TUBULIN-LIKE PROTEIN FROM ASPERGILLUS NIDULANS

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SUMMARY: [^{35}S] labeled extracts of the fungus *Aspergillus nidulans* were copolymerized with purified porcine brain tubulin. The [^{35}S] *A. nidulans* protein which copurified with porcine microtubules was found to be similar to [^{3}H] chick tubulin when the two were coelectrophoresed on several polyacrylamide gel electrophoresis systems. These results strongly suggest the presence in *A. nidulans* of a tubulin-like protein.

INTRODUCTION

Tubulin, the major component of microtubules, is a heterozygous dimeric protein whose subunits, tubulins 1 and 2, are acidic proteins with molecular weights of 55,000-60,000 (1). The ability of tubulin dimers, under appropriate conditions, to polymerize into microtubules *in vitro*, forms the basis of a purification method for this protein (1-3). Tubulin from higher organisms binds the drug colchicine; this feature has played an important part in the identification and characterization of tubulin (1).

Electron micrographs reveal the presence of microtubules in the spindle apparatus of fungi (4-7). However, the protein component of fungal microtubules has not yet been well characterized. Attempts to purify a colchicine-binding protein from fungi have been unsuccessful (8-10), but colcemid (a colchicine derivative) did bind weakly to a component in extracts of *Saccharomyces cerevisiae* (8). In this paper we describe some of the properties of a tubulin-like protein which was prepared from the fungus *Aspergillus nidulans* by copolymerization of radioactively labeled fungal extracts with purified porcine brain tubulin.

MATERIALS AND METHODS

Organism and Growth: The diploid strain of *A. nidulans* resulting from a mating of FGSC-154 and FGSC-99 was used for this study . This prototrophic strain was grown for 24 hr at 32° C in media containing (per 200 ml): 6 g glucose, 0.6 g $NaNO_3$, 0.2 g KH_2PO_4, 0.1 g $MgCl_2$, 0.1 g KCl, 0.002 g $FeSO_4$, 0.002 g $(NH_4)_2SO_4$, 0.004 g adenine, and 1 mCi $Na_2[^{35}S]O_4$ (825 mCi/mmol, New England Nuclear).

Preparation of Microtubular Protein: Fresh pig brains were homogenized in an equal volume of homogenization buffer consisting of 100 mM PIPES* (pH 6.95), 1 mM EGTA, 1 mM GTP, and 1 mM PMSF. Microtubular protein was purified according to the method of Dentler *et al.* (11). The microtubular pellet from the second cycle of polymerization was frozen in liquid nitrogen and stored at -80° C. Before use the pellet was resuspended in a small volume of homogenization buffer and centrifuged at 100,000 x g for 1 hr at 5° C. Labeled chick brain tubulin was prepared in the same manner from 1-2 day old chicks sacrificed 16 hr after an intracranial injection of 0.3 mCi per chick of [^{3}H]methionine (10.5 Ci/mmol, New England Nuclear).

[^{35}S] labeled *A. nidulans* (1 g wet weight) was suspended in 5 ml of homogenization buffer and disrupted using a Ten Broeck homogenizer. The homogenate was centrifuged at 100,000 x g at 5° C for 1 hr and the resulting supernatant was carefully decanted. To this crude extract was added porcine tubulin (20-25 mg) and an equal volume of polymerization buffer consisting of 100 mM PIPES (pH 6.95), 1 mM EGTA, 1 mM GTP, 1 mM $MgSO_4$, and 8 M glycerol. After incubation at 37° C for 20 min, the microtubules were collected by centrifugation at 100,000 x g for 1 hr at 30° C. The microtubules were depolymerized in homogenization buffer at 5° C and the polymerization cycle repeated to obtain a purified pellet of copolymerized porcine and [^{35}S] *Aspergillus* microtubular protein.

Polyacrylamide Gel Electrophoresis: SDS-urea polyacrylamide gels were prepared according to the method of Swank and Munkres (12) except that ethylenediacrylate was substituted for N,N methylenebisacrylamide. Discontinuous SDS polyacrylamide gels were prepared according to the method of Laemmli (13). Isoelectric focusing gels contained 6.25% acrylamide, 0.167% N,N methylenebis-acrylamide, 8 M urea, 2% Nonidet P-40, 1.8% ampholytes (pH 5-8, LKB), 0.2% ampholytes (pH 3-10, LKB), 0.0063% TEMED, and 0.02% ammonium persulfate. All gels contained 10% glycerol to facilitate handling during freezing. Frozen gels were sliced into 1 mm slices with a Mickle gel slicer (Brinkman Co.). Radioactivity was determined in a solution containing 4 g/l Omnifluor (New England Nuclear) in 1 part Triton X-100 and 2 parts toluene.

RESULTS

A crude [^{35}S] *Aspergillus* extract was copolymerized with purified porcine brain tubulin as described in Methods. After two polymerization cycles, the re-

*ABBREVIATIONS: PIPES, Piperazine-N,N' bis 2 ethane sulfonic acid; EGTA, ethylenebis oxyethylenenitrilo tetraacetic acid; GTP, guanosine 5' triphosphate; PMSF, phenylmethylsulfonyl fluoride; TEMED, N,N' tetramethylene diamine; SDS, sodium dodecyl sulfate.

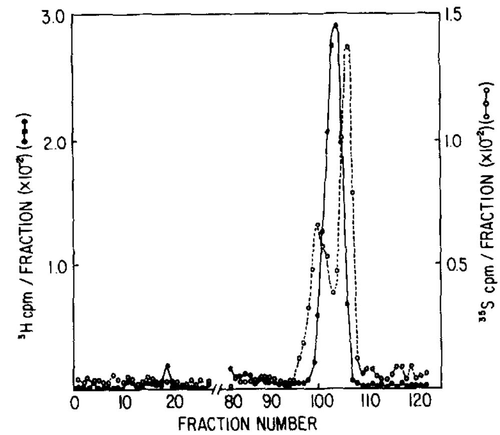

Figure 1. Coelectrophoresis of [^{3}H]chick tubulin and [^{35}S]*A. nidulans* copolymerized protein in an SDS-urea polyacrylamide gel. [^{3}H] chick tubulin (30 μg) and *A. nidulans* [^{35}S]copolymerized protein (10 μg) were coelectrophoresed for 16 hr through a 5% polyacrylamide gel. The gel was sliced into 1 mm sections, and the slices were counted in a liquid scintillation spectrometer to determine the presence of [^{3}H] (●) and [^{35}S] (o). The anode is on the right.

sulting pellet had a specific radioactivity of 140 cpm/μg protein. The total radioactivity in the microtubular pellet was approximately 1% of the total acid precipitable counts in the crude extract; the amount of radioactivity recovered represented 20-50 times more than expected solely by dilution. These microtubular pellets were coelectrophoresed with [^{3}H] chick tubulin on continuous SDS-urea polyacrylamide gels in order to determine the number of labeled components and their molecular weights. The [^{35}S] *A. nidulans* sample exhibited two electrophoretic components, one with slightly greater mobility, and one with slightly lower mobility, than [^{3}H] chick tubulin (Fig. 1). In the SDS-urea gel system, the apparent molecular weight of chick tubulin was 57,000 and the apparent molecular weights of the two *Aspergillus* proteins were 55,000 and 61,000. No other labeled components were found. Although chick tubulin exhibited only one electrophoretic component in the SDS-urea system, this protein can be resolved into its subunits by other gel systems (13).

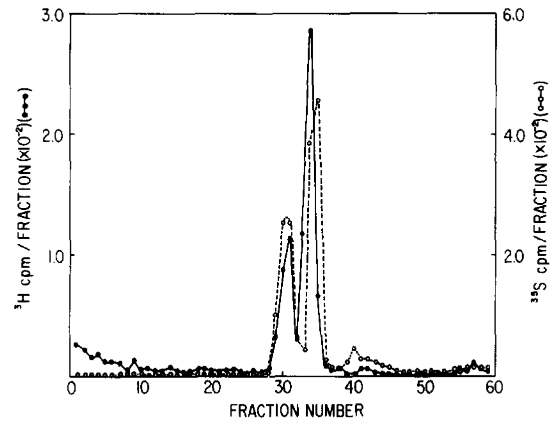

Figure 2. Coelectrophoresis of [^{3}H] chick tubulin and [^{35}S] A. nidulans copolymerized protein in a discontinuous polyacrylamide gel. [^{3}H] chick tubulin (20 μg) and [^{35}S] A. nidulans copolymerized protein (20 μg) were coelectrophoresed for 6 hr through a 10% polyacrylamide gel. The gel was sliced into 1 mm sections, and the slices were counted in a liquid scintillation spectrometer to determine the presence of [^{3}H] (•) and [^{35}S] (o). The anode is on the right.

To investigate whether the two Aspergillus components observed in the SDS-urea system were analogous to tubulins 1 and 2, we coelectrophoresed the [^{35}S] Aspergillus sample with [^{3}H] chick tubulin on discontinuous SDS gels. These gels are known to resolve tubulin into its two subunits (13). In this gel system, the [^{35}S] A. nidulans sample exhibited two components, one comigrating with chick tubulin 1, and the other migrating slightly faster than chick tubulin 2 (Fig. 2). The similar electrophoretic patterns of both the chick and the Aspergillus proteins suggest the tubulin-like nature of the A. nidulans protein.

To determine if the [^{35}S] Aspergillus protein and [^{3}H] chick tubulin were similar in charge, they were coelectrophoresed on isoelectric focusing gels. Both the Aspergillus and the chick proteins exhibited two main bands which focused in the region between pH 5 and 6 (Fig. 3). The similarity of the two profiles is apparent.

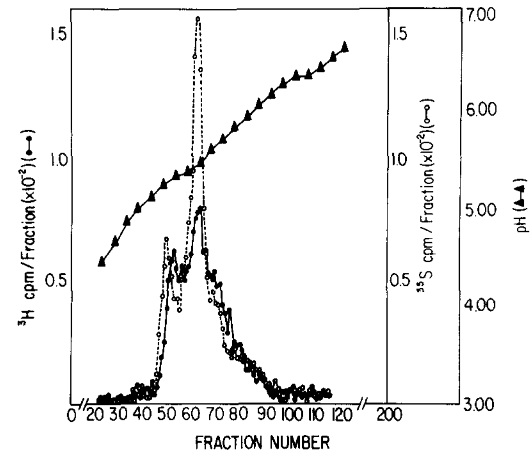

Figure 3. Coelectrophoresis of [^3H] chick tubulin and [^{35}S] A. nidulans copolymerized protein in an isoelectric focusing gel. [^3H] chick tubulin (40 μg) and [^{35}S] A. nidulans copolymerized protein (20 μg) were subjected to isoelectric focusing for 36 hrs. The gel was sliced into 1 mm sections, and the slices were counted in a liquid scintillation spectrometer to determine the presence of [^3H] (•) and [^{35}S] (o). The anode is on the right. A second gel, run without protein samples, was used to determine the pH gradient. This gel was sliced into 5 mm sections. Each section was soaked in 1 ml of distilled water and the pH (▲) of each section was determined.

DISCUSSION

We have developed a method for the identification of a tubulin-like protein from A. nidulans which depends upon copolymerization of radioactive fungal extracts with purified porcine brain tubulin. This method allows tubulin to be prepared from dilute solutions without the use of colchicine as a marker, and may be useful for identification of other tubulins which either are found in low cellular concentrations or have diminished ability to bind colchicine. The A. nidulans protein which was identified by this technique was similar to vertebrate tubulin in being composed of two acidic components with molecular weights of about 55,000-60,000. Preliminary experiments indicate that A. nidulans has at least one high molecular weight protein that can associate with microtubules (G. Sheir-Neiss, unpublished data). We are currently investigating tubulin-like proteins and microtubule-associated proteins in the bim mutants of A. nidulans which are blocked in mitosis at restrictive temperatures (14).

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