IDENTIFICATION OF α AND β TUBULIN IN YEAST

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SUMMARY

The similarity of yeast microtubular protein to the tubulins purified from other eukaryotic organisms has been open to question. This work involves the identification of two yeast proteins that resemble α and β tubulin prepared from other eukaryots in their ability to copolymerize with purified rat brain tubulin and co-migrate with α and β brain tubulin on a sodium dodecyl sulfate polyacrylamide slab gel.

INTRODUCTION

Fission yeast is an interesting organism for studying cell division because it possesses a mitotic process similar to higher organisms and a capability for genetic analysis and mutant isolation similar to bacteria. Although the presence of a mitotic spindle in yeast has been observed electron microscopically, little biochemical information is available concerning the nature of its microtubular protein. Unlike most eukaryotic organisms, yeast cell division is not inhibited by the alkaloid colchicine, and is sensitive to the antimitotic drug colcemid (N-methyl N-deacetyl colchicine) only when exceedingly high concentrations are employed (1,2). Because binding of colchicine and colcemid is thought to be a defining biochemical property of the microtubular protein tubulin (3), there is some uncertainty as to the nature of the microtubular protein of yeast and its similarity to the tubulin purified from higher organisms.

Abbreviations: PMSF, phenylmethylsulfonyl floride; SDS, sodium dodecyl sulfate.

Several investigators have recently shown that mammalian brain tubulin can be polymerized into microtubules in vitro by raising the temperature to 37° C in the presence of GTP (4-6). The microtubules formed can be collected by centrifugation, and subsequently disassembled into tubulin by lowering the temperature. By alternating cycles of assembly and disassembly, a rapid purification of tubulin can be achieved. We reasoned that if yeast contain tubulin similar to that of higher eukaryotes, it might be identified by its ability to copolymerize with purified mammalian tubulin in an assembly-disassembly assay. The present paper shows that such an approach has led to the identification of α and β tubulin in yeast.

MATERIALS AND METHODS

Growth of Cells and Labeling of Proteins

Saccharomyces cerevisiae diploid strain SCD12-1 was obtained from J. Adams. This strain was derived from single zygotes isolated by micromanipulation after conjugation between XC500A and XC500B. The cells were grown in a defined liquid minimal media as described by Adams (7). Cultures were incubated on a reciprocal shaker at 30° C and grew with a generation time of 135 min.

To label proteins, 50 ml of cells in mid log phase were filtered, washed with low-sulfate minimal media (minimal media in which sulfate salts were replaced by chloride salts), and resuspended in low-sulfate minimal media to which was added 1 mCi of ^{35}S-labeled sodium sulfate (carrier free). The cells were collected by centrifugation after one generation of growth, and washed two times with water and once with tubulin reassembly buffer (0.1 M MES, 1 mM EGTA, 1 mM GTP, 0.5 mM $MgCl_2$, pH 6.4). The washed pellet was resuspended in 1 ml of cold tubulin reassembly buffer; glass beads were added and the cells broken by 10 min agitation with a vortex mixer. The extent of cell breakage, which was monitored by phase contrast microscopy, appeared to be in excess of 80%. The sample was pipeted away from the glass beads and centrifuged at 100,000 x g for one hour at 4° C.

In Vitro Copolymerization of Rat Brain Tubulin and Yeast Proteins

Rat brain tubulin was purified by two cycles of assembly and stored as described by Shelanski et al. (6). Aliquots (0.1 ml) of the yeast high-speed supernatant were mixed with equal volumes of brain tubulin (1.4 mg/ml) in reassembly buffer containing 8 M glycerol. The samples were incubated at 37° C for 20 min and then centrifuged at 100,000 x g for one hour at 30° C. The pellets were either saved for electrophoresis or put through a second cycle of disassembly-assembly. In the latter case the pellets were resuspended in 0.1 ml of reassembly buffer, kept at 0° for 30 min to disassemble the tubulin, and centrifuged at 100,000 x g for 60 min at 4° C. The supernatant from this centrifugation was then mixed with brain tubulin (0.8 mg/ml) in reassembly buffer -8 M glycerol. The samples were incubated for 20 min at 37° C and the polymerized material collected via centrifugation at 100,000 x g for one hour at 30° C.

SDS-Polyacrylamide Slab Gel Electrophoresis

Purified rat brain tubulin and the pellets obtained from one and two cycles of copolymerization of radioactive yeast proteins with brain tubulin were dissolved in 0.2 ml of electrophoresis sample buffer (0.05 M tris, pH 6.8, 10% glycerol, 1% 2-mercaptoethanol, 0.001% Bromophenol Blue). These tubulin samples were mixed in a ratio of 20:1 (v/v) with a 40 mM stock solution (in 95% ethanol) of the protease inhibitor phenylmethylsulfonyl floride (PMSF) and heated to 95° C for 5 min.

A 0.1 ml sample of the yeast high-speed supernatant was mixed with PMSF stock solution (20:1) and dialysed against electrophoresis sample buffer-PMSF stock solution (20:1). After dialysis, the yeast high-speed supernatant sample was heated to 90° C for 5 min.

The polyacrylamide slabs (1.5 mm x 17.5 cm x 32 cm) and electrophoresis running buffer were prepared according to Laemmli (8). The slabs contained a 5% to 20% polyacrylamide gradient. Aliquots (0.03-0.04 ml) of sample were subjected to electrophoresis at 40 V for 24 hours. Gels were stained with Coomassie Brilliant Blue, dried, and exposed to X-ray film (Kodak RP 54) for one to 48 hours.

RESULTS AND DISCUSSION

Brain tubulin will undergo *in vitro* assembly into microtubule-like structures. By utilizing successive cycles of assembly and disassembly, tubulin from a high-speed supernatant can be purified by 95% (6). We have subjected brain tubulin purified in this way to SDS-polyacrylamide gel electrophoresis through a long slab (1.5 mm x 17.5 cm x 32 cm) containing a 5% to 20% polyacrylamide gradient. It was found that when the marker dye is allowed to migrate to about 80% the length of the gel, the α and β subunits of tubulin can be separated from each other (fig. 1).

We have used the tubulin assembly procedure and the slab gel electrophoresis technique to determine whether yeast proteins exist that will copolymerize with brain tubulin. A high-speed yeast supernatant, containing ^{35}S-labeled soluble protein, was mixed with brain tubulin, taken through a cycle of tubulin assembly, and the centrifuged product subjected to electrophoresis and autoradiography. When the autoradiogram of this sample was compared to the stained electrophoretic pattern of brain tubulin and an autoradiogram of total yeast supernatant protein, it appeared that two yeast proteins with the same molecular weights as α and β brain tubulin were enriched in the copolymerized sample relative to their amounts in the original yeast high speed supernatant (fig. 1).

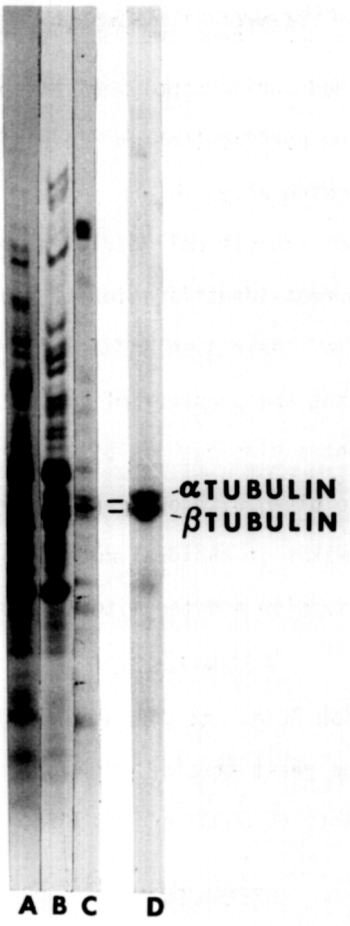

Fig. 1. Copolymerization of yeast tubulin with rat brain tubulin and separation of α and β tubulin subunits by SDS-polyacrylamide slab gel electrophoresis. (A) Autoradiogram of [^{35}S] labeled yeast proteins from a high speed supernatant. (B) Autoradiogram of [^{35}S] labeled yeast proteins taken through one cycle of copolymerization with rat brain tubulin. (C) Autoradiogram of [^{35}S] labeled yeast proteins taken through two cycles of copolymerization with rat brain tubulin. (D) α and β rat brain tubulin purified by the method of Shelanski et al. (6), stained with Coomassie Brilliant Blue.

When the pellet from one cycle of copolymerization was taken through a second cycle of disassembly and reassembly with the addition of fresh brain tubulin, the autoradiogram of the electrophoretic pattern indicated a further selection for these two yeast proteins, which now accounted for 30% of the radioactive yeast protein, compared to its original concentration of less than 1% in the original yeast supernatant. Although co-migration with authentic α and β tubulins

does not unequivocally prove that these two yeast proteins are tubulin, the fact that they become enriched during copolymerization with microtubules, a widely used procedure for the purification and identification of tubulin (6,9), strengthens the argument considerably.

Since colchicine does not inhibit cell division or cause microtubule breakdown in yeast (1,2), the current identification of yeast polypeptides similar to α and β tubulin in terms of their size and ability to polymerize into microtubules *in vitro* suggests that the property of colchicine sensitivity appeared later in evolution and is not a *sine qua non* of tubulin. The identification of α and β tubulin in yeast, combined with our relatively advanced genetic understanding of this organism, makes it an ideal model system for future studies on the regulation of cell division and the mitotic apparatus in eukaryotic cells.

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