubules sufficiently rich to be analyzed in detail by electron microscopy. As with brain tubulin, assembly of yeast tubulin is stimulated by polycations in the absence of MAPs (Erickson and Voter, 1976). Similar 'doubled-walled' microtubules are formed in both cases, although the outer wall with yeast tubulin appears to be less saturated with protofilaments. The effects of pH have been described previously for assembly of brain tubulin into microtubules (Burton and Himes, 1978), as well as for assembly into zinc-induced sheets (Rozycki et al., 1988). When we extended the pH range to 4.5, we observed open microtubule-based structures formed from brain tubulin. In contrast, yeast tubulin stability was altered more dramatically, as reflected by a significantly diminished number of microtubules. Moreover, the structures observed at low pH had a fragmented appearance, reflecting an unusual fragility. We also investigated the cold sensitivity of yeast tubulin, which was previously utilized for purification of tubulin from

S. uvarum by Kilmartin (1981). Our results indicated a rate of depolymerization in the cold for microtubules assembled with tubulin from *S. cerevisiae* comparable to the rate found with microtubules from *S. uvarum* or brain tubulin. In conclusion, the assembly studies with the new purification procedure demonstrate that yeast tubulin is similar to brain tubulin in most respects, although somewhat more sensitive to mildly acidic pH.

In certain cases, where the highest degree of purity is not essential, yeast tubulin can be obtained with a one-step preparation using only the immunoaffinity column. With this preparation microtubule assembly was readily achieved, but with a higher background on the electron microscope grids reflecting the poorer degree of purification. For all of the purification procedures, it was found to be essential to desalt the tubulin fractions rapidly in order to maintain assembly activity.

Overall, the work described in this report represents an important step in achieving purification of tubulin from *S. cerevisiae* in a functional form, while avoiding the conventional cycles of assembly – disassembly that in our hands lead to a significant loss of tubulin at each step. A reproducible and rapid purification of assembly-competent tubulin represents the first stage towards our goal of preparing yeast tubulin suitable for structural studies and we have initiated efforts to crystallize this tubulin. In addition, fractions of yeast proteins obtained during the purification procedure are currently being tested for 'MAP activity' with the routine assembly system that we have developed. Up to the present time no proteins that stimulate assembly related to the major classes of MAPs (or *tau*) have been isolated from yeast. However, several laboratories have identified proteins that interact with yeast microtubules, such as the kinesin-related protein (the product of the gene *KAR3*) in *S. cerevisiae* (Meluh and Rose, 1990), and a large number of proteins that bind to columns composed of microtubule matrices (Barnes et al., 1992). In addition to a general screening of yeast proteins for MAP activity, the seven proteins that copurify with tubulin in the peak fractions of the immunoaffinity column are also being examined to determine the extent to which they represent true MAP proteins.

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