Partial Purification and Characterization of Microtubular Protein from *Trypanosoma brucei*

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The tubulin proteins of the parasitic hemoflagellate *Trypanosoma brucei brucei* were purified and characterized. Cytoskeletal microtubules of trypanosomes do not disrupt under conditions used to solubilize brain tubulins. Trypanosomal tubulins, solubilized by extensive sonication, were partially purified from the crude cell extract by taxol-mediated polymerization. Taxol-induced microtubules were identified by electron microscopy and analyzed biochemically. They consist predominantly of two proteins of about 52,000 and 56,000 Da. Their mobilities on sodium dodecyl sulfate gels differ slightly from those of bovine brain tubulins. Immunological cross-reactivity with antibodies raised against bovine brain tubulins confirmed the nature of the trypanosomal proteins. Peptide mapping of bovine and trypanosomal α- and β-tubulins was performed by enzymatic digestion with staphylococcal protease V8 and chemical cleavage with N-chlorosuccinimide. In both cases, the peptide patterns generated from the trypanosomal α- and β-tubulins were closely related to each other. This suggests that the trypanosomal α- and β-tubulins may have remained more conserved during evolution than the tubulins from higher eukaryotes.

The trypanosomal α-tubulin is post-translationally modified in *vivo* by the reversible addition of a tyrosine residue at its COOH terminus. As in higher eukaryotes, this reaction is completely specific for the α-polypeptide chain. Our observation represents the first documentation of the occurrence of COOH-terminal tyrosination of α-tubulin in an eukaryotic microorganism.

African trypanosomes are the causative agents of human sleeping sickness, as well as of various disastrous diseases of cattle (1, 2). Closely related trypanosomatid flagellates, *Trypanosoma cruzi*, and Leishmania species are equally important etiological agents of Chagas disease in South America and of various Leishmanioses in many parts of the world. Therapy and prevention of these diseases are still in an unsatisfactory state, and the development of successful trypanocidal drugs is severely hampered by the limited knowledge of the cellular biology of these organisms.

In this context, considerable interest has recently been focused on the cytoskeleton and particularly the microtubular structures of trypanosomes. The cell body of trypanosomes is tightly enveloped by a compact single layer of microtubules, which are situated immediately beneath the surface membrane (3). These pellicular microtubules provide a high degree of flexibility to the cells, and presumably they are involved in motility as well. Microtubules are also found in the single flagellum, where they form one of the two prominent structures of this organelle, the axoneme and the paraxial rod. The flagellar axoneme consists of microtubules in the canonical 9+2 pattern while the paraxial rod is essentially a network of actin fibers, which extends along the axoneme and stays in close contact with it (3). A third domain of microtubule function in trypanosomes is the formation of the spindle apparatus in dividing nuclei (4, 5).

Trypanosoma tubulins, solubilized by extraction with taxol, were partially purified from the crude cell extract of *Trypanosoma brucei* by taxol-mediated polymerization (45). The tubulin proteins of the parasitic hemoflagellate *Trypanosoma brucei brucei* were purified and characterized. Cytoskeletal microtubules of trypanosomes do not disrupt under conditions used to solubilize brain tubulins. Trypanosomal tubulins, solubilized by extensive sonication, were partially purified from the crude cell extract by taxol-mediated polymerization. Taxol-induced microtubules were identified by electron microscopy and analyzed biochemically. They consist predominantly of two proteins of about 52,000 and 56,000 Da. Their mobilities on sodium dodecyl sulfate gels differ slightly from those of bovine brain tubulins. Immunological cross-reactivity with antibodies raised against bovine brain tubulins confirmed the nature of the trypanosomal proteins. Peptide mapping of bovine and trypanosomal α- and β-tubulins was performed by enzymatic digestion with staphylococcal protease V8 and chemical cleavage with N-chlorosuccinimide. In both cases, the peptide patterns generated from the trypanosomal α- and β-tubulins were closely related to each other. This suggests that the trypanosomal α- and β-tubulins may have remained more conserved during evolution than the tubulins from higher eukaryotes.

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ize the pellicular microtubules and, furthermore, by the problems inherent in the polymerization of tubulin from solutions containing low concentrations of tubulin monomers (20, 21, 23). In the present study, we have partially purified the tubulin from procyclic Trypanosoma brucei by in vitro polymerization of the solubilized tubulin in the presence of the alkaloid taxol (24). The tubulin was characterized by immunoblotting, peptide mapping, and by electron microscopic analysis of the microtubules formed in vitro. Furthermore, we have established that post-translational tyrosination of the COOH terminus of α-tubulin occurs in these lower eukaryotes.

EXPERIMENTAL PROCEDURES

Cell Culture—Procyclic trypanosomes (Trypanosoma brucei, stock STIP 366) were grown at 26 °C in SDM-79 medium (25) containing 5% fetal calf serum. Small cultures were grown in 25-cm² and 75-cm² tissue culture flasks, while for larger preparations, 0.5-ml batches of culture were grown in Fernbach flasks, maintained at constant slight agitation in a culture room at 26 °C. Cells were harvested during exponential growth, i.e. at densities of about 5 x 10⁶ to 1 x 10⁷/ml.

After in vitro labeling experiments, a simplified maintenance medium (ME medium) was developed. ME medium is composed essentially as follows: 1 x Hank's balanced salts (Gibco); 10 mM HEPES, pH 7.3; 1 x nonessential amino acids (Gibco); 50 μg/ml of menadione; 5% fetal calf serum; 0.2% sodium acetate; 100 μg/ml of penicillin and streptomycin, and the following additional amino acids (mg/ml): leucine (0.128), cysteine (0.024), glutamine (0.292), histidine (0.044), isoleucine (0.096), leucine (0.096), lysine (0.072), methionine (0.016), phenylalanine (0.032), proline (1.0), threonine (0.048), tryptophan (0.012), valine (0.048), and tyrosine (0.036). For labeling experiments with [3H]tyrosine, the supernatant was stored at -20 °C until use. For V8 peptide mapping, the gel slices were crushed in a potter with an equal volume of electrophoresis sample buffer. After incubation at 37 °C overnight, acrylamide particles were removed by centrifugation, and the supernatant was stored at -20 °C until use.

Gels with radioactively labeled samples were first fixed and stained with Coomassie blue in 45% methanol-10% acetic acid and destained by diffusion in the same solution without dye.

Immunoblotting—Taxol-polymerized trypanosomal tubulin and proteolyzed bovine brain tubulin were electrophoresed on 10% acrylamide gels and analyzed in duplicate on a 10% SDS-polyacrylamide gel. The proteins were electrophoretically (60 V, 200 mA, 2 h) transferred to nitrocellulose paper according to Towbin et al. (31). One pair of strips was directly stained with Coomassie brilliant blue. The other pair of strips was incubated (overnight, 20 °C) with polyclonal anti-bovine brain tubulin antibodies in the presence of 3% bovine serum albumin. After washing, the blot was incubated (3 h, 20 °C) with peroxidase-conjugated protein A (from Dr. Bommeli, Bern; used in a 1:200 dilution). Peroxidase activity was visualized with a freshly prepared solution of 10 mm Tris-Cl, pH 6.8, 3.3 mm 4-chloro-1-naphthol, 4 mm H₂O₂. The reaction was terminated after 5 min by washing with water.

Peptide Mapping—Partial peptide mapping using N-chlorosuccinimide/urea was carried out according to Lischwe and Ochs (32). Gel slices containing α- and β-tubulin were equilibrated in a solution of urea/H₂O/CH₃COOH (1:1:1, w/v/v) followed by digestion with 0.015 M N-chlorosuccinimide in the same solution for 30 min at room temperature. After equilibration in electrophoresis sample buffer to remove the cleavage agent, the slices were loaded into the slots of a 15% polyacrylamide gel. Limit proteolytic digestions with S. aureus V8 protease and electrophoresis on a 15% SDS gel were performed using the method of Cleveland et al. (33). Approximately 5 μg of each gel slice was applied per lane. Digestion with V8 proteinase was performed at 37 °C in 50 mM Tris-Cl, pH 6.8, 0.1 M β-mercaptoethanol, 2% SDS, 10% glycerol was digested with four different protease concentrations (1, 5, 15, and 50 μg/ml) at 57 °C for 30 min. The reaction was terminated by boiling the samples for 5 min. The resulting peptides were analyzed on a 15% polyacrylamide gel. The peptide patterns from both digestions were visualized by the silver staining technique as described by Wray et al. (34).

Tyrosination/Detyrosination—Exponentially growing trypanosomes were collected, washed twice, and finally resuspended in ME medium. The cells were preincubated for 30 min in the presence of 50 μg/ml of cycloheximide, 25 μg/ml of each of puromycin and chloramphenicol to inhibit any de novo protein synthesis. As checked by light microscopy, neither cell shape nor motility of the cells was disturbed by this procedure over a period of up to 16 h. The cells were labeled with 25 μCi/ml of [3H]tyrosine for 2 h and then processed for tubulin purification.

Detyrosination was done by incubating an aliquot of the 100,000 X g supernatant from sonicated trypanosomes in PEM buffer containing 1 mm GTP, 4 mM MgSO₄, and 30 μg/ml of pancreatic carboxypeptidase A at room temperature. After 10 min an aliquot was removed and the protein precipitated by addition of cold 10% trichloroacetic acid. The samples were analyzed on a 7% polyacrylamide gel as described in the electrophoresis section.

Electron Microscopy—10 to 20 μl of sedimented microtubules were placed on carbon-coated glow-discharged grids and negatively stained with 2% uranyl acetate. The grids were viewed under a Philips EM 300 electron microscope at 60 kV.
RESULTS

Partial Purification of Tubulin—Initial attempts to purify tubulin from trypanosomes failed to disrupt the pellicular microtubules. These are completely resistant to conditions favoring the dissociation of microtubules in most other organisms. Disruption of microtubules with urea or guanidinium chloride resulted in the dissolution of these structures, but upon removal of the chaotropic agents, tubulin, as well as a host of other proteins, precipitated. Attempts to purify the dissolved tubulins by ion exchange chromatography on DEAE-cellulose in the presence of 6 M urea were equally unsuccessful. Finally, a procedure was adopted in which cells were supended in a polymerization buffer (see under “Experimental Procedures” for details) and then extensively sonicated. This treatment resulted in the solubilization of a considerable fraction of the tubulin. Tubulin is considered soluble when it stays in the supernatant after centrifuging at 100,000 × g for 1 h.

Solubilized tubulin could not be polymerized in vitro, since the concentration of tubulin in the final S100 extract is still comparatively low. Polymerization of tubulin from crude cell extracts of other lower eukaryotes has already earlier been shown to be problematic (20, 21, 23). These difficulties have been circumvented by polymerizing the tubulins in the presence of 2 μg/ml of taxol. This alkaldoid from Taxus brevifolia has been shown to reduce the critical concentration for tubulin polymerization over 100-fold (24), allowing the quantitative polymerization of tubulin even from solutions of very low initial concentration. However, the resulting taxol-induced microtubules cannot depolymerize anymore, and that precludes a further purification of the microtubular proteins by additional cycles of polymerization.

Fig. 1A depicts the course of the isolation of trypanosomal tubulin by taxol-induced polymerization. Lane 1 demonstrates total trypanosomal proteins, while lane 2 contains the proteins recovered in the S100 supernatant of sonicated cells. Lane 3 presents the proteins recovered in the high speed pellet after treatment of the S100 with taxol, GTP, and 30 °C. A comparison of lane 2 with lane 3 indicates the virtually complete recovery of tubulin in the high speed pellet. This conclusion is also supported by an inspection of lane 4, which contains the proteins remaining in the supernatant after taxol-induced polymerization of tubulin. The results presented in Fig. 1A demonstrate that tubulin can indeed be recovered with good yield from crude cell lysate via taxol-induced polymerization. At present it is not possible to decide if the additional proteins found in the microtubular pellet represent bona fide microtubule-associated proteins, or if they are unrelated contaminants.

SDS-gel electrophoresis of the microtubular pellet reveals a prominent triplet of bands with an apparent molecular weight of 48,000, 52,000, and 56,000, respectively (lane 3), none of which comigrates with bovine brain tubulin (lane 5). In contrast, after reduction and carboxymethylation, the tubulins of both species show very similar migration properties (Fig. 1B, lanes 1 and 2). Their apparent molecular weights are increased to 66,000 and 60,000, respectively, concertedly with respect to the molecular weight markers. α- and β-subunits are well resolved. The third band of the triplet observed in Fig. 1A, lanes 1–3, migrates at a much changed position after reduction and carboxymethylation and was assumed to be a protein not related to tubulins. This assumption is further substantiated by immunoblotting (see below).

Electron Microscopical Examination of Polymerized Microtubules—Electron microscopic analysis of taxol-polymerized microtubules revealed the presence of abundant smooth microtubules of variable length (Fig. 2A). Different stages of microtubule assembly can be observed, as exemplified by Fig. 2B. A bent sheet of protofilaments, considered to represent an early stage of microtubule formation (35) can be seen to evolve gradually into an open sheet of greater width, i.e., consisting of a larger number of protofilaments, which sub-

![Fig. 1. SDS-polyacrylamide gel electrophoresis of trypanosomal tubulin.](http://www.jbc.org/) A, the 100,000 × g supernatant of sonicated trypanosomes was incubated (45 min at 30 °C) in PEM buffer in the presence of 1 mM GTP and 2 μg/ml of taxol to induce polymerization of tubulin. Microtubules were pelleted by ultracentrifugation at 100,000 × g for 30 min at 25 °C. Samples were analyzed on a 10% SDS-polyacrylamide gel according to the method of Laemmli (28) with minor modifications (for details see under “Experimental Procedures”). Lane 1, crude lysate of trypanosomes (40 μg of protein); lane 2, 100,000 × g supernatant (40 μg of protein); lane 3, pelleted microtubular protein after taxol treatment (40 μg of protein); lane 4, supernatant of the sample of lane 3 (30 μg of protein); lane 5, bovine brain tubulin (10 μg of protein). B, pelleted microtubular protein and bovine brain tubulin were reduced and carboxymethylated. α- and β-subunits were separated on a preparative 10% SDS gel. The bands were cut out, equilibrated in electrophoresis sample buffer, and re-electrophoresed on a 10% SDS gel. Lane 1, pelleted microtubular protein after taxol treatment (40 μg of protein); lane 2, bovine brain tubulin (10 μg of protein); lane 3, α-tubulin from trypanosomes (5 μg of protein); lane 4, β-tubulin from trypanosomes (5 μg of protein); lane 5, α-tubulin from bovine brain (5 μg of protein); lane 6, β-tubulin from bovine brain (5 μg of protein).
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FIG. 2. Electron micrographs of in vitro polymerized microtubules. The 100,000 × g supernatant of sonicated cells were incubated (45 min, 30 °C) in PEM buffer in the presence of 2 µg/ml of taxol and 1 mM GTP. Taxol-polymerized microtubules were sedimented by a second centrifugation and processed for electron microscopy. Bar represents 100 nm; magnification, × 105,000.

Fig. 3. Immunological cross-reactivity. In vitro polymerized microtubules of trypanosomes and tubulin from bovine brain were carboxymethylated. They were electrophoresed in duplicate on a 10% SDS-polyacrylamide gel. After transferring the protein electrophoretically onto nitrocellulose, one pair of strips was stained with Coomassie brilliant blue (lanes 1 and 2). The other pair was incubated with anti-bovine brain tubulin antibodies. Bound antibodies were visualized by activity staining with peroxidase-conjugated protein A (lanes 3 and 4). Lanes 1 and 3, bovine brain tubulin (10 µg of protein); lanes 2 and 4, taxol-polymerized microtubules from trypanosomes (40 µg of protein).

sequently rounds up into a complete microtubule. Taxol-induced microtubules are all smooth structures and were never found decorated with lateral projections, which are seen very prominently on the pellicular microtubules in situ.6

A background of spherical protein complexes is consistently observed in these preparations, as well as in control preparations without the addition of taxol. Hence, this material is considered to be unrelated to tubulin. The size of the complexes (14-nm diameter) and their similarity to controls of purified ferritin further suggested that they might represent trypanosomal ferritin particles.

Electron microscopic analysis of the structures induced by taxol clearly demonstrated the presence of microtubules. This observation further supports the identity of the trypanosomal tubulins.

Identification of Trypanosomal Tubulin by Immunoblotting—Observations from many laboratories have well established that tubulins from different organisms show extensive immunological cross-reactivity. For the purpose of this study, antibodies raised against DEAE-cellulose-purified bovine brain tubulins were used to identify the trypanosomal tubulins by immunoblotting.

Taxol-polymerized trypanosomal tubulins and purified bovine brain tubulins were carboxymethylated and further analyzed by immunoblotting. Fig. 3 shows a comparison between the protein pattern revealed by direct staining with Coomassie blue and by immunostaining. The anti-bovine antibodies readily detect the trypanosomal tubulins, both α- and β-chains. The reaction of the trypanosomal β-chain is reproducibly somewhat weaker than that of the α-chain. This slight difference may be due to greater intrinsic heterogeneity between bovine and trypanosomal β-chains. The results of the immunostaining experiments strongly support the identifi-

6 P. Gehr and T. Seebeck, unpublished observations.
cification of the trypanosomal tubulins. Furthermore, they indicate that the third strong protein band detected in taxol-induced microtubules (see Fig. 1B, lane 1) is a protein unrelated to tubulin.

Peptide Mapping—Taxol-polymerized trypanosomal tubulins and DEAE-cellulose-purified bovine brain tubulin were reduced, carboxymethylated, and fractionated into their subunits on preparative gels. The α- and β-bands (Fig. 1B, lanes 3-6) were excised and processed for peptide mapping as detailed under “Experimental Procedures.”

Fig. 4A presents the peptide pattern generated by N-chlorosuccinimide cleavage. α- and β-tubulins from bovine brain (lanes 1 and 3) yield a clearly distinct pattern with this cleavage procedure. The trypanosomal α-tubulin resolves into a peptide pattern which appears rather similar to the corresponding bovine pattern. In contrast, the trypanosomal β-tubulin is cleaved into a pattern which is strikingly different from its bovine counterpart. It much more closely resembles the trypanosomal α- or, for that matter, the bovine α-tubulin.

A gel slices containing isolated α- and β-tubulin from trypanosomes or from bovine brain were prepared as described in Fig. 1B. Chemical cleavage was accomplished by incubating (30 min at 20 °C) the gel slices in a solution containing urea/CH₃COOH/H₂O (1:1:1, v/v/v) with 0.015 M N-chlorosuccinimide added (32). The resulting peptide pattern was analyzed on a 15% SDS gel. Lane 1, α-tubulin from bovine brain; lane 2, α-tubulin from trypanosomes; lane 3, β-tubulin from bovine brain; lane 4, β-tubulin from trypanosomes. B, limited proteolytic digestion with S. aureus V8 protease was done according to Cleveland et al. (33). Isolated tubulin subunits in electrophoresis sample buffer were incubated at 4 different concentrations of V8 protease (1.5, 15, and 50 μg/ml) for 30 min at 37 °C. The resulting peptides were separated on a 15% SDS gel. 1, α-tubulin from bovine brain; 2, β-tubulin from bovine brain; 3, α-tubulin from trypanosomes; 4, β-tubulin from trypanosomes. The peptide pattern of both digestion procedures was visualized by silver staining.

Protease concentration (μg/ml)

Exponentially growing trypanosomes were transferred into ME medium lacking tyrosine. Protein synthesis inhibitors by two different procedures are in good mutual agreement, and they strongly point out a striking similarity between the trypanosomal α- and β-tubulins. The bovine α- and β-tubulins are much less similar to each other, though amino acid sequencing of these latter two proteins has clearly established a considerable degree of homology between them (11).

In Vivo Tyrosinolation of α-Tubulin—The amino acid sequence of the α-tubulins of all organisms analyzed to date contain a tyrosine residue at their COOH terminus. In vivo, this residue can be exchanged post-translationally by the joint action of a microtubule-associated tubulinyl tyrosine carboxypeptidase (36, 37) and an ATP-dependent tyrosine ligase (38). The biological significance of this exchange reaction is still unclear.

In order to investigate tubulin tyrosinolation in trypanosomes, the complex culture medium, which contains all amino acids in high concentrations, was replaced by a simplified maintenance medium (ME medium; see under “Experimental Procedures” for details). This medium supports normal cell growth for at least 36 h and allows the substitution of individual amino acids by their radioactive analogues. Protein synthesis can be blocked by more than 98% by a combination of 50 μg/ml of cycloheximide, 25 μg/ml of chloramphenicol, and 25 μg/ml of puromycin (39). Cycloheximide alone is not sufficient for complete suppression of protein synthesis. The arrest of protein synthesis is well tolerated by the trypanosomes in that they maintain cell shape and motility unchanged over a period of at least 16 h.

Exponentially growing trypanosomes were transferred into ME medium lacking tyrosine. Protein synthesis inhibitors

- J. Stieger, T. Wyler, and T. Seebeck, unpublished observations.
were added, and after 30 min, 25 μCi/ml [3H]tyrosine were added. After 2 h of incubation, cells were collected and processed for microtubule solubilization. The resulting S100 extract was then analyzed by gel electrophoresis and autoradiography. Lanes 1 of Fig. 5A illustrates the protein pattern of the S100 as visualized by staining with Coomassie blue. From an aliquot of this S100, tubulin was polymerized by the addition of taxol and GTP, and the resulting microtubules were pelleted. Lanes 2 and 3 represent the proteins recovered from the taxol-induced microtubular fraction and those remaining in the S100 after the removal of the microtubules, respectively. Upon fluorography of this gel, a single band of radioactivity was detected in both, the unfraccionated S100 (lane 1 of Fig. 5B) and the microtubular pellet (lane 2 of Fig. 5B). In contrast no radioactivity was detectable in the microtubule-depleted supernatant (lane 3). The radioactive bands in lanes 1 and 2 co-migrate exactly with the α-tubulin. No radioactivity could be detected in the region of β-tubulin, even after overexposure of the autoradiographs. Pulse-chase experiments have revealed a very slow turnover of the label incorporated into α-tubulin. In addition to the labeled α-tubulin, a small number of weakly radioactive bands are seen in the 10,000- to 20,000-Da size range. Their identity is unknown.

If the label incorporated into α-tubulin under our labeling protocol is indeed situated exclusively at the COOH terminus of the peptide chain, it should be very sensitive to the action of a carboxypeptidase. Aliquots of the S100 were digested to different extents with carboxypeptidase A, and the digestion products were fractionated on SDS gels which were subsequently fluorographed. Lanes 4 and 5 of Fig. 5A and B, illustrate that the radioactive label is indeed very rapidly removed from the α-tubulin. This observation strongly indicates that the label associated with α-tubulin is located exclusively at its COOH terminus. These observations support the conclusion that the post-translational tyrosinolation of α-tubulin does occur in trypanosomes, i.e. a lower eukaryote. In addition, the tyrosinolation reaction further confirms the identity of the trypanosomal α-tubulin.

**DISCUSSION**

The tubulins from the parasitic hemoflagellate *Trypanosoma brucei brucei* have been partially purified and identified. Standard procedures for the solubilization of tubulins have proved inefficient in disrupting the microtubular cytoskeleton. The abundant pellicular microtubules could only be disrupted by extensive sonication. The basis for this unusual stability of trypanosomal microtubules is not clear. It might be related to their predominantly structural function, which implies that they must be rigidly protected from depolymerization, lest the entire cellular architecture would collapse.

Tubulin solubilized by sonication can be repolymerized into microtubules in vitro in the presence of taxol and GTP. Preliminary observations suggested that in the taxol mediated polymerization also proceeds in the absence of added GTP, in close analogy to the observations reported for bovine brain tubulin (42). Electron microscopic analysis of the microtubules formed in vitro revealed microtubules in various stages of assembly (35), i.e. bent sheets of protofilaments, flattened sheets, sheets rounding up into microtubules, and completed microtubules.

Electrophoretic analysis of the proteins present in the microtubular pellet revealed three major protein bands with apparent M, in the range of 48,000-56,000. Two of these bands have subsequently been identified as tubulins by immunoblotting, while the identity of the third band and its relationship to microtubules in general are not established. Isoelectric focusing of the tubulins revealed the existence of several subclasses of both subunits.

Tubulins purified through preparative gel electrophoresis have been compared among themselves, as well as with bovine brain tubulin by two different methods of peptide mapping. Despite the different cleavage specificities of the two procedures, both approaches have lead to quite similar conclusions. The trypanosomal α- and β-tubulins yield very similar peptide patterns. With NCS cleavage, this pattern is in turn similar to the one generated from bovine α-tubulin, but entirely unrelated to the peptides derived from bovine β-tubulins. Using protease V8 for digestion, no obvious relationship can be observed between trypanosomal and bovine tubulins. However, the trypanosomal α- and β-tubulins are again very similar to each other. The results of ongoing DNA sequence studies of the tubulin genes will soon allow analysis of this apparent similarity between trypanosomal α- and β-tubulins with the bovine α-tubulin with much greater stringency. Taking the observed similarity of the trypanosomal α- and β-tubulins at face value and considering that both α- and β-tubulin genes are tightly clustered in the genome of trypanosomes (15, 16), but not in most other organisms analyzed to date, the trypanosomal tubulins might be hypothesized to represent an evolutionarily archaic form of tubulin, where neither the primary sequences of the α- and β-protein nor the arrangement of their genes have strongly diverged since the original duplication of the tubulin β gene.

The α-tubulin of many higher eukaryotes has been reported to be post-translationally modified by a reversible exchange of the COOH-terminal tyrosine residue. This process was generally believed to be restricted to higher eukaryotes. However, it has now been shown to occur in trypanosomes and to be specific for the α-tubulin. In the case of trypanosomes, the
specify of this tyrosine exchange mechanism for α-tubulin is not trivial, since the β-tubulin also contains a tyrosine residue at its COOH-terminus. The detection of post-translational tyrosination in trypanosomes, i.e., a lower eukaryote, is somewhat at variance with earlier studies on the distribution of the relevant enzyme, tubulin tyrosine ligase, throughout the animal kingdom (40, 41). However, the failure to detect the enzyme from lower eukaryotes may simply reflect the fact that the enzyme from lower eukaryotes is not active in the assay conditions employed.

The biochemical identification and partial characterization of the tubulins of Trypanosoma brucei constitutes a first step toward a more detailed analysis of the cytoskeleton of these organisms, in view of its potential as a target site for chemotherapeutic attack. Earlier work from this laboratory has demonstrated that the pellicular microtubules of trypanosomes are highly sensitive to many phenothiazine compounds, a group of drugs of which many members are in frequent clinical usage, e.g., as psychotherapeutic drugs or antihistamines (22). The isolation and characterization of the trypanosomal tubulin will hopefully allow a more detailed investigation of the mechanisms of action of these drugs as possible inhibitors of the trypanosomal cytoskeleton.

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REFERENCES
1. Hoare, C. A. (1972) The Trypanosomes of Mammals, Blackwell Scientific, Oxford
2. Ormerod, W. E. (1979) Pharmacol. Ther. 6, 1–40
3. Vickerman, K., and Preston, T. M. (1976) in Biology of the Kinetoplastida (Lumsden, W. H. R., and Evans, D. A., eds) Vol. 1, pp. 55–130, Academic Press, New York
4. Vickerman, K., and Preston, T. M. (1970) J. Cell Sci. 6, 365–383
5. Solarí, A. J. (1980) Chromosoma 78, 239–255
6. Gozes, I., and Sweadner, K. J. (1981) Nature (Lond.) 294, 477–479
7. Kemphues, K. J., Kaufman, T. C., Raff, R. A., and Raff, E. C. (1982) Cell 31, 655–670
8. Wallach, M., Pong, D., and Chang, K. P. (1982) Nature (Lond.) 299, 650–652
9. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J., and Kirschner, M. W. (1980) Cell 20, 85–105
10. Sánchez, F., Natke, J. E., Cleveland, D. W., Kirschner, M. W., and McCarthy, B. J. (1980) Cell 22, 845–854
11. Poon, H., Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R., and Ade, W. (1982) Cold Spring Harbor Symp. Quant. Biol. 46, 191–197
12. McKeithan, T. W., Lefebvre, P. A., Silflow, C. D., and Rosenbaum, J. L. (1983) J. Cell Biol. 96, 1060–1063
13. Lefebvre, P. A., Silflow, C. D., Wieben, E. D., and Rosenbaum, J. L. (1980) Cell 20, 469–477
14. Galle, J. M., and Anderson, B. H. (1983) Eur. Mol. Biol. 2, 479–483
15. Thomashaw, L. S., Milhausen, M., Rutter, W. S., and Agabian, N. (1983) Cell 32, 35–43
16. Seebeck, T., Whittaker, P. A., Imboden, M. A., Hardman, N., and Brau, R. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4634–4638
17. Mir, L., and Wright, M. (1978) Microbiol. Rev. 5, 39–41
18. Haber, J. E., Péloquin, J. G., Halvorson, H. O., and Borisy, G. G. (1972) J. Cell Biol. 55, 355–367
19. Messier, P. E. (1971) J. Protozool. 18, 223–231
20. MacKawa, S., and Sekai, H. (1978) J. Biochem. (Tokyo) 83, 1065–1075
21. Roobol, A., Pogson, C. I., and Gull, K. (1980) Exp. Cell Res. 130, 203–215
22. Seebeck, T., and Gehr, P. (1983) Mol. Biochem. Parasitol. 9, 197–208
23. Kilmartin, J. V. (1981) Biochemistry 20, 3629–3633
24. Schiiff, P. B., Fant, J., and Horwitz, S. B. (1979) J. Biol. Chem. 254, 665–667
25. Ruan, R., and Schönenberger, M. (1979) Acta Trop. 36, 289–292
26. Elpeo, B. A. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 2283–2287
27. Lüdewig, R. F., Shooter, E. M., and Wilson, L. (1977) J. Biol. Chem. 252, 7006–7014
28. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
29. Yang, D., and Criddle, R. S. (1979) J. Biochem. 90, 3063–3072
30. Crestfield, A. M., Moore, S., and Stein, W. H. (1963) J. Biol. Chem. 238, 622–627
31. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
32. Lischwe, M. A., and Ochs, D. (1982) Anal. Biochem. 127, 453–457
33. Cleveland, D. W., Fisher, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106
34. Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 297–298
35. Kirschner, M. W., Honig, L. S., and Williams, R. C. (1975) J. Mol. Biol. 99, 263–276
36. Kumar, N., and Flavin, M. (1981) J. Biol. Chem. 256, 7678–7686
37. Arce, C. A., and Barra, H. S. (1983) FEBS Lett. 157, 75–78
38. Haybin, D., and Flavin, M. (1970) Biochemistry 16, 2188–2194
39. Nath, J., Flavin, M., and Schiffmann, E. (1981) J. Cell Biol. 91, 232–239
40. Preston, S. F., Deanin, G. G., Hansen, R. K., and Gordon, M. W. (1979) J. Mol. Biol. 13, 233–244
41. Kobilayashi, T., and Flavin, M. (1983) Comp. Biochem. Physiol. 69B, 387–392
Partial purification and characterization of microtubular protein from *Trypanosoma brucei*.
J Stieger, T Wyler and T Seebeck

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