Biochemical Characterization of Tektins from Sperm Flagellar Doublet Microtubules

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Abstract. Tektins, protein components of stable protofilaments from sea urchin sperm flagellar outer doublet microtubules (Linck, R. W., and G. L. Langevin, 1982, *J. Cell Sci.*, 58:1–22), are separable by preparative SDS PAGE into 47-, 51-, and 55-kD equimolar components. High resolution two-dimensional tryptic peptide mapping reveals 63–67% coincidence among peptides of the 51-kD tektin chain and its 47- and 55-kD counterparts, >70% coincidence between the 47- and 55-kD tektins, but little obvious similarity to either α- or β-tubulin. With reverse-phase HPLC on a C_{18} column, using 6 M guanidine-HCl solubilization and a 0.1% trifluoroacetic acid/CH_3CN gradient system (Stephens, R. E., 1984, *J. Cell Biol.* 90:37*a* [Abstr.]), the relatively less hydrophobic 51-kD tektin elutes at >45% CH_3CN, immediately followed by the 55-kD chain. The 47-kD tektin is substantially more hydrophobic, eluting between the two tubulins. The amino acid compositions of the tektins are very similar to each other but totally distinct from tubulin chains, being characterized by a >50% higher arginine plus lysine content (in good agreement with the number of tryptic peptides) and about half the content of glycine, histidine, proline, and tyrosine. The proline content correlates well with the fact that tektin filaments have twice as much α-helical content as tubulin. Total hydrophobic amino acid content correlates with HPLC elution times for the tektins but not tubulins. The average amino acid composition of the tektins indicates that they resemble intermediate filament proteins, as originally postulated from structural, solubility, and electrophoretic properties. Tektins have higher cysteine and tryptophan contents than desmin and vimentin, which characteristically have only one residue of each, more closely resembling certain keratins in these amino acids.

Sea urchin sperm flagellar doublet microtubules can be fractionated into A- and B-subfiber components composed of chemically different tubulin subunits (21, 24). Doublet microtubules and purified A-subfiber microtubules can be further fractionated into sarkosyl-detergent-insoluble ribbons of 3-protofilaments composed of tubulin (19, 31, 32) and certain other polypeptides that are quantitatively retained in this fraction (12). Finally, it has been shown that doublet microtubules can be fractionated into an insoluble material that appears as filaments 2–3 nm in diameter and comprises three major polypeptides (components of the sarkosyl-insoluble protofilament ribbons) with molecular masses of 47-, 51-, and 55-kD (15, 17). These three polypeptides were originally proposed to form a related set, referred to as "tektins" (13); one or more of these tektins form the so-called tektin filaments, which have been shown to exist as longitudinal polymers in the walls of flagellar doublet microtubules (17). We have previously noted the similarity of the tektin filaments and proteins to another class of cytoskeletal elements, the intermediate filaments (IF)[1] and IF proteins. In an effort to understand the exact nature of the tektins and their molecular interactions with tubulin, we have begun a biochemical characterization of these novel polypeptides.

1. *Abbreviations used in this paper*: IF, intermediate filaments; TFA, trifluoroacetic acid.

Materials and Methods

Purification and Fractionation of Microtubules

Sperm flagellar axonemes were prepared from the sea urchin *Strongylocentrotus purpuratus*, as modified from Gibbons and Fronk (9), by demembranating and homogenizing whole sperm in 1% Triton X-100, 0.15 M KCl, 5 mM $MgSO_4$, 0.5 mM EDTA, 10 mM Tris, 1 mM ATP, and 1 mM DTT, pH 8.3, at 4°C, followed by differential centrifugations to separate sperm heads from axonemes. Axonemes were purified by repeated differential spins in this same solution minus Triton, and were subsequently dialyzed against 100 vol of 1 mM Tris, 0.1 mM EDTA, and 0.5 mM DTT (TED) for 48 h. The resulting purified doublet microtubules were collected by centrifugation at 100,000 *g* for 25 min and washed by resuspension and recentrifugation in TED. The doublet microtubule preparation was resuspended in TED to a final concentration of 10–15 mg/ml. Protein determinations were made by the Lowry procedure (18).

Fractionation of doublet microtubules into tektins was conducted at 4°C. 1 vol of doublet microtubules (typically 10 mg in 0.9 ml) was added to and quickly mixed with 9 vol of the desired solvent. After dilution the solvent consisted of 50 mM Tris, 50 mM lysine, and 1 mM EDTA, pH 8.3, plus varying concentrations of urea and sodium dodecyl sarcosinate (Sarkosyl,

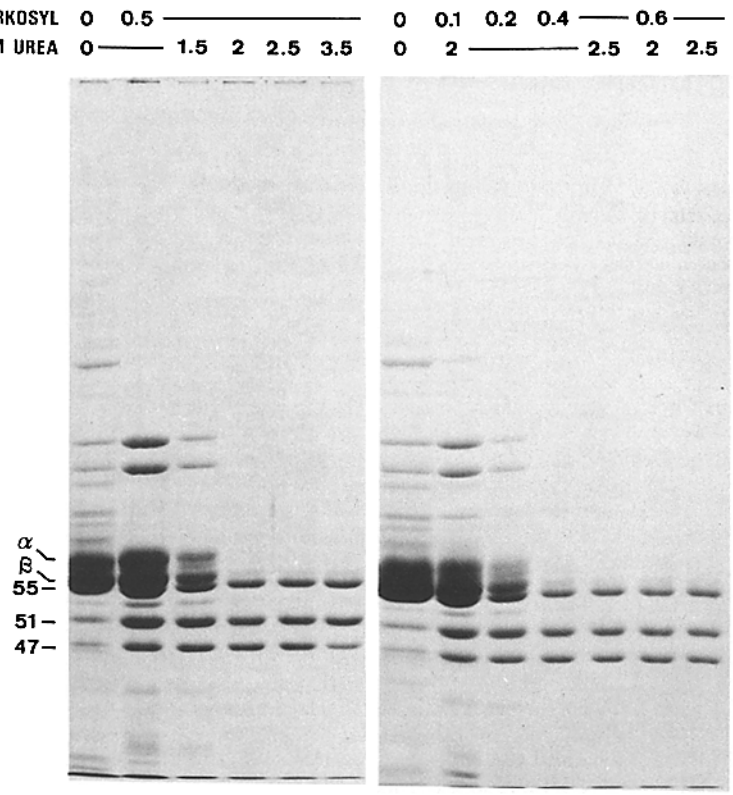

Figure 1. Quantitative SDS PAGE analysis of the fractionation of *S. purpuratus* doublet microtubules into tektin filament proteins by sarkosyl-urea. Maximum retention of the 47-, 51-, and 55-kD tektin polypeptide bands was obtained at 0.5% sarkosyl/2.0 M urea with minimal contamination by tubulin (αβ) and other microtubule-associated proteins; the 47-kD polypeptide was selectively solubilized above 4 M urea (data not shown). Equivalent volumes of microtubules at 14.9 mg/ml were added to a series of tubes containing 9 vol of solvent. After mixing, the tubes contained the indicated concentrations of urea (M) and/or sarkosyl (%), and buffer. After a single extraction and centrifugation, supernatants were discarded; pellets were freeze-dried and dissolved in 0.5 ml of SDS-sample medium. With the exception of the control lanes (0% sarkosyl, 0 M urea), which contained 2 μl (underloaded because of the excess tubulin), all other lanes contained equivalent amounts (6.5 μl) of the respective samples. Gels were stained with Serva Blue (Serva, Heidelberg, Federal Republic of Germany) according to Fairbanks et al. (8).

Ciba-Geigy Corp., Greenboro, NC); concentrations of the latter two agents were varied as described in Results. This procedure prevented the microtubules from experiencing local solvent concentrations significantly different from the final desired concentrations. The preparation was extracted for 1 h and centrifuged at 100,000 *g* for 90 min. Supernatants were discarded. Pellets were used directly or were reextracted with half the original volume of solvent at the final desired concentration, agitating during a 1-h period to loosen the pellet, and centrifugation at 100,000 *g* for 90 min. The insoluble pellets were rinsed briefly with deionized H_2O and stored frozen or freeze-dried at −30°C.

B(αβ)-Tubulin

Tubulin from the B-subfiber of flagellar doublet microtubules was prepared according to the procedures of Stephens (21), and Linck and Langevin (14). Purified doublet microtubules were thermally fractionated by heating for 6 min at 40°C and centrifuging at 100,000 *g* for 1 h to obtain the soluble B(αβ)-tubulin (21). This crude B-tubulin was twice polymerized into synthetic singlet microtubules (14) to yield a preparation of α- and β-tubulin that was 95% pure by analytical SDS gel electrophoresis.

SDS PAGE Separation and Purification of Proteins

Individual proteins were purified by SDS polyacrylamide slab gel electrophoresis (PAGE) according to the procedures of Laemmli (11) and Stephens (22). Proteins were dissolved by incubating for 1 h at 37°C in 2% SDS, 2.5 mM Tris, 19.2 mM glycine, and 5% 2-mercaptoethanol, pH 6.8, and held at 100°C for 5 min. For tektins, the Laemmli (11) discontinuous system was used: 7.5% acrylamide/0.2% bisacrylamide, running at 50 V until the tracking dye reached the bottom of the gel (135 mm) and continuing for 20% more volt-hours. For tubulins, the continuous Tris-glycine system (5, 22) was used: 6% acrylamide-0.16% bisacrylamide, running at 75 V until the tracking dye reached the bottom of the gel (135 mm) and continuing for 50% more volt-hours. For optimal separation of the tektins, Bio-Rad SDS (electrophoresis purity reagent, cat. No. 161-0302; Bio-Rad Laboratories, Richmond, CA) proved best; with Sigma SDS (cat. No. L-5750; Sigma Chemical Co., St. Louis, MO), which optimized α- and β-tubulin separation, the 51- and 55-kD tektins were less well resolved. After running, gels were soaked in 0.25 M KCl for 15 min to visualize the protein bands, which were then cut out. Gel strips were placed in electroelution tubes and the proteins collected as described by Stephens (22). Electroeluted proteins were dialyzed exhaustively for 3 d with multiple changes of deionized H_2O. Aliquots of the salt-free protein were taken for SDS PAGE analysis of purity and the remainder was freeze-dried. To free the protein of residual SDS, freeze-dried protein powders were extracted twice with 90% acetone at 4°C for 20 min and then once with 100% acetone, collecting the protein by centrifugation.

Reverse-Phase HPLC Purification and Analysis of Proteins

Proteins were also analyzed and purified by reverse-phase, HPLC, using a Waters system and a μBondapak C_{18} reverse-phase column (Waters Associates, Millipore Corp., Milford, MA). For HPLC purification, it was necessary to dissolve the samples at ~0.3 mg of individual protein per ml in 6 M guanidine-HCl, 5 mM DTT, and 10 mM Tris, pH 8.3, incubate them at 37°C for 1 h and then briefly sonicate. Afterwards, the samples at room temperature were made 0.1% in trifluoroacetic acid (TFA) and centrifuged (model TL-100; Beckman Instruments, Inc., Palo Alto, CA) at 50,000 *g* for 15 min. The clarified supernate (75–125 μl) was applied to the HPLC column, which was previously equilibrated with 30% acetonitrile (0.1% TFA). For separation, the conditions that proved optimal were modified from those of Stephens (27): a linear gradient from 30 to 45% acetonitrile followed by an exponential gradient from 45 to 60% acetonitrile in 0.1% TFA. Elution time and OD at 214 nm were continuously recorded in the elution profile. Eluted peaks were evaporated to dryness, using a Savant Speed Vac (Savant Instruments, Inc., Hicksville, New York), and subsequently analyzed by SDS PAGE, or further treated for peptide mapping (see below).

Carboxymethylation

Proteins were carboxymethylated with iodoacetate by a procedure similar to that of Weeds and Lowey (30). All solutions were made using O_2-free water, prepared by boiling deionized water for 1 h, bubbling with N_2 while cooling, and keeping tightly capped. A 1–5-mg sample of protein was dissolved in 5 ml of 6 M guanidine-HCl, 0.1 M Tris, 1 mM EDTA, and 1 mM DTT, pH 8.8, at room temperature. The tube was capped under nitrogen and left at room temperature for 6–12 h. After this period, an additional 1 mM DTT was added and mixed under nitrogen, followed by the addition of a two-fold excess of iodoacetate over the total thiol content of DTT. The sam-

ple was incubated at room temperature in the absence of light for 45 min, after which the reaction was stopped by the addition of a 200-fold excess of 2-mercaptoethanol. The protein was dialyzed against multiple changes of H_2O and then freeze-dried.

Tryptic Peptide Mapping

High-resolution, fluorescent, two-dimensional tryptic peptide mapping was carried out essentially as described by Stephens (25). Briefly, 200–500 μg amounts of carboxymethylated tektins and tubulin subunits were digested with TPCK (L-1-*p*-tosylamino-2-phenylethyl chloromethyl ketone)-treated trypsin (cat. No. T8642; Sigma Chemical Co.) in 0.1 M ammonium bicarbonate at a 1:100 enzyme/protein ratio for 24 h at 25°C. A second aliquot of enzyme was added and the incubation was continued for an additional 6 h at 37°C. The samples were evaporated to dryness in a SpeedVac (Savant Instruments, Inc.) and redissolved in 0.1 M ammonium bicarbonate to a final concentration of 2–3 μg/ul. Typically, 10–15 μl (~0.5 nmole of initial protein) was spotted on a 20 × 20 cm, 0.25-mm thick silica gel GHL plate (Analtech, Inc., Newark, DE) and chromatographed with chloroform/methanol/ammonium hydroxide (2:2:1) until the solvent front reached the top of the plate. The plates were air-dried overnight. They were then spray saturated with pyridine/acetic acid/water (1:10:489) and electrophoresed perpendicular to the chromatographic dimension at 1,000 V for 60 min at 10°C. The plates were oven-dried at 110°C for >1 h, cooled, and sprayed with 0.025% fluorescamine (Hoffman-LaRoche, Inc., Nutley, NJ) in acetone to derivatize the peptides, postsprayed with 3% pyridine in acetone to stabilize the image, and then sprayed lightly with water to hydrolyze background products. The maps were photographed under 360-nm UV light, using a No. 8 Wratten barrier filter and Kodak Plus-X 35-mm film. The resulting negatives were enlarged and printed as positive transparencies on Kodak 4489 electron image film and a negative transparency was produced from this positive by contact printing. These reciprocal transparencies were then superimposed 2% out of register and contact printed as bas-relief images. This process enhances peptide spots while "subtracting" unwanted background fluorescence.

Amino Acid Analysis

A reverse-phase HPLC "PICO-TAG" system (Waters Associates, Millipore, Corp.) was used. This proprietary system is derived from PITC-(phenylisothiocyanate)-amino acid separation methods evaluated by Heinrikson and Meredith (10). After drying and thorough evacuation-purging with high purity nitrogen, microgram amounts of carboxymethylated tektins and tubulin subunits were hydrolyzed with 6 N HCl containing 1% phenol for 24, 48, and 72 h at 105°C under vacuum. Samples were redried and neutralized with triethylamine, and then derivatized with PITC, using the PICO-TAG work station and prescribed protocol. The analysis was performed on a HPLC system (Waters Associates, Millipore Corp.) equipped with automated sample injector (WISP), permitting unattended sequential sample and standard analysis plus periodic recalibration. Serine and threonine were corrected for loss on hydrolysis. All other residues typically agreed within 2% among identical samples hydrolyzed for different times (or among replicate samples) and within 5% between protein samples prepared separately for hydrolysis.

Tryptophan was determined spectrophotometrically in 6 N guanidine-HCl and 20 mM phosphate, pH 6.5, according to the method of Edelhoch (7). In addition, the high pH method of Beaven and Holiday (3) was also used. The tyrosine/tryptophan ratio was obtained from the absorbance at 280 and 288 nm in the former case, and at 280 and 295.5 nm in the latter. The two methods agreed within 20%. The absolute amount of tryptophan was estimated using the known tyrosine content from amino acid analysis.

Results

Isolation and Purification of Tektins

As previously published (17), tektin filaments composed of three proteins (referred to herein as 47-, 51-, and 55-kD tektins) can be isolated by extraction of flagellar doublet microtubules with 0.5% sarkosyl and 2.5 M urea. Use of sarkosyl alone yields microtubule protofilament ribbons of which tubulin is still the major component, and extraction with urea alone incompletely solubilizes the tubulin while

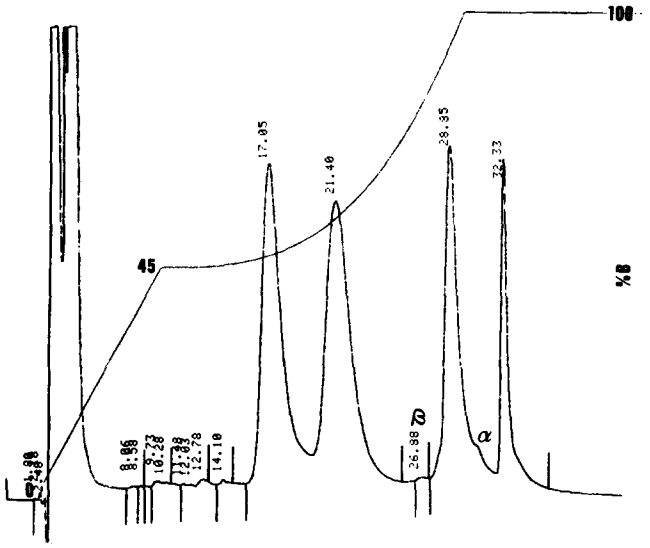

Figure 2. Separation of tektins by reverse-phase HPLC; elution profile of 150 μg of sarkosyl-urea extracted tektin filaments, solubilized in 6 M guanidine-HCl. The gradient profile axis (%B) denotes percent second solvent (60% acetonitrile, 0.1% TFA); the initial solvent is 30% acetonitrile, 0.1% TFA. Elution times for α-tubulin (30.4) and β-tubulin (26.9) subunits, demonstrated in a separate experiment, are indicated by Greek letters. The peak at 32.33 min is residual sarkosyl. Full scale = 1.0 OD. The 51-kD tektin elutes at 17.05 min, followed by the 55-kD tektin at 21.40; the 47-kD peak elutes at 28.85, flanked by the minor contaminating tubulin subunits.

solubilizing some of the tektin polypeptides. The rationale for a combined sarkosyl-urea solvent stemmed from the use of detergent (i.e., Triton X-100)-urea systems to separate proteins on the basis of their differential hydrophobicities (26, 33). Because the concentrations of sarkosyl-urea previously used led to the partial extraction of the 47-kD tektin polypeptide, a more careful study was made. The effect of varying the sarkosyl and urea concentrations is shown in Fig. 1. For the maximum retention of the three 47-, 51-, and 55-kD polypeptides in nearly equimolar amounts, the optimal concentration of sarkosyl was determined to be 0.5%, and that of urea was 2.0 M for a single extraction. Contamination by tubulin and several minor components was effectively reduced to zero by a second extraction.

Tektin filaments were prepared by these procedures and separated into their three polypeptide subunits by SDS PAGE. Before and after carboxymethylation, each polypeptide was cut out, electroeluted, and reanalyzed for its integrity and purity. Within the linear range of Serva blue staining, each polypeptide was pure, with undiscernible contamination from adjacent higher or lower molecular weight species (data not shown). The positive identity of the three polypeptides as tektins and not tubulins was made by showing that antitubulin antibodies do not recognize the three bands, that antibodies to the tektins do not recognize tubulin (17; manuscript in preparation), and that the tektins are biochemically different from tubulin (this report).

Tektin filament polypeptides could also be resolved by HPLC, resulting again in the separation of three major protein peaks of equal area (Fig. 2). The HPLC peaks were subsequently identified by SDS PAGE; the peaks, in order of

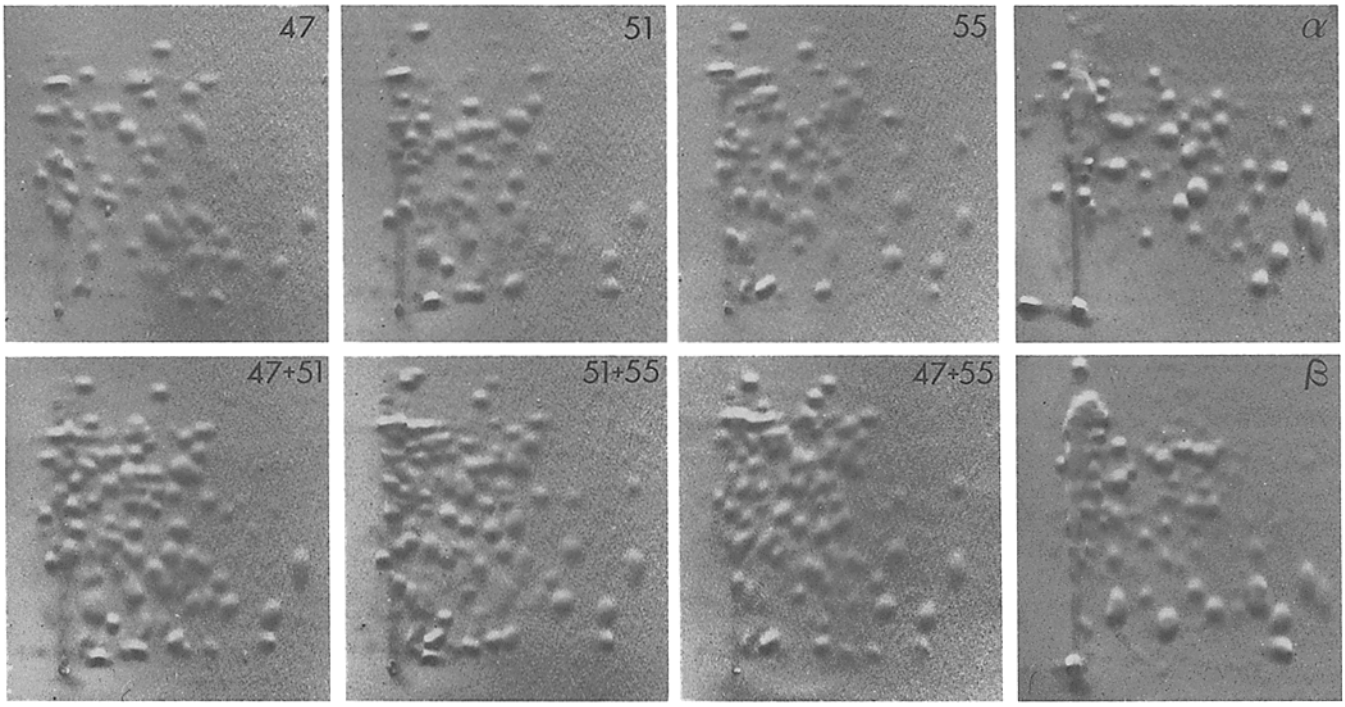

Figure 3. Comparative two-dimensional tryptic peptide mapping on silica gel GHL plates. Origin at lower left corners. Vertical axis: chromatographic dimension (chloroform, methanol, ammonium hydroxide); least polar peptides migrate farthest. Horizontal axis: electrophoretic dimension (aqueous acetic acid, pyridine, pH 3.5); smallest, most charged peptides migrate farthest. The numerical designation on each map denotes the tektin type by molecular mass (in kD); the numerical sum designates equal amounts of the two respective tektins run as a mixture. Greek letters designate the tubulin chain type.

their elution, correspond to the 51-, 55-, and 47-kD polypeptides, and these were present in equimolar amounts as judged by quantitative staining with acid-fast green. Separately purified α- and β-tubulin from *S. purpuratus* B-subfibers were found to elute with distinctly different retention times (Fig. 2), indicating not only a difference in the relative hydrophobicity of the tektins and tubulins, but also demonstrating the purity and apparent homogeneity of the three *S. purpuratus* tektins in the original preparations. Yields of tektins separated by this method were typically >80%, given that the initial sample was salt-free and had not undergone oxidization during storage.

Peptide Mapping

The three tektins were compared with each other and with α- and β-tubulin (from thermally fractionated B-subfibers) by two-dimensional peptide mapping of tryptic digests, as described in Materials and Methods. Individual maps were run, as were composite maps made by running two combined samples for direct comparison (Fig. 3). The 47-kD tektin yielded 60–61 distinct spots, the 51-kD produced 57–59 spots, and the 55-kD tektin gave 69–71 spots. The number of tryptic peptides for each tektin is in good agreement with the number predicted from the total arginine and lysine contents (Table I). To produce an objective estimate of relative coincidence among peptides of these proteins, equal amounts were cochromatographed and the total number of peptides present was scored. The mixture of 47- and 51-kD tektins produced 94–95 spots, the 51- and 55-kD gave 94–96 spots, and the 47- and 55-kD tektins yielded 89–91 spots. Using the average number of peptides in the two individual, paired maps and the total scored in the composite map, ~63% coincidence was found between the 47- and 51-kD tektins, 67% between the 51- and 55-kD tektins, and 72% between the 47- and 55-kD tektins (Table II).

Tryptic peptide maps of the two tubulin chains are very distinctive and are diagnostic of the chain type, having many distinguishing sets of spots (cf. 25); none of these are evident in any of the tektin maps. In spite of having a moderate degree of coincidence of peptides, the maps of the three tektins are each distinct from the other. There is no evidence for the presence or absence of entire sets of spots between maps, negating any possible precursor–product or proteolytic cleavage relationship among the tektins or tubulins.

Amino Acid Composition

The three tektins were carboxymethylated and sbusequently hydrolyzed in preparation for amino acid analysis, using the PICO-TAG method. The amino acid compositions are presented in Table I. The three tektins are quite similar in amino acid composition, more so than might be expected from the peptide map differences. Major differences in histidine content distinguish the 47-kD tektin from the other tektins. Differences in alanine, methionine, and valine distinguish the 51-kD tektin from its counterparts; differences in arginine, lysine, and phenylalanine set the 55-kD tektin apart. The lysine plus arginine content and the respective molecular weights would predict a maximum of 61, 64, and 70 tryptic peptides respectively for the 47-, 51-, and 55-kD tektin, quite close to what was found. The hydrophobic amino acid content is in the order of 51- < 55- < 47-kD, coinciding with the elution order from reverse-phase HPLC.

Table I. Amino Acid Compositions (Mole Percent) of the 47-, 51-, and 55-kD Tektins and Alpha and Beta Tubulins from S. purpuratus

Residue	47-kD		51-kD		55-kD		Alpha		Beta
Ala	6.84	*	8.10	*	7.41		8.95	*	7.04
Arg	7.85		7.38	*	6.55	>	4.24		4.93
Asx[1]	16.1		14.1		13.3	>	9.67	*	11.0
Cys	1.27	*	1.99		2.01		1.93		1.67
Glx[1]	17.0		17.2		16.2	>	14.2		14.6
Gly	5.23		6.05		5.52	≪	11.4		10.03
His	0.67	*	1.17		1.26	≪	2.28		1.95
Ile	4.44	*	3.73		3.38		4.16	*	3.13
Leu	9.61		9.17		9.02	>	6.68		7.08
Lys	7.03		7.01	*	8.02	≫	3.94		3.44
Met	2.67	*	1.16	*	2.67		1.81	*	3.47
Phe	1.54		1.37	*	2.27	<	3.78	*	4.61
Pro	2.03		2.63		2.56	≪	4.30		4.53
Ser	4.99		6.16		5.82		7.20		6.16
Thr	5.98		6.75		7.05		5.51		6.18
Trp	1.24		1.19		1.10	>	0.87		0.86
Tyr	1.56		1.79		1.81	≪	3.47		3.42
Val	3.99	*	3.09	*	4.08	<	5.69		5.68

* Denotes differences among tektins or between tubulins.
< > and ≪≫ Denote differences between tektins and tubulins as protein classes; significant (< >) and major (≪≫).
Asx[1] combines Asn and Asp; Glx[1] combines Glu and Gln.

As is well established, α- and β-tubulin chains are similar to one another, differing characteristically in methionine by a factor of about two, while lesser differences occur in alanine, aspartic plus asparagine, isoleucine, and phenylalanine (cf. 24). Major differences distinguish the tektins from the tubulins, particularly in glycine, histidine, lysine, proline, and tyrosine, and to a lesser degree in arginine, aspartic plus asparagine, glutamic plus glutamine, leucine, phenylalanine, tryptophan, and valine.

Discussion

Uniqueness of the Tektins

The central questions of this work concern the identity and nature of the proteins we have called tektins: Are these proteins tubulin variants (real or artifactual), breakdown products of other flagellar proteins, or unique unto themselves? The main conclusions from this work are that the three tektins are clearly distinct from each other but nevertheless related, and that they are dramatically different from either tubulin subunit. Results from peptide mapping and amino acid analysis eliminate the possibility that one of the tektins is derived from the others or from the tubulins by modification, natural or artifactual, and these findings are consistent with the fact that antibodies to tubulins or tektins do not cross-react with the opposite proteins (17). The question of whether the tektins result from the breakdown of a larger flagellar protein is not addressed here, but in separate studies we have found that tektin antibodies cross-react in SDS PAGE immunoblots with only tektins and not with higher molecular weight components (17; manuscript in preparation). Thus, we must now consider the tektins as a unique set of proteins.

This study and previous reports have shown the tektins to be isolated as a reproducible set of relatively insoluble proteins that remain in a filamentous form in sarkosyl and urea at concentrations as high as 1% and 5 M, respectively. The tektin filaments are resolved by SDS PAGE and by reverse-phase HPLC into three equimolar components, with each peak corresponding to a different molecular mass polypeptide, i.e., 47-, 51-, and 55-kD; thus, each of the tektin polypeptide chains is homogeneous with respect to size and hydrophobicity. We cannot rule out the possibility that each tektin is heterogeneous by other criteria (see reference 15), although our peptide mapping and amino acid analysis do not suggest significant heterogeneities. The molecular masses given here for the tektins must be regarded as tentative and nominal, since they were determined using Bio-Rad SDS (Bio-Rad Laboratories), which maximally resolves them, and since other grades of SDS yield less separation; obviously, the degree of separation in SDS PAGE does not necessarily reflect true size differences. In spite of these differences, other results point to the relatedness of the tektins.

Table II. Percent Coincidence of Tryptic Peptides

Tektins A	Tektins B	Peptides per tektin or tektin pair	% Coincidence of peptides*	
47	–	60–61 (60.5)	ND	
51	–	57–59 (58)	ND	
55	–	69–71 (70)	ND	
47	51	94–95 (94.5)	64.0	62.7
51	47	"	61.4	
51	55	94–96 (95)	61.0	67.4
55	51	"	73.7	
47	55	89–91 (90)	67.2	72.5
55	47	"	77.8	

$$* \% = \frac{\text{Number of peptides in tektin A}}{\text{Number of peptides in composite maps of tektins A + B}} \times 100.$$

Relatedness of the Tektins

There are other differences and also similarities among the three tektins, based on tryptic and cyanogen bromide (CnBr) peptide mapping and amino acid analysis. The largest differences appear in the CnBr peptides (data not shown), where the three chains are clearly distinct but each tektin yields certain peptides with the same size but in different amounts. With tryptic peptide mapping the degree of sequence homology depends on how one sets up the calculations. The only concrete information that we can objectively present is Table II, listing the numbers of peptides derived from each tektin, the sum of the paired peptides, and the maximum number of coincident peptides contributed in the paired peptides. From these data one may estimate the relative amount of homology (i.e., shared peptides) between one tektin and another (although two values are obtained for each pair, since different numbers of peptides are produced by each protein, and since they have different apparent molecular weights). When one calculates homologies in this way, the values vary from an average of 63% for tektins 47- vs. 51-kD, 67% for 51- vs. 55-kD, and 73% for 47- vs. 55-kD, and range from a low of 61% to a high of 78%. In previous work, the tektins were compared by limited digestion with *Staphylococcus*

aureas V8 protease, which showed that the 47- and 51-kD tektins were similar to each other but unlike 55-kD (15). These results, which differ from those of the CnBr and tryptic mapping, can be accounted for by the fact that the *S. aureas* protease cleaves at aspartic and glutamic acid residues, which may be more conserved.

In spite of the distinct differences seen by peptide mapping (where one could judge the tektins not to be closely related), the amino acid analysis data show striking similarities among the tektins. Even where large differences are seen (i.e., the two-fold differences in histidine, methionine, or cysteine), a given difference occurs in only one of the three tektins. The basic and hydrophobic residue contents of the tektins are comparable, and in their HPLC elution times the tektins follow their hydrophobic amino acid contents regardless of how one tallies hydrophobicities. The tubulins do not fit this observation, probably because they have very differently organized hydrophobic surface and intramolecular domains.

Biological Significance

As previously shown with immunolabeling and electron microscopy, one or all of the tektins are organized as longitudinal filaments in the walls of the A-tubules of doublet microtubules (17). These results raise two interesting implications. First, there may be a direct association between tektins and tubulins. Although the tektins and tubulins represent two very different classes of proteins based on our work, tektins may have certain local interactive domains in common with tubulin chains, depending on how they intercalate into the microtubule surface lattice; these interactive domains could, for example, be α-helical regions (see also 2, 6, 15).

A second implication arises, given that native flagellar microtubules appear to be parallel polymers of tubulin and tektin. Based on the original identification of the polypeptides comprising the sarkosyl-resistant protofilament ribbons (12, 16), Stephens (23) found that one of the polypeptides ('Component 20') was synthesized *de novo* and in quantal amounts during sea urchin (*S. droebachiensis*) embryonic ciliary regeneration and proposed that the synthesis of Component 20 might act as a control for ciliary and flagellar assembly and/or elongation. From subsequent work on *S. purpuratus* embryonic cilia, we now know that Component 20 is a tektin (1, and unpublished observations), thus strengthening this hypothesis.

The final point of discussion concerns the possible relatedness of tektins to IF proteins and keratins (28). As previously shown, tektins are similar to IF proteins in their relative solubility properties, molecular weights, isoelectric points, α-helical content, and fibrous substructure (13, 15). With regard to the latter two properties, tektin filaments show strong α-type x-ray diffraction patterns (4), and filaments composed of 51- and 55-kD tektins have an average α-helical content of ~70% (15); these properties are consistent with the low proline contents we have measured here. In addition, our amino acid analyses and tryptic peptide mapping suggest that tektins may have a 63–73% primary sequence homology with each other, a feature also characteristic of the different IF proteins (20). Nevertheless, our studies also reveal features of tektins that set them apart from classes of IF proteins: (*a*) While the amino acid compositions of tektins and IF proteins correlate rather well (cf. 29), tektins have 4–5 tryptophan residues per subunit, whereas IF proteins and keratins characteristically have a single tryptophan at a conserved site in their sequence (29). (*b*) The tektins also possess several cysteines unlike desmin and vimentin that have only one such residue, but they do closely resemble certain keratins in their higher cysteine content. (*c*) Finally, while the tektins appear as individual or bundles of 2–4-nm fibrils with an apparent coiled-coil arrangement (2, 15, 17), formed IFs have diameters of 7–12 nm (28). Thus, the tektins would seem to represent a distinctive class of proteins; their properties suggest they could be evolutionary forbearers of IFs.

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