Purification and Characterization of Kinesin from Bovine Adrenal Medulla*

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Kinesin was purified from bovine adrenal medulla. The sedimentation coefficient was 8.8 S. Sedimentation equilibrium ultracentrifugation studies showed the molecular weight of kinesin to be 300,000. The calculated axial ratio was 1:16. The Stokes radius was estimated to be 8.9 nm by gel filtration. Circular dichroism showed the α-helix content to be about 50%. Purified kinesin preparation contained a major polypeptide with a molecular weight of 120,000 and minor ones with molecular weights of 71,000, 68,000, and 65,000. Bovine adrenal kinesin had an ATPase activity which was stimulated severalfold by microtubules to a specific activity of about 0.1 µmol/min·mg. Kinesin molecules adsorbed to a glass slide promoted the movement of microtubules on the glass surface at a rate of about 0.5 µm/s.

Immunostaining of EBTr (bovine embryonic trachea fibroblast) cells and bovine adrenal chromaffin cells in interphase with an affinity-purified antibody against the major polypeptide of kinesin showed that some kinesin was located on microtubules and the rest distributed throughout the cytoplasm in a diffuse manner. EBTr cells in mitotic phase gave a staining pattern showing that kinesin was present throughout the cytoplasm with higher concentration in the region of mitotic apparatus.

Many data have been reported showing that microtubules have an important role in fast axonal transport. For instance, microtubule poisons such as colchicine and vinblastine inhibit fast axonal transport (1). It has also been reported that local chilling of axons, which destroys microtubules, makes axonally transported particles accumulate on both sides of the chilled region (2). By video-enhanced light microscopy particle movement along microtubules was visualized in dissected squid giant axons (3) and axoplasm extruded from them (4–6). A protein termed kinesin, which mediates microtubule-based transport of latex beads, was purified from axoplasm of squid giant axons, squid optic lobes, and mammalian brains (7), taking advantage of the higher and lower affinity to microtubules in the presence of AMP-PNP and ATP, respectively (8, 9). This protein induces movement of microtubules on the surface of glass coverslips in the presence of ATP (5, 10). Experiments using microtubules with definite polarity reconstituted from tubulin in the presence of centrosomes as nucleation sites revealed that particles move from the minus to plus ends of microtubules (11). Although it has been established that kinesin-mediated particle and microtubule movements are ATP-dependent, there is a controversy on the ATPase activity of kinesin. Vale et al. (7) reported that kinesin prepared from squid giant axon and optic lobes did not contain measurable ATPase activity. On the other hand, Penningroth et al. (12) and Cohn et al. (13) showed that kinesin prepared from bovine brain and sea urchin eggs, respectively, had ATPase activities with lower specific activities than those of myosin and dynein. According to the report of Kuznetsov and Gelfand (14) bovine brain kinesin has a microtubule-stimulated Mg²⁺-ATPase with a specific activity higher than that of dynein. They also reported high Ca²⁺-ATPase activity in their kinesin preparation. A high specific activity of Mg²⁺-ATPase in the presence of microtubules and that of Ca²⁺-ATPase in the absence of microtubules were found by Kachar et al. (15) in a kinesin-like translocator protein purified from Acanthamoeba. The presence of kinesin is not restricted to neural cells. For instance, kinesin was partially purified from sea urchin eggs (16–18). Chick embryo fibroblast (19) and Drosophila embry (20) have been reported to contain kinesin.

Chromaffin cells, the major constituent of adrenal medulla, are developmentally related to neurons. Secretion of catecholamines by chromaffin cells has been used as the model system for the study on exocytosis of neurotransmitters by neurons. In search of possible functions of microtubules in secretion, we have been studying MAPs in adrenal medulla and have found that it contained large amounts of MAP1 and a MAP with molecular weight of 190,000, termed 190-kDa MAP, but smaller amounts of MAP2 and τ protein (21). It is possible that kinesin has a major role in the functions of microtubules in chromaffin cells such as transport of secretory granules from the central region to the periphery of the cell. Therefore, it is important to study kinesin in adrenal medulla in detail.

In this study we developed a method for the purification of kinesin from bovine adrenal medulla in fairly large amounts using column chromatography and co-sedimentation with microtubules.

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The abbreviations used are: AMP-PNP, 5′-adenylylimidodiphosphate; MAPs, microtubule-associated proteins; Pipes, 1,4-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetaacetic acid; PMSF, phenylmethylsulfonyl fluoride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; SDS, sodium dodecyl sulfate.
crotubules. We determined the physical properties of kinesin including molecular weight, sedimentation coefficient, Stokes radius, and α-helix content. We also determined the catalytic nature of adrenal kinesin, such as ATPase activity and an ability to promote the movement of microtubules on the surface of kinesin. We also produced an affinity-purified antibody against bovine adrenal kinesin and studied the localization of this protein in culture cells.

MATERIALS AND METHODS

Preparation of Tubulin—Microtubule proteins were prepared from porcine brains according to the method of Shelanski et al. (22). Tubulin was purified by phosphocellulose column chromatography according to the method of Weingarten et al. (23).

Purification of Kinesins—Bovine adrenal glands obtained from the local slaughterhouse were chilled on ice and processed within 1 h after the death of the animals. All the procedures unless otherwise mentioned were carried out at 0-4 °C. Adrenal medulla was carefully dissected with surgical blades. 150 ml of PEM buffer (0.1 M Pipes (pH 6.8), 2 mM EGTA, 1 mM MgCl₂) containing 0.5 mM NaCl, 20 mM HEPES (pH 7.6), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, 0.2 mM diethiothreitol was added to about 100 g of fresh adrenal medulla. The homogenate was centrifuged at 100,000 g for 1 h, and the supernatant was dialyzed against 0.15 M NaCl, 20 mM HEPES (pH 7.6), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, 0.2 mM dithiothreitol. After removing the small amount of precipitates formed at 20,000 g for 30 min, the supernatant was applied to a phosphocellulose column (2.5 X 15 cm) pre-equilibrated with the above buffer. Washing the column with the same buffer solution was followed by an application of a linear gradient of NaCl (from 0.15 to 0.8 M) in 20 mM HEPES (pH 7.6), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, 0.2 mM diethiothreitol (total 400 ml) at a flow rate of 10 ml/min. Kinesin was identified by co-sedimentation with microtubules. Aliquots from several phosphocellulose fractions were dialyzed against PEM buffer. After removing precipitates by ultracentrifugation, supernatants were supplemented with 20 μM taxol, 0.5 mM GTP, 1 mg/ml tubulin, and 5 mM ATP or 5 mM AMP-PNP. Incubation of the mixtures at 37 °C for 10 min was followed by centrifugation to collect microtubules. Proteins co-sedimented with microtubules were analyzed by electrophoresis. Fractions rich in kinesin were combined and dialyzed against 50 mM NaCl, 25 mM HEPES (pH 7.4), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, 0.2 mM dithiothreitol. The dialysate was applied to a DEA-cellulose column (1.5 X 16 cm) pre-equilibrated with the above buffer. After washing the column with the same buffer solution, kinesin was eluted with a linear gradient of NaCl (from 50 mM to 0.55 M) in 25 mM HEPES (pH 7.4), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, 0.2 mM diethiothreitol, and fractions of 5 ml were collected. Fractions containing kinesin were combined, and affinity purification (0.39 g/ml) was added. Precipitates formed were collected by centrifugation and dissolved in about 3 ml of PEM buffer. After dialysis against PEM buffer the fraction was ultracentrifuged at 200,000 g for 1 h. The supernatant was supplemented with 0.5 mM GTP, 30 μM taxol, and 1 mg/ml tubulin and incubated at 37 °C for 5 min to allow the tubulin to polymerize. After the addition of 3 mM AMP-PNP the solution was further incubated at 37 °C for 10 min to allow kinesin to bind to the microtubules. The microtubule suspension was chilled on ice and layered onto PEM buffer containing 10% (w/v) sucrose, 30 μM taxol, 0.1 M AMP-PNP, and 0.55 mM GTP. Microtubules containing kinesin were collected by centrifugation at 30,000 g for 20 min, and the pellet was washed with PEM buffer containing 20 μM taxol and 0.5 mM GTP. Kinesin was eluted by suspending the microtubules in 1.5 ml of PEM buffer containing 15 mM ATP, 0.2 M NaCl, 20 μM taxol, and 0.5 mM GTP and incubating the suspension at 37 °C for 10 min. After removing the microtubules by centrifugation at 50,000 X g for 30 min, the supernatant was applied to a high performance liquid chromatography system equipped with TSK G4000SW gel filtration columns (two columns of 0.75 X 60 cm in series) pre-equilibrated with 0.4 M NaCl, 25 mM MES (pH 6.8), 1 mM EGTA, 1 mM MgCl₂, 0.2 mM PMSF, and eluted with the same buffer solution collecting fractions of 1 ml.

ATPase Assay—The ATPase activity of kinesin was assayed in a reaction mixture containing 0.1 M Pipes (pH 6.8), 2 mM EGTA, 0.3 mM ATP, 2 mM MgCl₂, and kinesin fraction with or without 0.5 mg/ml taxol in a final volume of 0.2 ml. The reaction was allowed to proceed at 37 °C for 15 min. Trichloroacetic acid, 0.1 ml of 15% (w/v), was added to terminate the enzyme reaction, and the protein precipitates were removed by centrifugation. Aliquots from the supernatants were used for inorganic phosphate assay according to the method of Amos and Dubin (24).

Motility Assay—Movement of microtubules on a kinesin-coated glass surface was measured by the method of Porter et al. (25) with some modifications. Purified kinesin was dialyzed against PEM buffer. After removing a small amount of insoluble materials in the dialysate, 5 μl of the kinesin fraction was put onto a clean slide glass and incubated at room temperature for 10 min in a moisture box to allow kinesin molecules to bind to the glass surface. 2 μl of 5 mM ATP, 0.1 M Pipes (pH 6.8), 2 mM EGTA, 5 mM MgCl₂, and 3 μl of microtubules suspended in 50 μl of tubulin and 10 μM taxol in PEM were added. After putting a coverslip on the mixture, microtubules were observed with a Nikon microscope (Optiphot) equipped with a dark field condenser and a 100-watt mercury arc lamp. Pictures of microtubules on the surface of the glass slide were taken with exposure times of 1 s using Kodak Tri-X film at an ASA value of 1,600.

Electrophoresis—SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (26) using 7.5% (w/v) polyacrylamide gels. Molecular weight markers used were rabbit skeletal muscle myosin (205,000), rabbit skeletal muscle phosphorylase b (94,000), bovine serum albumin (68,000), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (35,000), and bovine erythrocyte carbamoyl anhydride (29,000). Gels were stained with Coomassie Blue R.

Preparation of Bovine Adrenal Kinesin—EBT cells (bovine embryonic trabeca fibroblast, obtained from Japanese Cancer Research Resources Bank, Tokyo) were grown in Eagle's minimum essential medium supplemented with 10% (v/v) newborn calf serum, 0.1 mM nonessential amino acids, 0.2 mg/ml kanamycin, and 100 units/ml penicillin. Adrenal chromaffin cells were isolated from fresh bovine adrenal medulla by the collagenase digestion method (26) and plated on collagen-coated coverslips and maintained for 5 days in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 40 μg/ml gentamycin, 10 μM 5-fluorodeoxyuridine, 10 μM cytosine arabinoside, and 0.5 μM uridine.

Preparation of Anti-Kinesin Antibody—About 0.1 mg of purified kinesin was electrophoresed on an SDS-polyacrylamide slab gel without wells in the spacer gel. After brief staining of the gel with Coomassie Blue, the part of the gel containing the major component of kinesin with molecular weight of 120,000 was dissected and washed with phosphate-buffered saline. The gel pieces were homogenized with a small amount of phosphate-buffered saline and emulsified with an equal volume of Freund's complete adjuvant and injected into a rabbit. Booster injections were performed three times with 2-week intervals in the same way as the first injection except that complete adjuvant was replaced with incomplete adjuvant. Antibody against kinesin was purified from the immune rabbit serum according to the method of Talian et al. (27).

Immunoblotting—SDS extracts of culture cells were prepared according to the reported method (28). Crude extract was obtained by sonication of the cells in the presence of PEM buffer (5 ml/ml packed cells) and the ultracentrifugation of the homogenate at 100,000 X g for 1 h. Aliquots of 1 ml from the crude extract were supplemented with 0.5 mM GTP, 20 μM taxol, 0.25 mg/ml tubulin, and 3 mM ATP or 3 mM AMP-PNP. After incubation at 30 °C for 10 min, the mixtures were overlaid on sucrose cushions and centrifuged at 20,000 X g for 40 min. Supernatants and pellets were removed, and the pellets were suspended in the same volume of TEM buffer as supernatants. Samples were electrophoresed with 7.5% (w/v) polyacrylamide gels in the presence of SDS. Electrophoretic transfer of proteins was carried out by the method of Towbin et al. (29) with slight modifications that 0.1% (w/v) SDS was added to the transfer buffer and that Durapore sheets (GVHP, Millipore Corp., Bedford, MA) were substituted for nitrocellulose paper. Affinity-purified anti-kinesin antibody was applied as the first antibody, and horseradish peroxidase-conjugated goat IgG against rabbit IgG (Miles Laboratories, Naperville, IL) was used for the second antibody.

Immunofluorescence—EBT cells and bovine chromaffin cells were grown on coverslips and were labeled with affinity-purified anti-kinesin antibody and a rat monoclonal antibody against tubulin (VOL 1/34, Sera-Lab, Crawley Down, Sussex, United Kingdom) as first antibodies and fluorescein isothiocyanate-conjugated goat IgG against rabbit IgG and rhodamine isothiocyanate-conjugated goat IgG against rat IgG which had been pre-absorbed.
with formaldehyde-fixed and Triton-permeabilized EBTr cells as second antibodies.

**Estimation of Stokes Radius of Kinesin by Gel Filtration**—Purified kinesin and standard proteins, α2-macroglobulin, thyroglobulin, apo-ferritin, γ-globulin, catalase, and glyceraldehyde-3-phosphate dehydrogenase, were gel-filtered separately on TSK G4000SW columns pretreated with 0.4 M NaCl, 25 mM MES (pH 6.8), 1 mM EDTA, 1 mM MgCl₂, 0.2 mM diithiothreitol monitoring the absorption of the eluate. Stokes radii of the proteins versus (Kd)² were plotted.

**Analytical Ultracentrifugation**—A Beckman Spinco Model E analytical ultracentrifuge equipped with a photoelectric scanner and an An F rotor was used for the determination of the sedimentation coefficient and the molecular weight of bovine adrenal kinesin. Purified kinesin at a concentration of 0.21 mg/ml in 0.4 M NaCl, 25 mM MES (pH 6.8), 1 mM EGTA, 1 mM MgCl₂ was used for the measurements. Sedimentation velocity ultracentrifugations were performed at 40,000 rpm and 25°C. In the sedimentation equilibrium study, overspeeding at 15,000 rpm for 3 h was followed by centrifugation at 4,788 rpm and 26°C for 24 h. In order to check the equilibrium, the patterns of protein concentration in the cell were monitored at 18 and 24 h by a photoelectric scanner.

**Circular Dichroism Measurement**—Purified kinesin, 0.21 mg/ml, was put into a cell with a light path of 1 mm and was subjected to measurement of circular dichroism using a Jasco J-40S spectropolarimeter at room temperature.

**Protein Measurement**—Concentrations of proteins were determined according to the method of Lowry et al. (30) with bovine serum albumin as a standard.

**Chemicals**—Taxol was generously supplied by Dr. M. Sufliness, Natural Product Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. All other chemicals used were of reagent grade.

### RESULTS

**Purification of Kinesin**—A polypeptide with molecular weight of 120,000 co-sedimenting with microtubules in a AMP-PNP-dependent manner was eluted from a phosphocellulose column with 0.35–0.45 M NaCl. Judging from the molecular weight and its affinity for microtubules, this protein was considered to be kinesin. Fractions containing kinesin were combined and applied to a DEAE-cellulose column. Kinesin was eluted with 0.13–0.27 M NaCl and concentrated by ammonium sulfate precipitation. Fig. 1 demonstrates the results of affinity purification of kinesin, consisting of co-sedimentation with microtubules in the presence of AMP-PNP and elution with ATP. More than 90% of the polypeptide with molecular weight of 120,000 in the ammonium sulfate fraction co-sedimented with microtubules, and about 70% of the polypeptide was released from microtubules by ATP. The ATP eluate fraction was subjected to high performance liquid chromatography using gel filtration columns. In this step, tubulin and high molecular weight polypeptides which are considered to be MAPs were removed to yield purified kinesin fraction (Fig. 2a). About 0.5 mg of purified kinesin was routinely obtained from 150 g of adrenal medulla.

**Stokes Radius of Kinesin**—The Stokes radius of kinesin was estimated by gel filtration. The Stokes radii of the standard proteins versus (Kd)² gave a straight line. A value of 8.9 nm was obtained for bovine adrenal kinesin. This is similar to that of a kinesin-like protein purified from *Acanthamoeba* (8.5 nm) (15).

**Sedimentation Coefficient and Molecular Weight of Kinesin**—The sedimentation coefficient of kinesin was measured by analytical ultracentrifugation. Kinesin showed a single sharp sedimentation boundary with an s₂₀,ₐ of 8.8 S (two determinations gave the same value). In the sedimentation equilibrium experiments, centrifugation at 4,788 rpm for 18 and 24 h gave the same linear plots of ln A₂₅₀ versus r². A slope of 0.370 ± 0.027 was obtained. The molecular weight of kinesin was calculated to be 300,000 ± 20,000 (same values from two analyses), on the assumption of a partial specific

**ATPase Activity of Bovine Adrenal Kinesin**—The ATPase activities of the fractions obtained by gel filtration were
FIG. 2. TSK G4000SW gel filtration of the ATP eluate fraction. a, electrophoresis of gel filtration fractions. ATP-eluate fraction obtained by the affinity purification step was applied to a high performance liquid chromatography system equipped with G4000SW gel filtration columns. 20 μl from each fraction was electrophoresed. M, marker proteins. b, ATPase activity of gel filtration fractions. ATPase activity was measured in the absence or presence of 0.5 mg/ml tubulin and 20 μM taxol. 50 μl from each fraction was used for the assay.

assayed in the presence and absence of microtubules (Fig. 2b). A very low level of Mg²⁺-ATPase activity was observed in the absence of microtubules. On the other hand, higher activity was observed in the presence of microtubules. Microtubules at concentrations higher than 0.5 mg/ml gave the maximal activity of Mg²⁺-ATPase. These data show that bovine adrenal kinesin has a microtubule-dependent Mg²⁺-ATPase activity. Average of the specific activities of Mg²⁺-ATPase in four different kinesin preparations was 0.095 ± 0.005 μmol/min·mg and 0.018 ± 0.006 μmol/min·mg in the presence and absence of microtubules, respectively. When MgCl₂ was replaced with 1, 2, or 5 mM CaCl₂, ATPase activity in the
was measured by a Jasco J-40s spectropolarimeter. Dichroism of bovine adrenal kinesin at a concentration of 0.21 mg/ml in 0.4 M NaCl, 25 mM MES (pH 6.8), 1 mM EGTA, 1 mM MgCl₂ was measured by a Jasco J-40S spectropolarimeter.

absence of microtubules was 0.025 ± 0.012, 0.015 ± 0.010, and 0.041 ± 0.009 μmol/min·mg, respectively, and in the presence of microtubules 0.121 ± 0.009, 0.110 ± 0.008, and 0.157 ± 0.013 μmol/min·mg, respectively (average of two preparations). Thus, the Ca²⁺-ATPase activity of bovine adrenal kinesin was not so different from the Mg²⁺-ATPase activity, and the rate of activation of the former by microtubules was similar to that of the latter. This is quite different from the very high Ca²⁺-ATPase activities, in the absence of microtubules, reported for bovine brain kinesin (14) and the kinesin-like protein purified from Acanthamoeba (15). Almost no effect of free calcium ion in the range of less than 10⁻⁸ M to 0.2 mM was observed on the ATPase activity of bovine adrenal kinesin measured with the assay mixture containing 2 mM MgCl₂ in the presence or absence of microtubules.

Movement of Microtubules on Glass Surface Coated with Kinesin—Microtubules reconstituted from tubulin moved on a glass surface previously coated with kinesin. Motility activity in the gel filtration fractions corresponded to the ATPase activity. The percentage of moving microtubules using the peak fractions was more than 90%. The rate of movement with two different kinesin preparations was 0.52 ± 0.12 and 0.43 ± 0.15 μm/s at room temperature (average of 50 microtubules). These values are close to the rate of microtubule movement reported earlier (5, 7, 10, 18).

Immunoblotting of Proteins in Culture Cells with Anti-Kinesin Antibody—Fig. 4 shows the results of immunoblotting of the proteins in cultured cells with affinity-purified anti-kinesin antibody. Lane 8 of Fig. 4a represents the staining pattern of purified kinesin. It is clear that only the heavy chain of kinesin was stained. No staining was observed of the light chains even when a heavier loading of kinesin was carried out (data not shown). Total proteins of EBTr cells obtained by direct extraction of the cells with SDS were electrophoresed and immunoblotted. Only a single band with the same mobility as that of the heavy chain of authentic kinesin was observed (Fig. 4a, lane 9), showing that the antibody used in this study was highly specific to kinesin. It was also shown that the polypeptide reactive to the anti-kinesin antibody co-sedimented with microtubules in a AMP-PNP-dependent manner (compare lanes 13 and 14 in Fig. 4a) and that immuno-reactive band was hardly observed in microtubule-deprived supernatant obtained in the presence of AMP-PNP (Fig. 4a, lane 12). From these data it is reasonable to conclude that the protein in EBTr cells recognized by the antibody against bovine adrenal kinesin is EBTr kinesin. SDS extracts from bovine adrenal chromaffin cells showed a single band with a molecular weight of about 120,000 when immunostained with anti-kinesin antibody (Fig. 4b, lanes 4).

Immunostaining of Culture Cells with Anti-Kinesin Antibody—To localize kinesin in culture cells, indirect immunofluorescence microscopic observation was performed using anti-kinesin and anti-tubulin antibodies. Fig. 5 shows the staining pattern of EBTr cells. Interphase cells were doubly-labeled with anti-kinesin and anti-tubulin antibodies. Fig. 5b shows that anti-kinesin antibody stained interphase microtubules. However, the staining of microtubules with anti-kinesin antibody was less specific than that with anti-tubulin antibody (Fig. 5a). Diffuse staining with anti-kinesin antibody all over the cytoplasm was also observed. In the case of the study on mitotic cells, single labelings with anti-tubulin (Fig. 5c) and anti-kinesin (Fig. 5d) antibodies were carried out because the staining of the mitotic apparatus with anti-tubulin antibody was so strong that leaking of rhodamine fluorescence to the fluorescein channel could not be ignored. Fig. 5d shows that kinesin was present in the cytoplasm of mitotic cells in a diffuse manner. The staining intensity of the mitotic apparatus was higher than the other part of the cell. It is concluded that in EBTr cells a part of kinesin was bound to interphase and mitotic microtubules and the rest was diffusely distributed in the cytoplasm.

Staining of chromaffin cells with anti-tubulin and anti-kinesin antibodies gave the same pattern as EBTr cells (Fig. 6). Staining of microtubules and other cytoplasmic parts in a diffuse pattern was observed also in these cells.

**DISCUSSION**

Kuznetsov and Gelfand (14) first described the large scale purification of kinesin by column chromatography and co-sedimentation with microtubules. Our method is different from theirs in that the phosphocellulose column chromatography was applied to the first step of the purification. Because the major part of the proteins in the crude extract is removed...
into the flow-through fraction, it was easy to detect fractions containing kinesin by electrophoresis throughout the steps of the purification.

Sedimentation equilibrium ultracentrifugation revealed the molecular weight of kinesin to be 300,000. A value of 320,000 was calculated from the sedimentation coefficient and Stokes radius. It has been reported that the Stokes radius of filamentous proteins measured by the gel filtration method is a little larger than the actual value (32). Judging from this fact, the value of 300,000 is considered to be reasonable for the molecular weight of bovine adrenal kinesin. Recently Kuznetsov et al. (31) reported a value of 390,000 for the molecular weight of bovine brain kinesin measured by sedimentation equilibrium study. This value is significantly different from that of kinesin purified from bovine adrenal medulla and may be due to either the difference in the source of kinesin or the difference in the purification procedure.

Densitometric analysis of purified kinesin showed that the molar ratio of kinesin heavy chain and the sum of light chains was about 1:0.5. It can be considered that a kinesin molecule consists of heavy and light chains at a ratio of 2:1. The sum of the molecular weights of two heavy chains (120,000 × 2) and a light chain (about 70,000) is almost equal to that of native kinesin obtained by sedimentation equilibrium ultracentrifugation (300,000). Kuznetsov et al. (31) reported that bovine brain kinesin consisted of two 120-kDa heavy chains and two 62-kDa light chains. Therefore, adrenal medulla and brain probably have different types of kinesins. In fact, our preliminary experiment showed that the molecular weights of the major light chains of adrenal medulla and brain kinesins purified in the same way were clearly different from each other (adrenal medulla, 71,000; brain, 65,000). It is not clear at present why three kinds of light chains were present in the purified kinesin prepared from bovine adrenal medulla. It is possible that some of the light chain with a molecular weight of 71,000 suffered limited proteolysis by endogenous proteases in the course of the purification giving rise to the light chains with molecular weights of 68,000 and 65,000. However, it is also possible that the kinesin preparation obtained from bovine adrenal medulla was a mixture of three subtypes of kinesins consisting of a 2 to 1 complex of two common heavy chains and one of three types of light chains.
The circular dichroism data strongly suggest that kinesin molecules have a more rigid structure than MAPs. We obtained an $\alpha$-helix value of about 50% on kinesin. In contrast, it has been reported that MAP2 (33) and $\tau$ protein (34) have a very low content of $\alpha$-helix (7% for MAP2 and 12% for $\tau$ protein). This view is supported by the data of electron microscopy, that is in contrast to the very flexible long thin structures of MAPs (35–38), kinesin appears to have a molecular shape of a relatively rigid rod with branched structure at one end and a small fork at the other (39).

Our experiments showed that purified bovine adrenal kinesin has an ATPase activity which is stimulated by microtubules. The specific activity in the presence of microtubules was higher than the values reported by Penningroth et al. (12) and Cohn et al. (13) on bovine brain and sea urchin egg kinesins, respectively. However, the specific activity of bovine adrenal kinesin was much lower than that obtained by Kuznetsov and Gelfand (14) on bovine brain kinesin and that of Acanthamoeba kinesin-like protein reported by Kachar et al. (15) (4.6 and 3.4 $\mu$mol/min/mg, respectively, in the presence of microtubules). A high Ca$^{2+}$-ATPase activity in the absence of microtubules reported on bovine brain kinesin (1.6 $\mu$mol/min/mg) (14) and Acanthamoeba kinesin-like protein (1.7 $\mu$mol/min/mg) (15) was not observed in the preparation of bovine adrenal kinesin. At present we cannot explain the discrepancy of the difference in the specific activities of kinesin preparations. It may be related to the difference in the source of kinesin or the procedure of purification.

Indirect immunofluorescence studies showed that a part of kinesin in EBTr and chromaffin cells is bound to microtubules. It is plausible that secretory materials such as $\alpha$-macroglobulin. We wish to express our thanks to the Japanese Cancer Research Resources Bank, Tokyo, for providing EBTr cells.

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