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Characterization of a Microtubule-Stimulated Adenosinetriphosphatase Activity Associated with Microtubule Gelation–Contraction†

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ABSTRACT: A microtubule-stimulated ATPase is associated with particles that are responsible for microtubule gelation–contraction in vitro. These particles have been proposed to be slow axonal transport, component a, particulates (SCAPs) [Weisenberg, R. C., Flynn, J. J., Gao, B., Awodi, S., Skee, F., Goodman, S., & Riederer, B. (1987) *Science (Washington, D.C.) 238*, 1119–1122]. The SCAP ATPase activity is stimulated approximately twofold by microtubules. The microtubule-stimulated ATPase activity correlates with the occurrence of microtubule gelation–contraction. Both microtubule-stimulated ATPase activity and microtubule gelation–contraction are inhibited by millimolar calcium, 0.3 M KCl plus 2 mM ethylenediaminetetraacetic acid (EDTA), 5 μM vanadate, and millimolar *N*-ethylmaleimide (NEM). Neither the ATPase activity nor microtubule gelation–contraction is affected by high magnesium concentrations (up to 8 mM) or by the anti-ATPase drugs ouabain, oligomycin, sodium azide, and *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA). Magnesium is required for both ATPase activity and microtubule gelation–contraction. Microtubule-stimulated hydrolysis of GTP, CTP, ITP, and UTP is less than 50% of ATP hydrolysis, and microtubule gelation–contraction is reduced in these nucleotides. On the basis of these results we propose that the microtubule-stimulated ATPase activity associated with SCAPs is a previously undescribed enzyme that is responsible for microtubule gelation–contraction in vitro and that is the likely motor for component a of slow axonal transport.

Slow axonal transport is generally divided into two major categories according to the rate of transport and the proteins involved. Component a of slow axonal transport (SCa) has a rate of 0.2–1.1 mm/day and consists primarily of cytoskeletal proteins, including tubulin, neurofilament proteins, and brain spectrin (Tytell et al., 1981; Lasek et al., 1984). These proteins appear to be transported as an insoluble complex (Grafstein et al., 1970; Lorenz & Willard, 1978; Tashiro et al., 1984; Filliatreau & De Giamberadino, 1985). Component b of slow transport consists primarily of soluble proteins and has a rate of 2–8 mm/day (Lasek et al., 1984). The mechanism of SCa transport, the form of the transported protein, and the "motor" involved have not been clearly demonstrated.

Crude calf brain microtubule proteins can undergo gelation–contraction in the presence of ATP (Weisenberg & Cianci, 1984). During microtubule gelation–contraction, movement of particulate material along microtubules occurs at a rate of about 1 μm/min (Weisenberg et al., 1986). Recently a particulate fraction has been isolated from crude microtubule proteins which is required for gelation–contraction of microtubules (assembled from purified tubulin) in the presence of ATP (Weisenberg et al., 1987). These particulates have a protein composition consisting primarily of tubulin, neurofilament, and spectrin polypeptides, which is similar to the composition of SCa. Movement of these particulates along microtubules, at a rate of about 1 μm/min, occurs in the presence of ATP. Because of similarities in their rate of movement, protein composition, and solubility, we have proposed that the particles responsible for microtubule gelation–contraction in vitro are the transported components of SCa

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and that, therefore, microtubule gelation–contraction is a cell-free model for SCa transport. We proposed the name SCAPs,[1] for SCa particulates, for the particles responsible for microtubule gelation–contraction (Weisenberg et al., 1987). In this paper, we describe a microtubule-stimulated ATPase activity associated with SCAPs and its correlation to microtubule gelation–contraction.

Materials and Methods

Microtubule proteins were prepared by three cycles of temperature-dependent assembly–disassembly as described previously (Weisenberg & Cianci, 1984; Weisenberg et al., 1987). For the isolation of SCAPs and tubulin, third cycle microtubule proteins were centrifuged in a Beckman type 65 rotor at 45 000 rpm (175000*g*) for 15 min at 4 °C. Both the supernatant, containing free tubulin and MAPs, and the pellet, containing SCAPs, were recovered. The supernatant was applied to a slurry of phosphocellulose (PC), equilibrated with MES–DTT buffer [0.1 M 2-(*N*-morpholino)ethanesulfonic acid, 1 mM dithiothreitol, pH 6.6], in a double centrifuge tube as described by Neal and Florini (1973). The unbound protein, containing nearly pure tubulin, was recovered by centrifugation in a table-top centrifuge.

To isolate SCAPs, the pellet from the 175000*g* centrifugation was rinsed gently with MES–DTT buffer containing 1 M NaCl and 10% glycerol (in some experiments 1% Triton X-100 was included) and was then resuspended in the same buffer and homogenized in a piston homogenizer. The dispersed pellet was layered on top of a 2–3 mL cushion of MES–DTT buffer made up to 25% glycerol and centrifuged in a Beckman 65 rotor at 45 000 rpm (175000*g*) for 15 min at 4 °C. The pellet, containing SCAPs, was rinsed gently with MES–DTT buffer plus 15% glycerol and was resuspended and homogenized in the same buffer. The homogenized pellet was further dispersed by sonication for 1–2 s in a Braunsonic Model 1510 sonicator operated at 100 W.

ATPase activity was measured according to the method described by Heinonen and Lahti (1981). The proteins to be assayed were incubated at 37 °C in a standard assay buffer consisting of 0.1 M MES, 3 mM $MgCl_2$, 1 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM DTT, and 15% glycerol, at pH 6.6. All reactions were carried out in this buffer unless otherwise specified. At each designated time point 100 μL of each sample was withdrawn from the incubation mixture and added to 800 μL of freshly made AAM solution (10 mM ammonium molybdate–5 N sulfuric acid–acetone in a ratio of 1:1:2). The color was allowed to develop for 1 min and 80 μL of 1 M citric acid was added to stop the reaction. Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as a standard.

Gelation–contraction activity was determined by incubating the samples in microcuvettes at 37 °C with 1 mM GTP in gelation–contraction buffer (0.1 M MES buffer containing 1 mM DTT, 15% glycerol, 1 mM EGTA, and 3 mM $MgCl_2$, pH 6.6) for 30 min to assemble microtubules, after which 2 mM ATP was added. Photographs of contracted microtubule gels were taken 1–2 h after ATP addition.

Sodium dodecyl sulfate (SDS) slab gels containing a 5–15% continuous acrylamide gradient were prepared according to the procedure described by Walker (1984). Gels were stained with Coomassie Blue R-250.

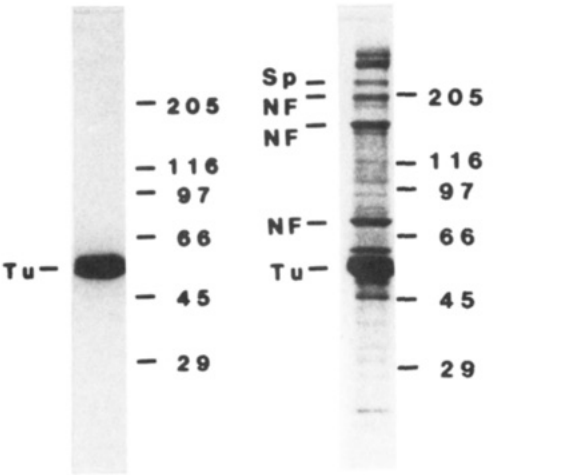

FIGURE 1: Gel electrophoresis of purified tubulin and SCAPs was done on 5–15% acrylamide gradient gels and stained with Coomassie blue. The left lane is phosphocellulose-purified tubulin; the right lane shows the composition of SCAP proteins isolated by high-salt centrifugation as described under Materials and Methods. The numbered lines mark the positions of molecular weight markers. The positions of tubulin (Tu), neurofilament proteins (NF), and spectrin (Sp) are indicated.

Nucleotides were analyzed according to the procedure of Arezzo (1987). Mixtures of SCAPs and phosphocellulose-purified tubulin in gelation–contraction buffer, containing 1 mM GTP, were divided into several Eppendorf tubes (100 μL/tube) and incubated at 37 °C for 30 min, and 2 mM ATP was added. A parallel protein sample was incubated in a microcuvette to monitor the process of gelation–contraction. At each designated time point 50 μL of a sample was assayed for phosphate, the remainder of the sample was brought to 5% trichloroacetic acid (TCA), and the precipitated protein was removed by centrifugation. TCA was extracted with ethyl ether, and the sample was filtered for HPLC analysis on a Synchropak AX-100 column from Synchrom. The relative amount of each nucleotide was estimated by cutting the area under each peak from the recording chart and weighing the paper removed. Each nucleotide was identified by its spectrum and by its peak position compared to a standard. All chemicals were of reagent grade, and solutions were made up in distilled, deionized water. EHNA was a gift from Steven Penningroth. Taxol was provided by the National Institutes of Health.

Results

SCAPs Contain a Microtubule-Stimulated ATPase. SCAPs are isolated from crude calf brain microtubule proteins by differential centrifugation in the presence of high salt concentrations. Isolated SCAPs plus phosphocellulose-purified tubulin (PC-tubulin) undergo microtubule gelation–contraction in the presence of ATP (Weisenberg et al., 1987). Neither SCAPs nor PC tubulin alone undergo gelation–contraction under the same conditions. Purified tubulin alone forms smooth-walled, undecorated microtubules. SCAPs consist of roughly 20-nm globules and aggregates of these globules (Weisenberg et al., 1987). No microtubules were observed in SCAPs. In mixtures of purified tubulin and SCAPs, microtubules are heavily decorated with particles of various sizes.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of isolated SCAPs reveals a unique protein profile (Figure 1). The major proteins in SCAPs are tubulin, neurofilament proteins, spectrin, two high molecular weight proteins (one comigrates with MAP2, and another migrates

[1] Abbreviations: SCAP(s), slow component a particulate(s); MAP, microtubule-associated protein; MES, 2-(*N*-morpholino)ethanesulfonic acid; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine.

Table I: ATPase Activity of Different Fractions[a]

sample	% of total protein	total ATPase (nmol/min)	sp act. (nmol mg^{-1} min^{-1})	% of total activity
third cycle	100	208	13 ± 1.0	100
supernatent	63	70	7 ± 1.5	33
tubulin	38	6	1 ± 1.0	3
SCAPs	10	94	59 ± 5.8	45

[a] Results are the average of 10 separate preparations. About 45% of the total ATPase activity is associated with SCAPs, which make up only 10% of the total protein in the third cycle microtubule proteins.

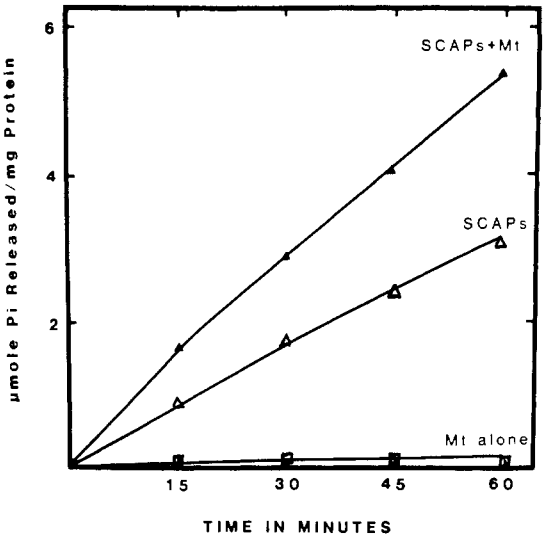

FIGURE 2: Stimulation of SCAP ATPase by the presence of microtubules. ATPase activity was measured in standard buffer (see Materials and Methods) without GTP and in the presence of 10 μM taxol to promote microtubule assembly. Purified tubulin was incubated at 37 °C for 15 min to assemble microtubules, and SCAPs and 2 mM ATP were then added (zero time). The protein concentrations of SCAPs and purified microtubules were 0.1 mg/mL and 0.8–1.0 mg/mL, respectively. The data used are the average of 10 experiments. The standard error was less than 10%. (□) Microtubules alone; (△) SCAPs alone; (▲) SCAPs plus microtubules.

between MAP1 and MAP2), and proteins at about 45K, 50K, and 62K (Weisenberg et al., 1987). Tubulin isolated by the method described in this paper (see Materials and Methods) is essentially pure. No band other than tubulin was detected by Coomassie Blue staining even on heavily loaded gels (Figure 1).

Approximately 10% of the total protein and 45% of the total ATPase activity present in the starting material (third cycle microtubule protein) are associated with the SCAP particulate fraction (Table I). Phosphocellulose-purified tubulin, on the other hand, contains insignificant ATPase activity. Approximately 30% of the initial activity remains in the supernatant fraction, and another 20% of the initial activity is lost. The identity of the supernatant and unrecovered portion of the ATPase activity is not known.

To examine the properties of the SCAP ATPase in the presence of microtubules, PC-tubulin was assembled in the absence of GTP, with 10 μM taxol to promote assembly. Microtubule gelation–contraction is not significantly altered by taxol (Weisenberg & Cianci, 1984). The SCAP ATPase activity was stimulated approximately twofold in the presence of microtubules (Figure 2). The microtubule-stimulated ATPase activity was calculated by subtracting the total ATPase activity of SCAPs alone plus microtubules alone from the ATPase activity of the mixture of SCAPs plus microtubules. The PC-purified tubulin and SCAP protein samples

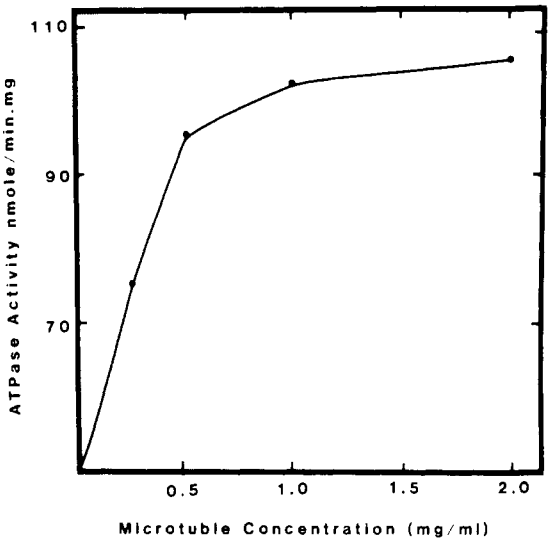

FIGURE 3: Dependence of the SCAP ATPase activity upon microtubule concentration. The concentration of SCAPs was fixed at 0.1 mg/mL. Microtubule stimulation appears to plateau above approximately 1 mg/mL tubulin (polymerized in the presence of 10 μM taxol).

were incubated separately at 37 °C for 15 min to assemble microtubules before mixing and the addition of 2 mM ATP. Neither unpolymerized tubulin nor taxol alone stimulated SCAP ATPase activity. Gelation–contraction of microtubules can also be induced by particulates that are isolated directly from brain homogenates without the use of cycles of microtubule assembly (Weisenberg et al., 1987). These particulates also contain a microtubule-stimulated ATPase (data not shown), but in the present work we have only examined the activity of particulates isolated from cycled microtubule proteins.

The maximum level of stimulation of the ATPase by microtubules was about twofold under conditions used to induce microtubule gelation–contraction. To determine if this was the maximum level of stimulation attainable, the ATPase activity was measured while the microtubule and SCAP concentrations were varied. At a constant SCAP concentration of 0.1 mg/mL, ATPase activity appeared to plateau at about 1 mg/mL tubulin (Figure 3). Similar results were obtained when the concentration of tubulin was maintained at 0.5 mg/mL and the SCAP concentration was varied. In this case the maximum specific ATPase activity was observed at SCAP concentrations below 0.1 mg/mL. In both cases the maximum extent of stimulation of ATPase activity by microtubules was about twofold.

The kinetics of the SCAP ATPase was investigated in the presence and absence of microtubules (three experiments were performed, in which there was less than 10% variation in the results obtained). The SCAP ATPase in the absence of microtubules has a V_{max} of about 59 nmol min^{-1} mg^{-1}, while in the presence of microtubules the V_{max} is increased to about 110 nmol min^{-1} mg^{-1}. The K_m of the SCAP ATPase is about 55 μM). No significant change is observed in the value of K_m in the presence of microtubules.

Microtubule-Stimulated ATPase Activity Correlates with Gelation–Contraction. It seemed likely that the SCAP microtubule-stimulated ATPase is the "motor" that is responsible for microtubule gelation–contraction, and to test this conclusion we compared microtobule-stimulated ATPase activity to gelation–contraction under a variety of conditions. Unfortunately it is difficult to make a quantitative measurement of microtubule gelation–contraction. The criteria we used to judge the extent of gelation–contraction were the time required

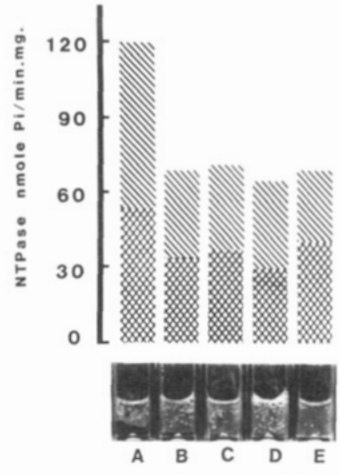

FIGURE 4: Nucleotide specificity of the microtubule-stimulated ATPase and microtubule gelation–contraction. Gelation–contraction was determined after the addition of 2 mM of each nucleotide to mixtures of SCAPs and tubulin in standard gelation–contraction buffer (see Materials and Methods) with 1 mM GTP. The contracted gels were photographed 60 min after the addition of nucleotide. Contraction of the microtubule gel is clearly visible in (A), while limited contraction is barely visible in (B), (C), and (D). Microtubule-stimulated ATPase activity was measured by polymerizing purified microtubules in standard buffer (see Materials and Methods) with 10 μM taxol. All nucleotides tested gave a microtubule-stimulated ATPase activity less than 50% of that obtained with ATP and yielded weak, or undetectable, microtubule gelation–contraction. (A) ATP; (B) GTP; (C) CTP; (D) ITP; (E) UTP. Single-hatched bars indicate microtubule-stimulated ATPase activity. Cross-hatched bars indicate the activity in the absence of microtubules.

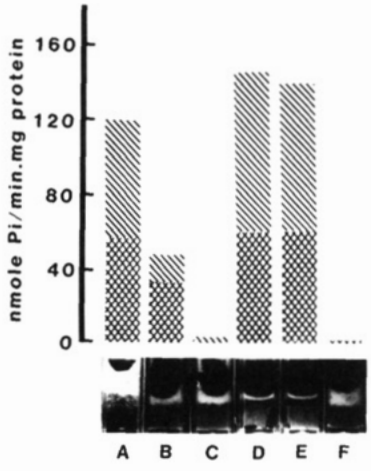

FIGURE 5: Ionic effects on microtubule-stimulated ATPase activity and gelation–contraction. Microtubule-stimulated SCAP ATPase activity correlates with the occurrence of microtubule gelation-contraction. The experiment indicates that the SCAP ATPase requires magnesium and is inhibited by calcium. Contraction is visible in cuvettes A, D, and E. (A) Control (standard gelation–contraction buffer); (B) 1 mM $CaCl_2$; (C) no $MgCl_2$; (D) 5 mM $MgCl_2$; (E) 8 mM $MgCl_2$; (F) 0.3 M KCl–2 mM EDTA. Single-hatched bars indicate microtubule-stimulated ATPase activity. Cross-hatched bars indicate the activity in the absence of microtubules.

to complete contraction and the final volume of the contracted gel. We considered inhibition to have occurred when microtubule polymerization appeared normal (routinely checked by electron microscope) but no contraction occurred or contraction was delayed and less contraction occurred compared to the experimental control. Using these criteria in examining microtubule gelation–contraction and the ATPase activity of the same SCAP preparation, we have found that the microtubule-stimulated ATPase activity correlates well with the occurrence of microtubule gelation–contraction (Figures 4–6).

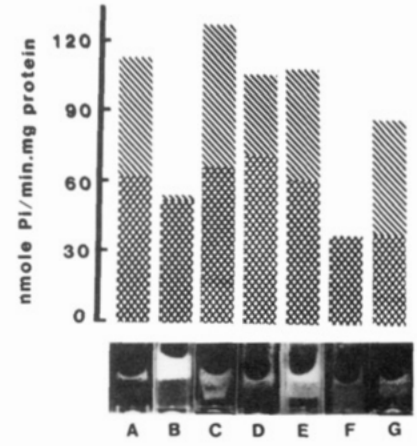

FIGURE 6: Effects of various agents on microtubule-stimulated ATPase activity and gelation–contraction. No DTT was present when vanadate and NEM were used. Absence of DTT did not affect ATPase activity and microtubule gelation–contraction of experimental controls. The ATP concentration was 0.1 mM in the presence of EHNA. Agents that inhibit microtubule-stimulated ATPase activity also inhibit microtubule gelation–contration. (A) Control (standard gelation–contraction buffer); (B) 5 μM vanadate; (C) 25 μg/mL oligomycin; (D) 0.2 mM ouabain; (E) 2 mM sodium azide; (F) 1 mM NEM; (G) 0.5 mM EHNA. Single-hatched bars indicate microtubule-stimulated ATPase activity. Cross-hatched bars indicate the activity in the absence of microtubules.

The nucleotide specificity of the microtubule-stimulated SCAP NTPase and microtubule gelation–contraction are shown in Figure 4. All other nucleotides tested (GTP, CTP, ITP, and UTP) give delayed gelation–contraction and a microtubule-stimulated NTPase activity less than 50% of that obtained with ATP. Limited gelation–contraction (barely visible in Figure 4) was observed in GTP, CTP, and ITP.

The microtubule-stimulated ATPase is inhibited by 1 mM calcium, 0.3 M KCl–2 mM EDTA, 5 μM vanadate, and 1 mM NEM (all of these were added to the standard contraction buffer which contains 3 mM $MgCl_2$ and 1 mM EGTA). Microtubule gelation–contraction is inhibited by these same agents (Figures 5 and 6). Both ATPase activity and microtubule gelation–contraction are unaffected by high concentration of magnesium (up to 8 mM) or by the ATPase-inhibiting drugs ouabain (0.2 mM), oligomycin (25 μg/mL), and sodium azide (2 mM) (Figures 5 and 6). To test the effects of EHNA, a competitive inhibitor of dynein (Penningroth et al., 1982), the ATP concentration was reduced to 0.1 mM, and 0.5 mM EHNA was added. No inhibition of either ATPase or gelation–contraction was observed with EHNA under these conditions (Figure 5). Magnesium is required for both ATPase activity and microtubule gelation–contraction (Figure 5).

SCAP ATPase Is Not Extracted by Nonionic Detergent. Because of possible contamination by vesicles or other membrane components, which may contain ATPases, we examined the possibility that the SCAP ATPase could be membrane associated. We were particularly concerned because of reports that purified "vesicle" fractions from squid axoplasm may contain tubulin and neurofilment proteins (Gilbert & Sloboda, 1986; Pratt, 1986). However, we rarely observe identifiable vesicles by negative stain electron microscopy of SCAPs. When SCAPs were loaded at the bottom of 15–30% sucrose gradients and centrifuged as described by Pratt (1986), no protein floated above the pellet. Pratt reported the presence of globular elements of various sizes in her preparations, and it is possible that these were SCAPs. This could account for the presence of cytoskeletal proteins in the squid vesicle fraction.

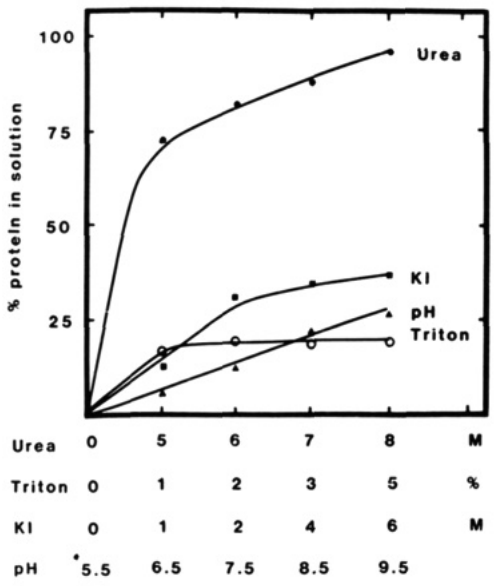

FIGURE 7: Solubility of SCAPs in various solvents. SCAPs were suspended in each indicated solution and dialyzed in the same solution for 15 h. The protein concentration was measured before and after an airfuge centrifugation (about 100000*g* for 10 min) after dialysis. The result shows that only about 20% of the protein was dissolved in 5% Triton, 25% dissolved in pH 9.5 Tris buffer, and 30% dissolved in 6 M KI. (*) The protein had precipitated after overnight dialysis in the pH 5.5 buffer.

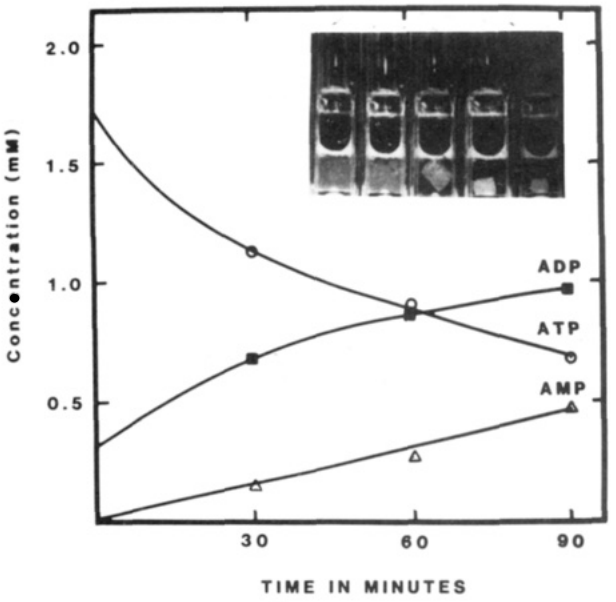

FIGURE 8: Changes in ATP, ADP, and AMP concentrations during microtubule gelation–contraction. (Insert) The photographs show the extent of gelation–contraction at (from left) 0, 30, 60, 90, and 120 min after ATP addition. The increase in AMP concentration suggests the existence of adenylate kinase in the SCAP preparation. (△) AMP; (■) ADP; (○) ATP.

The nonionic detergent Triton X-100 was used in attempts to extract the SCAP ATPase. The composition of SCAPs is not significantly changed by Triton extraction. SCAPs can still induce microtubule gelation–contraction after Triton extraction, and a microtubule-stimulated ATPase activity remains after Triton treatment. However, the Triton-treated SCAPs show about a 75% decrease in ATPase activity and give delayed gelation–contraction as compared to experimental controls (incubated with the same microtubule preparation). This difference may be due to inhibition by remaining Triton residues in the Triton-treated SCAPs, rather than to the loss of proteins or other components from SCAPs after Triton treatment. The ATPase activity of SCAPs and their ability to cause microtubule gelation–contraction showed roughly the same degree of inhibition when 0.1% Triton was present during incubation as when SCAPs were first extracted with 1% Triton. In both cases no ATPase activity is observed in the SCAP-free supernatant. This suggests that the presence of Triton, rather than extraction of protein, was sufficient to cause inhibition of SCAP activity.

The solubility of SCAPs in Triton and in other solvents was investigated. Isolated SCAPs were suspended in Tris buffer containing 1–5% Triton, 2–6 M KI, or 5–8 M urea or Tris buffer alone with a pH range of 5.5–9.5. The suspended SCAPs were then dialyzed against the same buffer for 15 h. The protein concentrations of the dialyzed SCAPs were measured before centrifugation at 100000*g* for 10 min. The protein concentrations of the supernatants were measured after centrifugation, and the percentage of dissolved protein was calculated (Figure 7). Only 20% of the total protein was dissolved in 5% Triton, 36% dissolved in 6 M KI, and 30% dissolved in Tris buffer of pH 9.5. Even in 5 M urea the SCAPs were not rapidly solubilized. Overnight dialysis was required to get 70% of the protein in solution. The ATPase activity recovered in the supernantant from these solubilization experiments was insignificant.

Nucleotide Changes during Microtubule Gelation–Contraction. To understand the nucleotide changes that occur during the process of microtubule gelation–contraction, which normally occurs in the presence of GTP, we examined the total nucleotide content of contracting microtubule gels by HPLC analysis. Parallel samples were incubated at 37 °C in gelation–contraction buffer; aliquots of one sample were removed and subjected to nucleotide extraction (see Materials and Methods for details) at designated times. The remainder of the sample was allowed to contract normally. HPLC analysis revealed that a steady increase in AMP and ADP concentrations occurs following ATP addition (Figure 8). The concentration of ATP drops and reaches about 25% of its initial value after 90-min incubation. Similar, but more complex, changes were observed in the content of guanine nucleotides (data not shown). As expected, GTP is hydrolyzed during microtubule assembly, but the content of GTP increases transiently following ATP addition. These observations suggest the presence of two enzymes in our preparations in addition to the ATPase: nucleosidediphosphate kinase, which uses ATP and GDP to produce GTP and ADP, and adenylate kinase, which converts two ADPs into one ATP and one AMP. Both of these enzymes have been previously reported to be associated with isolated microtubule proteins (Jacobs & Huitorel, 1979; Gaskin & Cantor, 1974; Weisenberg et al., 1976).

Gels may continue to contract up to 2 h after ATP addition while ATP hydrolysis had dropped to less than 25% of the initial rate. The ATPase activity during normal microtubule gelation–contraction conditions (about 40 nmol min^{-1} mg^{-1}) is low compared to the V_{max} of the microtubule-stimulated SCAP ATPase activity (about 110 nmol min^{-1} mg^{-1}). Microtubule gelation–contraction generally takes longer than the time required for the complete hydrolysis of the ATP present, assuming that the SCAP ATPase acts at a rate close to its V_{max}. The low ATPase activity during microtubule gelation–contraction may be due to inhibition by guanine nucleotides present and the accumulation of the ADP and AMP produced during the process of microtubule gelation–contraction. The calculated number of moles of inorganic phosphate released (about 3.0 μmol/mL) during the course of microtubule gelation–contraction is roughly equal to that of ATP and GTP

consumed plus AMP and GMP produced (about 2.8 μmol/mL).

Discussion

Our results demonstrate that a microtubule-stimulated ATPase activity is associated with a particulate fraction that is responsible for microtubule gelation–contraction in vitro. These particles have been identified, on the basis of similarities in composition, rate of movement, and solubility, as slow component a particulates or SCAPs (Weisenberg et al., 1987).

The microtubule-stimulated ATPase activity of SCAPs closely correlates with the occurrence of microtubule gelation–contraction. Both are inhibited by calcium, vanadate, and NEM, and both require magnesium and display similar nucleotide specificity. We have never observed gelation–contraction in the absence of a microtubule-stimulated ATPase activity. Nor have we observed normal levels of microtubule-stimulated ATPase activity without gelation–contraction activity. It is likely from these results that the SCAP microtubule-stimulated ATPase is the mechanochemical transducer responsible for microtubule gelation–contraction.

The SCAP microtubule-stimulated ATPase appears to differ from other known microtubule-stimulated ATPases. Although inhibition by micromolar vanadate suggests a similarity to dynein (Kobayashi et al., 1978), its nucleotide specificity, the inhibition of its activity by Triton X-100, and its insensitivity to EHNA distinguish the SCAP ATPase from dynein (Gibbons et al., 1976; Asai & Wilson, 1985; Penningroth et al., 1982). Inhibition of the SCAP microtubule-stimulated ATPase by EDTA and its requirement for magnesium indicate that the SCAP ATPase is a Mg-ATPase. It is unlikely that the SCAP ATPase is the proposed fast anterograde protein kinesin (Vale et al., 1985; Brady, 1985). The ATPase activity of kinesin is inhibited by 2–3 mM $MgCl_2$ and stimulated by millimolar $CaCl_2$ (Kuznetsov & Gelfand, 1986; Cohn et al., 1987). Unlike kinesin, the K_m of the SCAP ATPase is not altered by the addition of microtubules. This probably indicates different mechanisms of microtubule stimulation for the two ATPases. The addition of microtubule stimulation for the two ATPases. The addition of microtubules to SCAPs does not seem to change the affinity of enzyme–substrate binding; instead, it increases the rate of ATP hydrolysis by the enzyme. In the case of kinesin, a decrease of K_m was observed when the ATPase was measured in the presence of microtubules (Kuznetsov & Gelfand, 1986).

The SCAP ATPase displays some similarities to the soluble ATPase isolated from bovine spinal nerve roots by Hollenbeck and Chapman (1986) which displays ATP-sensitive binding to microtubules. Both ATPases are inhibited by calcium and are insensitive to EHNA. SDS–PAGE of SCAPs shows a high molecular weight protein of about 300K, similar in size to the ATPase identified by Hollenbeck and Chapman. The two enzymes differ in solubility, but it is possible that the SCAP ATPase exists in soluble form in the brain and spinal nerve. However, we have not observed ATP-sensitive binding of SCAPs to microtubules (Weisenberg et al., 1986). The SCAP ATPase also appears to differ, in solubility and sensitivity to EHNA, from MAP 1C, which has recently been proposed to be the fast retrograde transport motor (Paschal & Vallee, 1987; Paschal et al., 1986).

A particulate ATPase has previously been reported to be associated with microtubules by Murphy et al. (1983). The protein composition of the microtubule-associated particulates isolated by Murphy et al. is similar to that of SCAPs. An ATPase of 50K in molecular weight and with similarities to mitochondrial ATPase was isolated, but in very low yield, from these particulates. A protein similar in molecular weight to the 50K ATPase is present in our preparation. Protein blots using a polyclonal antibody kindly provided by Dr. Murphy confirmed the presence of this protein. However, neither the SCAP ATPase activity nor gelation–contraction was inhibited by the presence of the antibody (data not shown). Although it is possible that the binding of the antibody to the 50K protein does not affect its ATPase activity or its ability to promote gelation–contraction, the insensitivity of the SCAP microtubule-stimulated ATPase to azide and oligomycin and its stability at low temperatures make it different from both the 50K ATPase isolated by Murphy et al. and mitochondrial ATPase (Huiying & Slayter, 1961; Vigers & Ziegler, 1968).

It is not yet known for certain if any of the ATPases discussed above are present in SCAPs. Even if it could be shown that a particular ATPase were present, it could be part of the "cargo" of slow axonal transport rather than the motor. Unless the ATPase can be solubilized to allow its isolation and characterization, it will be difficult to identify it for certain. Unfortunately, solubilization has not yet been achieved. The amount of protein in SCAPs represented by the major species, including tubulin, spectrin, the neurofilament triplet, and MAP2, makes up about 70% of the total SCAP protein. No single unidentified component makes up more than 20% of the total protein remaining. This indicates that the specific activity of the SCAP ATPase, if purified, would be much higher than is reported in this paper, and comparable to the activity of other mechanochemical enzymes.

Recently, the presence of an ATPase that interacts with microtubules has been reported in isolated vesicles (Gilbert & Sloboda, 1986; Pratt, 1986). These preparations contained tubulin and neurofilament proteins and "globular masses of various sizes" (Pratt, 1986). We were concerned that our activity could be vesicle associated. However, the SCAP ATPase activity does not seem to be membrane associated because it does not float on sucrose gradients and cannot be removed from the SCAP protein complexes by nonionic detergent treatment. SDS–PAGE shows the same protein composition in both 1% Triton treated and non Triton treated SCAPs. Although Triton-treated SCAPs have lower ATPase activity and give slower contraction, the ATPase activity is still stimulated by purified microtubules. The decreases in SCAP ATPase activity and in their ability to induce gelation–contraction after Triton extraction are probably due to inhibition by Triton residues left in the Triton-treated SCAPs, rather than to the removal of material from SCAPs. Our results indicate that ATPase activity and microtubule gelation–contraction of SCAPs undergo the same degree of inhibition when only 0.1% Triton is present. After exposure to either 0.1 or 1% Triton, no activity was detected in the soluble fraction.

Finally, the close correlation of the SCAP ATPase activity to the occurrence of microtubule gelation–contraction shown by the results in this paper suggests that the SCAP ATPase is directly responsible for microtubule gelation–contraction and the particle movements that occur during gelation–contraction. On the basis of the similarities we have noted between microtubule gelation–contraction and slow axonal transport (Weisenberg et al., 1987), it is reasonable to postulate that the microtubule-stimulated ATPase in SCAPs is involved in the movement-generating mechanism of component a of slow axonal transport.

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High-Resolution Analysis of a Histone H1 Binding Site in a Rat Albumin Gene†

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ABSTRACT: Interaction of rat liver histone H1 fraction with the 5′-end of the rat serum albumin gene was localized within a 346 base pair (bp) restriction fragment. Sequence analysis of the fragment showed the fragment was 72 mol % adenosine–thymidine, which is significantly greater than the mole percent adenosine–thymidine composition of the rat genome. Gel retardation assays of the histone H1–DNA interaction indicate the complex formed behaves as previously characterized H1–DNA and shows a high-affinity H1 binding site within the enriched albumin restriction site. Deoxyribonuclease I (DNase I) protection assays on the H1 binding site define three protected regions only on the template strand of the DNA fragment. The three sites lie 55 and 110 bp apart (165 bp between the first and third binding site) with a consensus binding sequence of 5′-GA-ATA-CTGGCTT-C-TT-CTA-G-3′. The sequences between the protected DNA regions are highly enriched in adenosine–thymidine bases (79.3 and 86 mol % adenosine–thymidine, respectively). The functional significance is not understood.

Histone H1 has been shown to interact with high preference toward several restriction fragments of the rat albumin gene (Berent & Sevall, 1984). Previous observations of lysine-rich histone–DNA interactions have shown that H1 histones differ in binding with adenosine–thymidine-rich or guanosine–cytosine-rich DNA (Sponar & Sormova, 1972; Hwan et al., 1975). Subfractions of histone H1 differ in their interaction with a given DNA (Welch & Cole, 1979; Corbett et al., 1980), and H1 binds more strongly to supercoiled than to linear DNA (Vogel & Singer, 1976).

The functional role of histone H1 is to maintain the higher order structure of chromatin (Allan et al., 1980; Thoma & Koller, 1981) in part by defining the repeat length between

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