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Drosophila kinesin: Characterization of microtubule motility and ATPase

(cell motility/microtubule-activated ATPase)

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ABSTRACT **Preparations of kinesin, a microtubule-based force-producing protein, have been isolated from *Drosophila melanogaster* embryos by incubation of microtubules with a nonhydrolyzable ATP analogue and gel filtration of proteins released from the microtubules by ATP. These preparations induced MgATP-dependent microtubule gliding *in vitro* with a K_m for MgATP of 44 μM and a V_{max} for gliding of 0.9 μm/sec. Samples of *Drosophila* proteins that were active in motility assays possessed an average ATPase activity in solution of 17 nmol/min per mg that increased to an average of 106 nmol/min per mg in the presence of microtubules. The major polypeptides that copurified with these activities showed relative molecular masses of 115 kDa and 58 kDa. An antiserum raised against the 115-kDa polypeptide also recognized the 110-kDa component of squid kinesin preparations and the 130-kDa component of sea urchin kinesin preparations.**

Many examples of subcellular motility such as pronuclear migration, mitosis, saltatory organelle movements, and some components of axonal transport depend on microtubules (1, 2). How microtubules participate in these processes is not well understood but there is increasing evidence that they can serve as tracks upon which force-producing "motor molecules" can move (3, 4). Given the importance of cytoplasmic motility to the basic functions of eukaryotic cells and to the higher order functions of embryonic development, the interactions between microtubules, force-producing proteins, and motile organelles are of great interest.

Vale *et al.* (5) have identified kinesin, a cytoplasmic molecule able to induce microtubule-based motility *in vitro* in the presence of ATP. Preparations containing high levels of kinesin activity have been isolated from squid axoplasm and optic lobes (5), bovine brain (5), and sea urchin eggs (6). It has been presumed that kinesin-induced microtubule motility is driven by the hydrolysis of ATP since motility is halted by the addition of agents that interfere with ATP hydrolysis (5, 7). An ATPase activity for kinesin-like proteins from chicken neural tissue (8) and a microtubule-activated ATPase activity for kinesin preparations from bovine neural tissue (9) have been reported. More recently, Cohn *et al.* (10) have shown that sea urchin egg kinesin preparations possess a microtubule-activated ATPase activity, and they have presented evidence that the ATPase activity is coupled to kinesin-induced microtubule motility.

The quaternary composition of the functional kinesin molecule has not been precisely defined. Stoichiometry estimations by gel densitometry of the polypeptides in highly purified kinesin preparations suggest that the kinesin molecule is a complex of two or three different polypeptides (5), but conclusive experiments showing the functional contribution of each of these components have not been reported. In the work described here an effort is made to maintain a distinction between protein preparations that contain kinesin and the actual kinesin molecule.

The properties displayed by kinesin preparations *in vitro* clearly suggest that the kinesin molecule is involved in some aspects of microtubule-based motility *in vivo*. Consistent with this hypothesis, antibodies that recognize the major component of kinesin preparations bind to mitotic spindles of sea urchin embryos (6, 11) and various tissue culture cells (12). Thus, kinesin may participate in some of the motility events involved in mitosis. The presence of kinesin in axonal and neural tissues that are essentially nonmitotic implies that kinesin also participates in interphase transport phenomena such as fast axonal transport (4).

Classical and molecular genetic approaches may prove useful in defining the cellular and developmental functions of kinesin. Toward that end, we have isolated a kinesin preparation from *Drosophila melanogaster*, studied its motility characteristics and ATPase activity, and raised an antiserum against its major polypeptide. In another paper Yang *et al.* (13) report on the use of this antibody to isolate and characterize a *Drosophila* gene that encodes the major polypeptide. The microtubule-binding properties of the *in vitro* synthesized gene product suggest that the major polypeptide is an important part of the functional kinesin molecule possessing microtubule- and ATP-binding sites (13).

MATERIALS AND METHODS

Tissue Collection and Preparation. Schneider's tissue culture cells, line S2/M3, were cultured as described by Lindquist *et al.* (14) in medium developed by Shields *et al.* (15). Cells were collected by centrifugation and washed twice in pH 7.2 phosphate-buffered saline (PBS; 140 mM NaCl/10 mM Na_2HPO_4, pH 7.2). *Drosophila* adults were grown at 25°C with standard cornmeal/molasses/agar medium. Embryos were collected from egg-lay trays 24 hr after placement in population cages. They were rinsed first on graded copper screens with room temperature water, rinsed repeatedly on a nitex filter with 0.4% NaCl/0.03% Triton X-100, and then dechorionated with 50% commercial bleach for 2 min. Dechorionated embryos were rinsed extensively with the NaCl/Triton solution prior to disruption.

Protein Preparation. For preparation of *Drosophila* microtubule proteins, collected tissues were rinsed once in microtubule assembly buffer (0.1 M Pipes, pH 6.9/0.9 M glycerol/5 mM EGTA/0.5 mM EDTA/2.5 mM $MgSO_4$ and protease inhibitors: 0.1 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin per ml, 1 μg of pepstatin per ml, 1 μg of

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Abbreviation: p[NH]ppA, 5'-adenylyl imidodiphosphate.
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aprotinin per ml, and 1 mg of *p*-tosyl-L-arginine methyl ester per ml). The tissues were resuspended in 1 vol of assembly buffer and disrupted at 4°C in a Wheaton glass homogenizer. The homogenate was then clarified by centrifugation at 15,000 × *g* for 20 min; this was followed by centrifugation at 30,000 × *g* for 30 min. The supernatant was used immediately or stored at −70°C for up to 6 months.

Microtubule polymerization was induced in tissue homogenates by addition of GTP to 0.3 mM and taxol to 20 μM with gentle agitation at room temperature for 20 min. The microtubule suspensions were incubated 15 min more after addition of either a nonhydrolyzable ATP analogue, 5′-adenylyl imidodiphosphate (p[NH]ppA), to 2.5 mM or $MgSO_4$ and ATP (MgATP) to 2.5 mM each. Microtubules were then sedimented through a sucrose cushion (20% sucrose/10 μM taxol/0.3 mM GTP in assembly buffer) at 23,000 × *g* for 50 min. Pellets were resuspended in 10% of the original homogenate volume of assembly buffer with 10 μM taxol/0.3 mM GTP/75 mM NaCl and then sedimented at 23,000 × *g* for 30 min. Pellets from this salt wash were resuspended in 2.5% of the original homogenate volume of extraction buffer with 20 μM taxol/0.3 mM GTP/150 mM NaCl/10 mM MgATP. The suspension was then clarified by sedimentation at 120,000 × *g* for 20 min. The supernatant (referred to as "ATP extract") was stored at −70°C until used.

Gel-Filtration Chromatography. The ATP extract was fractionated at 4°C by passage over a 2.5 × 35 cm Bio-Gel A5M column that had been preequilibrated with assembly buffer. The flow rate was maintained at ≈15 ml/hr. Partition coefficients were estimated by comparison to the migration of blue dextran 2000 (excluded) and phenol red (included). Pooled column fractions were sometimes concentrated by means of a Centricon filtration unit (Amicon).

Electrophoresis and Electrophoretic Transfer Blots. The polypeptides in various tissue fractions were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis as described by Laemmli (16) and stained with Coomassie brilliant blue. Unstained gels were electrophoretically transferred to nitrocellulose filters as described by Towbin *et al.* (17). Filters were rinsed and then probed with rabbit antiserum as described by Goldstein *et al.* (18). An alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (Zymed) was used at a dilution of 1:5000. Phosphatase activity was localized by development in 150 mM Tris·HCl (pH 8.2) with 0.006% 5-bromo-4-chloro-3-indolyl phosphate and 0.006% nitroblue tetrazolium.

Microtubule Motility *in Vitro*. Protein fractions were tested for the ability to induce microtubule gliding by the method of Vale *et al.* (19) as modified by Porter *et al.* (7) and Cohn *et al.* (10). Briefly, 15 μl of the protein solution to be tested was applied as a drop to the surface of a glass coverslip in a moist chamber and incubated for 20–30 min to allow adsorption of proteins to the glass. Then 2 μl of a 0.2 mg/ml suspension of taxol-stabilized microtubules assembled from phosphocellulose-purified tubulin (20) or demembranated, salt-stripped, sea urchin flagellar axonemes (7) was added. Two microliters of 25 mM MgATP was added and the coverslip was inverted on a glass slide. Microtubules or axonemes were observed by using video-enhanced differential interference-contrast microscopy (21, 22). The polarity of movement was judged by using axonemes as described by Porter *et al.* (7). Velocities of microtubule movement used in estimating V_{max} and a K_m for ATP were measured as described by Cohn *et al.* (10).

ATPase Assays. Protein fractions were tested for the ability to hydrolyze ATP as described by Cohn *et al.* (10). Briefly, 300 μl of test solution was preincubated for 5–10 min with 80 μl of assembly buffer containing 35 μM taxol and 0.5 mM GTP. Then 20 μl of a solution of 50 mM MgATP with 150 nM [γ-^{32}P]ATP (10 mCi/ml; 1 Ci = 37 GBq) in assembly buffer was added. Aliquots were removed at timed intervals and assayed for the amount of free ^{32}P in a scintillation counter. The effect of microtubules on rates of phosphate release was determined by including taxol-stabilized, phosphocellulose-purified microtubules in the preincubation mixture at 2.1 mg/ml.

Production of Rabbit Antiserum. New Zealand White rabbits were immunized with 100–200 μg of the 115-kDa polypeptide of ATP extract cut from Coomassie-stained $NaDodSO_4$/polyacrylamide gels. Gel bands were macerated between two glass plates and mixed with adjuvant for the primary immunization and for booster injections at 4-week intervals. Sera were collected before immunization and after two booster injections.

RESULTS

Isolation of *Drosophila* Microtubule Proteins. Fig. 1 shows samples taken from various steps during the isolation of putative kinesin proteins. Microtubule pellets prepared from homogenates of 0- to 24-hr embryos in the presence of p[NH]ppA contained a polypeptide of 115 kDa apparent molecular mass (lane 4). This 115-kDa protein was not seen when ATP was substituted for p[NH]ppA (lane 2). p[NH]ppA-treated microtubules retained the 115-kDa protein when resuspended and pelleted in 75 mM NaCl (lane 6). It was released along with a 58-kDa protein and other, less prominent proteins when the salt-washed microtubules were resuspended and pelleted in 10 mM MgATP/150 mM NaCl (lanes 7 and 8).

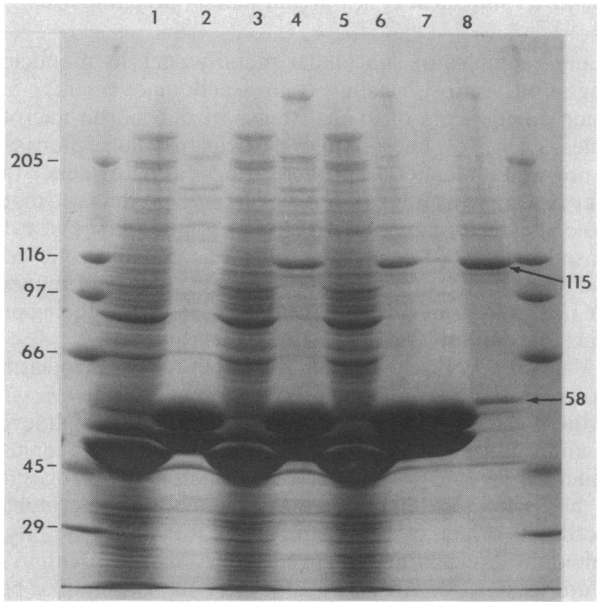

FIG. 1. Preparation of *Drosophila* embryo proteins that bind to microtubules in the presence of p[NH]ppA. Shown is a 5–11% polyacrylamide gradient gel stained with Coomassie blue. Molecular masses are given in kDa. Lane 1, clarified homogenate of 0- to 24-hr embryos; lane 2, pellet of taxol-induced microtubules from clarified homogenate that were incubated in 2.5 mM MgATP; lane 3, supernatant from the pellet shown in lane 2; lane 4, pellet of taxol-induced microtubules that were incubated with 2.5 mM p[NH]ppA; lane 5, supernatant from pellet shown in lane 4; lane 6, pellet of a 75 mM NaCl wash of the p[NH]ppA microtubule fraction; lane 7, pellet of the salt-washed microtubules after extraction in 150 mM NaCl/10 mM MgATP; lane 8, supernatant of MgATP extraction (ATP extract). Note the presence of 115-kDa and 58-kDa polypeptides in the supernatant of the ATP extract. Relative to the concentrations of microtubule proteins in clarified homogenate (lane 1), the concentrations seen in lanes 2, 4, 6, and 7 are 20-fold greater and in lane 8 are 40-fold greater.

Kinesin Characteristics of Polypeptides in the ATP Extract. An *in vitro* microtubule motility assay was used to test the *Drosophila* proteins in the ATP extract. When adsorbed to a glass coverslip, the putative kinesin preparation induced the gliding of microtubules (taxol-polymerized, phosphocellulose-purified tubulin) and of salt-stripped flagellar axonemes over the glass surface. The polarity of movement was determined by following the movements of axonemes that had been used as seeds for the growth of microtubules. Axonemes treated in this way nucleate the growth of long microtubules from one end (the "plus end") and short microtubules from the other (the "minus end"). Gliding of the axonemes proceeded with the minus ends leading, as has been reported for kinesin preparations from other organisms (7, 23, 24).

The motility factor was further purified and characterized by passage of ATP extract through a gel-filtration column. The polypeptide compositions of the collected fractions are shown in Fig. 2*A*. Fig. 2*B* shows that the motility-inducing ability copurified with the 115-kDa and 58-kDa polypeptides, suggesting the participation of one or both of these proteins in microtubule movement. The possibility that other, less prominent polypeptides were also involved was not eliminated. The 115-kDa and 58-kDa proteins coeluted from the column with an average partition coefficient of 0.39, suggesting that they were associated as macromolecular complexes. The similarities between this polypeptide profile and elution behavior and those of squid and sea urchin egg kinesin preparations (5, 6) suggest that the column fractions contained *Drosophila* kinesin.

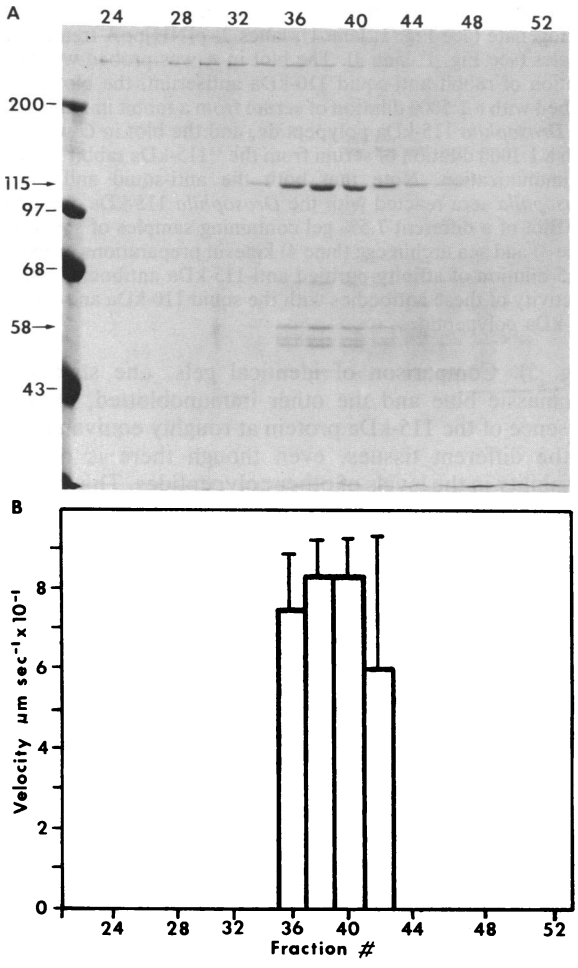

FIG. 2. Gel filtration of ATP extract and motility assays of the fractions. ATP extract (Fig. 1, lane 8) was passed through a Bio-Gel A5M column. (*A*) Polypeptide profiles of the even-numbered fractions that were collected. Molecular masses are given in kDa. (*B*) Rates of microtubule gliding induced by the even-numbered fractions. Each bar represents the mean velocity of 10 randomly selected microtubules. Note that the peak motility activity copurifies with the 115-kDa and 58-kDa polypeptides.

***Drosophila* Kinesin Motility: Inhibitors and Kinetics.** Recent work strongly suggests that the energy for kinesin-induced microtubule gliding is produced by the hydrolysis of MgATP (10). The most active fractions from gel-filtration chromatography were pooled and tested in the motility assay under various solution conditions. Microtubule gliding induced by 2.5 mM MgATP was completely inhibited by 5 units of apyrase per ml, 2.5 mM p[NH]ppA, or 2.5 mM ATP[γ-S]. Gliding induced by 5 mM MgATP was completely inhibited by 10 mM Mg^{2+}-free ATP plus 10 mM EDTA, and mean velocity was reduced to $\approx$50% by 10 mM Mg^{2+}-free ATP alone. The ATPase inhibitor sodium vanadate also inhibited gliding at concentrations >50 μM. These data suggest that the *Drosophila* motility factor drives microtubule gliding by ATP hydrolysis and that Mg^{2+} is an essential cofactor.

Fig. 3 shows a double-reciprocal plot of the effect of MgATP concentration on the mean rate of microtubule gliding. Linear regression analysis yields a K_m for ATP of 44 μM and a V_{max} for gliding of 0.9 μm/sec. Sea urchin egg kinesin fractions were tested under identical conditions, yielding a K_m of 120 μM and a V_{max} of 0.7 μm/sec (S.A.C., A. L. Ingold, and J.M.S., unpublished results). In the preparation of *Drosophila* and sea urchin egg proteins, ATP extracts were fractionated on gel-filtration columns that were free of ATP. Trailing fractions of the motility peak were discarded to avoid ATP contamination from the ATP extract.

Microtubule-Activated ATPase Activity in Preparations of *Drosophila* Kinesin. Pooled, active column fractions were tested for ATPase activity in solution. Two separate *Drosophila* preparations were each assayed in duplicate in the presence and absence of microtubules. Preparation 1 was prepared as described above and in *Materials and Methods* and then concentrated from 39 μg of protein per ml to 210 μg of protein per ml. Preparation 2 was prepared by the method of Cohn *et al.* (10) using hexokinase/glucose to deplete ATP

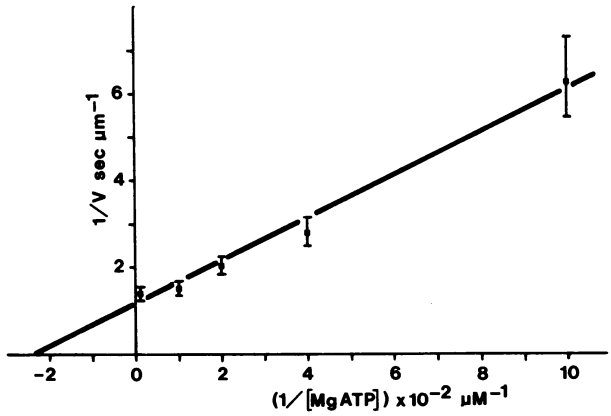

FIG. 3. Double-reciprocal plot of the effect of MgATP concentration on microtubule gliding velocity. Each point represents the mean velocity of 15 randomly selected microtubules that was induced by a pool of the peak fractions shown in Fig. 2 at one of six different MgATP concentrations. The value for the highest concentration used (1/0.77 μm/sec, 1/10,000 μM) is not shown since it is obscured by the *y* axis. The line was fit to the data by linear regression, yielding a *y* intercept of 1.14 sec/μm and an *x* intercept of -0.023 μM^{-1}. These values correspond to a V_{max} of 0.9 μm/sec and a K_m of 44 μM.

and pellet actomyosin from clarified homogenate; this was followed by p[NH]ppA to bind kinesin to microtubules. The protein concentration of this second preparation was 99 μg/ml. As shown in Table 1, the two different solutions displayed similar ATPase properties. The average specific activity for the putative kinesin fractions was 17 nmol/min per mg. This value increased ≈6-fold to an average rate of 106 nmol/min per mg in the presence of microtubules.

Immunoreactivity of the Major Components of Squid, Sea Urchin, and *Drosophila* Kinesin Preparations. The 115-kDa protein, which is the major component of the *Drosophila* fractions that induce motility, is antigenically similar to the major components of squid (110 kDa) and sea urchin (130 kDa) kinesin preparations. Fig. 4*A* shows the reactivity of a rabbit anti-squid 110-kDa serum (generously provided by Ronald D. Vale, University of California, San Francisco, and Michael P. Sheetz, Washington University, St. Louis) with clarified *Drosophila* embryo homogenate and with p[NH]ppA-treated microtubules. The antiserum recognized the *Drosophila* 115-kDa polypeptide in both samples. An anti-sea urchin 130-kDa serum also recognized the 115-kDa polypeptide, but faintly (data not shown).

An anti-*Drosophila* 115-kDa serum was produced by immunization of rabbits with the 115-kDa protein band cut from Coomassie-stained preparative gels of ATP extract. Fig. 4 *B* and *C* show the reactivity of the immune and preimmune sera with *Drosophila* proteins. The preimmune serum showed little reactivity with any polypeptides (Fig. 4*C*), whereas the immune serum reacted strongly with the 115-kDa band in the homogenate and the p[NH]ppA-treated microtubule samples (Fig. 4*B*). The immune serum also reacted with other bands in p[NH]ppA-treated microtubule samples. Some or all of these may be proteolytic fragments of the 115-kDa polypeptide.

Homology between the squid 110-kDa protein, the sea urchin 130-kDa protein, and the *Drosophila* 115-kDa protein was tested with affinity-purified anti-115-kDa antibodies. The 115-kDa band was excised from nitrocellulose blots of ATP extract and used as an affinity matrix as described by Olmsted (25). Fig. 4*D* shows that the purified antibodies react well with 110-kDa squid and 130-kDa sea urchin polypeptides.

The 115-kDa Polypeptide Is a Ubiquitous Component of *Drosophila* Tissues. Anti-115-kDa serum was used to probe blots of homogenates of third-instar larvae, adult heads, adult female bodies, adult male bodies, and Schneider's cultured cells, type S2/M3, as well as 0- to 24-hr embryos (Fig. 5). Comparison of identical gels, one stained with Coomassie blue and the other immunoblotted, shows the presence of the 115-kDa protein at roughly equivalent levels in the different tissues, even though there is noticeable variability in the levels of other polypeptides. This points out that kinesin might be prepared from any convenient *Drosophila* tissue source and provides a preliminary suggestion that kinesin functions are not developmentally restricted in *Drosophila*.

Table 1. Microtubule-activated ATPase assays

Sample	nmol/min per ml	nmol/min per mg
Microtubules	1.1	—
Kinesin preparation 1	1.9	25.7
	1.2	16.2
+ microtubules	10.3 −1.1 = 9.2	124.3
	11.3 −1.1 =10.2	137.8
Kinesin preparation 2	1.3	17.6
	0.7	9.4
+ microtubules	7.9 −1.1 = 6.8	91.9
	6.3 −1.1 = 5.2	70.3

Two *Drosophila* kinesin preparations (nos. 1 and 2) were tested in duplicate for ATPase activity in the presence and absence of taxol-stabilized, phosphocellulose-purified, bovine brain microtubules. Preparation 1 employed p[NH]ppA and column chromatography (210 μg of protein per ml). Preparation 2 employed an initial hexokinase/glucose incubation to deplete endogenous ATP and precipitate actomyosin before the p[NH]ppA and chromatography steps (99 μg of protein per ml). The bovine brain microtubules alone showed some ATPase activity. This value was subtracted from those shown by the mixtures of *Drosophila* proteins and microtubules.

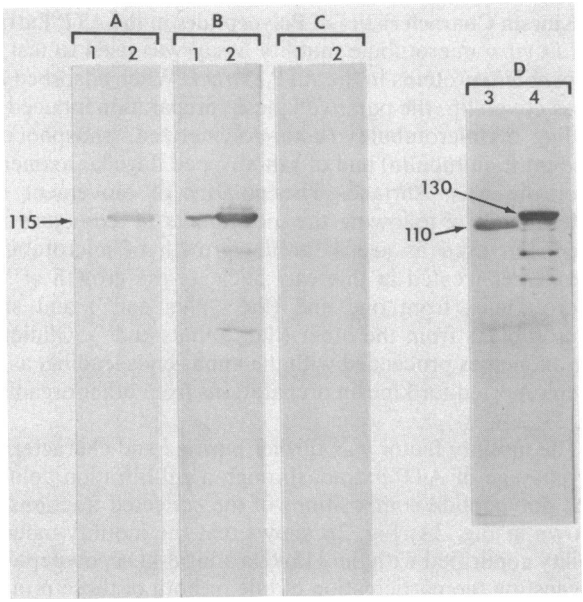

FIG. 4. Cross-reactivity of the major polypeptides of squid, sea urchin, and *Drosophila* kinesin preparations. Molecular masses are given in kDa. (*A–C*) Immunoblots of 7.5% $NaDodSO_4$/polyacrylamide gel slices cut from the same gel. Lanes 1, clarified *Drosophila* homogenate (see Fig. 1, lane 1); lanes 2, p[NH]ppA-treated microtubules (see Fig. 1, lane 4). The blot in *A* was probed with a 1:500 dilution of rabbit anti-squid 110-kDa antiserum, the blot in *B* was probed with a 1:5000 dilution of serum from a rabbit immunized with the *Drosophila* 115-kDa polypeptide, and the blot in *C* was probed with a 1:1000 dilution of serum from the "115-kDa rabbit" just prior to immunization. Note that both the anti-squid and the anti-*Drosophila* sera reacted with the *Drosophila* 115-kDa polypeptide. (*D*) Blot of a different 7.5% gel containing samples of squid axonal (lane 3) and sea urchin egg (lane 4) kinesin preparations probed with a 1:5 dilution of affinity-purified anti-115-kDa antibodies. Note the reactivity of these antibodies with the squid 110-kDa and sea urchin 130-kDa polypeptides.

DISCUSSION

We have isolated and characterized a protein fraction from *Drosophila* that contains a microtubule-based translocator. The characteristics of the microtubule gliding that is induced by this preparation are similar to those of squid and sea urchin kinesins (7). Immunoblot analysis shows that the 115-kDa component of the *Drosophila* fraction is antigenically similar to the 110-kDa component of squid and the 130-kDa component of sea urchin kinesin preparations. On the basis of these data, we suggest that the protein fraction we have isolated contains *Drosophila* kinesin and that the major 115-kDa polypeptide is a homologue of what are thought to be the major components of the squid and sea urchin kinesin molecules.

The kinetics of *Drosophila* and sea urchin kinesins in motility assays appear to be slightly different. *Drosophila* kinesin displays a lower K_m for ATP and induces slightly faster microtubule gliding. A K_m for MgATP and a V_{max} of microtubule gliding were calculated at 44 μM and 0.9

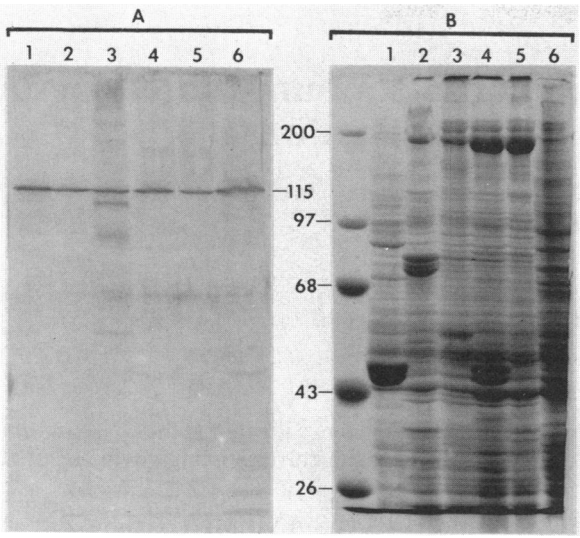

FIG. 5. Presence of the 115-kDa polypeptide in various *Drosophila* tissue sources. Two identical 7.5% $NaDodSO_4$/polyacrylamide gels were run as follows. Lanes 1, homogenates of 0- to 24-hr embryos; lanes 2, third-instar larvae; lanes 3, adult heads; lanes 4, adult female bodies; lanes 5, adult male bodies; lanes 6, Schneider's cells, line S2/M3. Molecular masses are given in kDa. (*A*) Nitrocellulose blot of one of the gels probed with anti-115-kDa serum at a dilution of 1:1000. (*B*) Second gel stained with Coomassie blue.

μm/sec, respectively, for *Drosophila* kinesin compared to values of 120 μM and 0.7 μm/sec for sea urchin egg kinesin under identical conditions (S.A.C., A. L. Ingold, and J.M.S., unpublished results). These differences may be trivial or may signal a real functional heterogeneity of kinesins from different organisms.

Our results further indicate that *Drosophila* kinesin preparations have a definite microtubule-activated MgATPase activity in solution. It initially appeared that the kinesin preparations for which motility activity had been demonstrated (squid and sea urchin proteins) did not have any substantial microtubule-activated ATPase activity, but recent work suggests otherwise. The results of Cohn *et al.* (10) show that sea urchin egg kinesin does display a microtubule-activated ATPase activity under appropriate assay conditions. We have confirmed here, by using *Drosophila* embryo proteins, that nonneuronal, motile kinesin preparations possess a microtubule-activated ATPase activity.

The quaternary composition of a functional kinesin molecule is not yet clear. That it has quaternary structure is suggested by the finding that the kinesin motility factor migrates in gel-filtration columns with an apparent molecular mass that is severalfold larger than that of any of the polypeptides present (5). Polypeptides of 110, 70, and 65 kDa comigrate with the squid motility factor with approximate stoichiometry of 2:0.5:0.5, respectively (5). Polypeptide profiles are similar in kinesin preparations from other organisms (5, 6), including *Drosophila* (described here). Although the participation of the lower molecular mass components of kinesin preparations is uncertain, antigenic homology between the squid 110-kDa protein, sea urchin 130-kDa protein, and *Drosophila* 115-kDa protein supports the hypothesis that these major components perform similar functions in generating microtubule movement.

The results of Yang *et al.* (13) also support the participation of the major kinesin component in motility. They have isolated a *Drosophila* gene that encodes the 115-kDa protein. The *in vitro*-synthesized gene product displays p[NH]ppA-dependent binding to microtubules, suggesting that two of the properties required for microtubule translocation in motility assays (microtubule binding and ATP hydrolysis) are possessed by the 115-kDa polypeptide. It is likely that a third property is necessary for kinesin function in cells—the ability to bind the organelles or structures that are destined to be translocated along microtubules. It is possible that this function also resides in the 115-kDa protein. If not, this would be a possible role for the lower molecular mass components in kinesin preparations.

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