Characterization of the Microtubule Movement Produced by Sea Urchin Egg Kinesin*

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We have used an in vitro assay to characterize some of the motile properties of sea urchin egg kinesin. Egg kinesin is purified via 5′-adenylyl imidodiphosphate–induced binding to taxol-assembled microtubules, extraction from the microtubules in ATP, and gel filtration chromatography (Scholey, J. M., Porter, M. E., Grissom, P. M., and McIntosh, J. R. (1985) Nature 318, 483–486). This partially purified kinesin is then adsorbed to a glass coverslip, mixed with microtubules and ATP, and viewed by video-enhanced differential interference contrast microscopy. The microtubule translocating activity of the purified egg kinesin is qualitatively similar to the analogous activity observed in crude extracts of sea urchin eggs and resembles the activity of neuronal kinesin with respect to both the maximal rate (>0.5 μm/s) and the direction of movement. Axonemes glide on a kinesin-coated coverslip toward their minus ends, and kinesin-coated beads translocate toward the plus ends of centrosome microtubules. Sea urchin egg kinesin is inhibited by high concentrations of SH reagents ([N-ethylmaleimide] > 3–5 mM), vanadate > 50 μM, and [nonhydrolyzable nucleotides] ≥ [MgATP]. The nucleotide requirement of sea urchin egg kinesin is fairly broad (ATP > GTP > ITP), and the rate of microtubule movement increases in a saturable fashion with the [ATP]. We conclude that the motile activity of egg kinesin is indistinguishable from that of neuronal kinesin. We propose that egg kinesin may be associated with microtubule-based motility in vivo.

Many types of intracellular movements are dependent upon microtubules, but the molecular mechanism of force production for these movements is not yet established. The recent application of video-enhanced light microscopy (Allen et al., 1985; Inoué, 1981) to the study of microtubule-mediated motility in squid axoplasm (Allen et al., 1985; Brady et al., 1985; Gilbert et al., 1985; Schnapp et al., 1985; Vale et al., 1985a, 1985b) has resulted in the description of at least two microtubule-based mechanochanical factors (Vale et al., 1985c, 1985d). The first factor, which has been purified and given the name kinesin, induces both gliding of microtubules on a glass surface and unidirectional translocation of beads along microtubules in an ATP-dependent fashion (Vale et al., 1985c). The motility induced by neuronal kinesin exhibits a distinct microtubule polarity; microtubules glide on a kinesin-coated coverslip towards their "minus" or slowly assembling end, and kinesin-coated beads are transported towards the "plus" or fast assembling end of microtubules at ~0.5 μm/s (Vale et al., 1985d). Since axonal microtubules are oriented with their plus ends toward the nerve terminal (Burton and Paige, 1981; Heidemann et al., 1981), kinesin is believed to act as the anterograde vesicle motor in the squid axon. A second activity with different immunological and pharmacological properties is thought to move vesicles in the retrograde direction at ~1.5 μm/s (Vale et al., 1985d), but the polypeptide composition of this factor has not yet been identified.

In previous work, we have identified a form of kinesin in sea urchin eggs by virtue of its AMPPNP-dependent association with microtubules, its microtubule translocating activity, and its immunological cross-reactivity with squid kinesin (Scholey et al., 1985). Antibodies to the 130-kDa polypeptide of egg kinesin were observed to stain the mitotic spindle of dividing embryos by immunoperoxidase light microscopy (Scholey et al., 1985), suggesting that egg kinesin may mediate some form of microtubule-based motility associated with mitosis. Mitotic motility is, however, quite complex. Chromosomes move both toward and away from the spindle poles during prometaphase and then move poleward during anaphase A. Pole to pole elongation (anaphase B) is associated with both microtubule polymerization and sliding in the interzone. Finally, granules in the spindle move in both directions throughout mitosis (Rebun, 1960, 1972). Thus, both chromosomes and granules proceed with various velocities and in both directions relative to spindle microtubule polarity. Furthermore, several studies have suggested that the various mitotic movements exhibit different nucleotide requirements and different sensitivities to enzyme inhibitors (Cande and Wolniak, 1978; Cande, 1982; Cande and MacDonald, 1985).

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1 The abbreviations used are: AMPPNP, 5′-Adenylyl imidodiphosphate; ATPγS, adenosine 5′-O-(3-thiotriophosphate); DIC, differential interference contrast; EGA, [ethylenebis(oxyethyl)enedinitri]) tetracetic acid; 8-azido-ATP, 8-azidoadenosine-5′-triphosphate; MAPs, microtubule associated proteins; SDS, sodium dodecyl sulfate; Pipes, 1,4-piperazineethanesulfonic acid; pI30, 130-kDa polypeptide.
1986; Spurck et al., 1986). One may therefore gain insight into the possible roles of kinesin in mitotic movements from a knowledge of the velocity, directionality, and pharmacology of motility induced by egg kinesin in vitro.

In this report, we characterize the motile properties of sea urchin egg kinesin. As described below, we find that kinesin activity accounts for virtually all the microtubule gliding activity observed in crude extracts of sea urchin eggs. Furthermore, we find that the motility of egg kinesin is almost indistinguishable from that of neuronal kinesin. These findings are discussed with respect to the different types of microtubule-based motility seen in vivo.

**EXPERIMENTAL PROCEDURES**

Materials—Sea urchins (S. purpuratus and L. pictus) were obtained from Marinus (Los Angeles, CA) and handled as described previously (Salmon, 1981). Taxol was the generous gift of Dr. Matthew Saffran at the National Cancer Institute. Latex beads were purchased from Polysciences, Inc. (Warrington, PA), Bio-Gel A-5m from Bio-Rad, ultrapure sucrose from Schwarz/Mann, and all other chemicals from Sigma.

Isolation of Kinesin from Sea Urchin Eggs—Kinesin was isolated from sea urchin egg extract supernatants as outlined in Fig. 1. The extracts were prepared by homogenization of packed, dejellied eggs in 2 volumes of PEME extraction buffer (0.9 M glycerol, 0.1 M Pipes, pH 6.9, 5 mM EGTA, 2.5 mM MgCl2, 0.5 mM EDTA, 1 mM diithiothreitol, 0.1 mg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, aprotinin, and pepstatin), and centrifugation at 57,000 × g for 45 min, then at 140,000 × g for 45 min, using a Beckman T50.5 or Ty265 rotor (at all steps at 0–4°C). Microtubule assembly in egg extract supernatants was induced by the addition of 1 mM GTP plus 20 μM taxol and incubation on ice for 30–45 min (Vallee and Bloom, 1983; Scholey et al., 1984). The extract was then divided into 3 aliquots with the following additions: 10 mM MgSO4, 10 mM MgATP, or 10 mM MgAMPPNP. All aliquots were kept on ice for an additional 0–30 min and then centrifuged in a Sorvall HB-4 swinging bucket rotor at 23,000 × g for 60 min at 4°C to pellet the assembled microtubules and their associated MAPs. The supernatant fractions were loaded onto 5–20% sucrose density gradients in PEME extraction buffer and centrifuged at 115,000 × g for 16 h in a Beckman SW41 rotor. The gradients were collected in 20 × 20 drop fractions and assayed for kinesin activity by video microscopy as described below.

The microtubule pellets were resuspended and pelleted in extraction buffer containing 75 mM NaCl, then extracted twice in 0.15 M NaCl, 0.1 M MgATP (ATP extract) or once in 0.5 M NaCl plus 0.1 mM ATP (high salt extract) for 10 min at 0°C, and finally centrifuged at 40,000 × g for 20 min in a Beckman Type 65 rotor (Beckman Instruments, Palo Alto, CA). The extract supernatants were concentrated when necessary in a Centricon 30 ultrafiltration apparatus (Amicon Corp., Danvers, MA). Duge (1965) had been precautions taken with 1 mg/ml bovine serum albumin or ovalbumin, and then fractionated by either Bio-Gel A-5m chromatography in PEME containing 0.1 mM ATP (Scholey et al., 1986) or by centrifugation on 5–20% sucrose density gradients. Fractions were tested for kinesin activity by video microscopy as described below. The peak of kinesin activity was determined by serial 2-fold dilutions to identify the fractions that would continue to support microtubule movement after the largest dilution.

Preparation of Microtubules and Axonemes—Bean brain tubulin was prepared by double assembly and by phosphocellulose chromatography (Williams and Detrich, 1979) and stored as droplets at −10 mg/ml in liquid N2. The droplets were later diluted to 2 mg/ml in PEME containing 1 mM GTP and 20 mM taxol and stored as 20-μl aliquots at −70°C for use in the motility assays. These aliquots were diluted 10-fold with the appropriate experimental buffer on the day of the experiment and used at a further 1:1 dilution in the final assay (see below).

Sea urchin flagellar axonemes were prepared by osmotic shock of live sperm and differential centrifugation (Bell et al., 1962). Flagellar dynein was extracted by incubation of axonemes in high salt (0.6 M NaCl) for 30 min and centrifugation at 10,000 × g for 20 min. The resulting axonemal pellet was further extracted in 0.6 M NaCl and 0.1–1% Nonidet P-40 to remove all of the dynein outer arms. The stripped axonemes were then washed three times to remove residual detergent and high salt and stored in 50% glycerol in buffer at −20°C until use. On the day of the experiment, axonemes were pelleted from glycerol (100,000 × g, 30 min) and washed three times in the appropriate experimental buffer.

Other Biochemical Procedures—SDS-polyacrylamide gel electrophoresis was performed on 7.5%–15% slab gels using the buffer system of Laemmli (1970). The histidyl/cysteinyl ratio was reduced to 1:100 (w/w) to allow better visualization of high molecular weight polypeptides (Porter and Johnson, 1983). Polypeptides were blotted to nitrocellulose (Towbin et al., 1979) and probed with blot affinity purified antibodies via the immunoperoxidase reaction (Olins, 1981). The sea urchin kinesin antibody has been previously described (Scholey et al., 1985), and the squid kinesin antibody was raised against the 110-kDa subunit electrophoresed from polyacrylamide gels.

Protein concentrations were determined by the method of Bradford (1976).

Motility Assays—Kinesin activity was monitored using an in vitro motility assay (Vale et al., 1985b) and video-enhanced DIC microscopy (Allen et al., 1981; Inoué, 1981). Five μl of the test samples were applied to a number 1 glass coverslip and incubated for 5 min in a humid chamber, during which time protein and other soluble molecules could adsorb to the glass. Then 5 μl of taxol-stabilized brain microtubules or salt and detergents stripped flagellar axonemes were added in the appropriate experimental solution. The sample was inverted onto a glass slide, sealed with vaseline/parafin/lanolin, 1:1:1 and viewed by video-enhanced DIC microscopy (see below).

Samples were scored for motility in "blind" assays in which the observer was unaware of the identity of the fraction or the inhibitor being tested. Motility was scored as positive if several microtubules were attached to and gliding on the coverslip surface, plus/minus if the microtubules were attached and undergoing bending and flexing movements, negative if no microtubules were attached to the coverslip surface, and frozen if the microtubules were attached but not moving. In the initial experiments, motility assays were performed in the "nontility buffer" described by Vale et al. (1985a) or the "egg buffer" described by Pryer et al. (1986). However, the majority of the assays were performed in the PEME extraction buffer described above. No significant differences were seen with control 3% sucrose conditions. All assays were performed at room temperature (20–25°C).

Determination of Polarity.—The polarity of microtubule gliding induced by kinesin was determined by means of flagellar axonemes that had been elongated with purified brain tubulin as described by Pryer et al. (1986). The polarity of bead translocation along centrosomal microtubules was determined as described by Vale et al. (1985d).

Microscopy.—Individual microtubules were visualized on the coverslip surface using a Zeiss Universal microscope equipped with DIC optics. The specimen was critically illuminated by a 100-watt mercury arc through a heat-cut filter and a wide band-pass green (λ = 546 nm) interference filter. The image of the specimen was magnified via a 100 × (N.A. = 1.25) plan objective lens, a 2 × optivar, and a 1 × TV tube or a Dage 68 Newvicon video camera with external gain and offset controls. The optics were initially set to extinction, then the upper Wollaston prism was adjusted to give optimal contrast on the video monitor with both the camera gain and offset on "auto." The final adjustment was made by changing the offset level of the video camera to "manual" and optimizing the image. Motility was recorded at real time using a ⅛-inch videocassette recorder (NEC) for further analysis and subsequent photography.

Rate Measurements—Previously recorded video images were played back into a Microtime time-base corrector (Bloomfield, CT), digitized, averaged, and stored using a video processing system (Hannaway & Associates, Boulder, CO). This system consists of an Imaging Technologies IP-512 Multibus digital video image processor controlled by a Silicon Graphics IRIS 2400 computer and a package of software for image management. The time coordinate for each image was recorded at the time of collection and stored with the image in a disc file. For the description of this system see Stemple et al. For analysis of microtubule movement, the stored images were recalled and the positions of the ends of 10 or more individual microtubules were indicated with a digitizing tablet. These microtubules were then followed throughout a series of 3–5 images of the time-lapse recorded at different times. The position and time data from each set of microtubules were converted into a graph of distance.
moved as a function of time. The mean rate of microtubule gliding was then determined from the slope of the line which was fitted to the data by the method of least squares.

Photography—Images of microtubule movement recorded on videotape were displayed on a flat-face video monitor (Tektronix number 634, Beaverton, OR) and photographed with a Nikon FM 35-mm camera using Plus-X film and a 0.5-s exposure. Axoneme gliding was displayed from the original videotape without modification of the image. In order to improve visualization, images of singlet microtubules were first collected and stored by the video processor, and then an out-of-focus background image was subtracted from the stored images. Contrast on these subtracted images was digitally increased by the video processor before display on the video monitor.

RESULTS

Isolation of Kinesin Activity from Crude Extracts—Kinesin activity in crude extracts of sea urchin egg was examined by comparing supernatant and pellet fractions from three different microtubule preparations as described under "Experimental Procedures" and Fig. 1. As reported previously, microtubules treated with MgAMPPNP contained significantly greater quantities of the ~130-kDa kinesin polypeptide than did control microtubules treated with MgSO₄ (see Fig. 2 and Scholey et al., 1985). However, control microtubule pellets occasionally contained low but detectable levels of the 130-kDa polypeptide when assayed on immunoblots. ATP extracts of these preparations also showed low levels of microtubule translocating activity as detected by video microscopy. These results suggested 1) that the kinesin present in the crude extract was in equilibrium between a microtubule-associated form and a soluble form and 2) that this equilibrium could be shifted by changes in the nucleotide level of the extract. In support of this hypothesis, we found that the removal of endogenous nucleotides by the addition of apyrase promoted binding of kinesin to microtubules (Scholey et al., 1985), and that addition of 10 mM MgATP to microtubule-containing cell extracts inhibited kinesin binding to the microtubules (Fig. 2). ATP-treated microtubule pellets contained no detectable p130 antigen, and subsequent ATP extraction of these microtubule pellets released no microtubule translocating activity. However, both translocating activity and the p130 antigen were detected in the supernatant of the ATP-treated, crude extract (Fig. 2). This soluble activity was further fractionated by sucrose density centrifugation, yielding a single peak of activity sedimenting at 9.5 S. This peak coincided with the peak of the 130-kDa kinesin polypeptide detected by immunoblotting (Fig. 2).

Isolation of Kinesin Activity from AMPPNP-treated Microtubules—The microtubule translocating activity present in crude extracts was purified efficiently by virtue of its AMPPNP-induced binding to microtubules (Vale et al., 1985c; Scholey et al., 1985). AMPPNP-treated microtubules were washed in extraction buffer without additional nucleotide, leaving both the kinesin activity and 130-kDa polypeptide in the pellet (Figs. 1 and 3A, Scholey et al., 1985). The kinesin activity and 130-kDa polypeptide were subsequently extracted from the microtubules by the addition of 0.1 M NaCl + 10 mM MgATP. This effect was specific for ATP; similar extractions with 0.1 M NaCl and 10 mM MgAMPPNP did not release kinesin.³

The ATP extract of AMPPNP-treated microtubules was highly enriched in both cytoplasmic dynein-like (HMR-3) and kinesin (p130) polypeptides and contained abundant microtubule translocating activity (see Fig. 3A, this report, and Figs. 1 and 2 of Scholey et al., 1985). When the ATP extract was fractionated by Bio-Gel A-5m chromatography, the peak of microtubule translocating activity routinely coincided with the peak of the 130-kDa polypeptide. This peak fraction also contained variable amounts of other contaminating polypeptides, including HMR-3, the 80-kDa MAP, and tubulin. The latter polypeptides were also present in ATP extracts of control microtubule pellets that lacked microtubule translocating activity and the 130-kDa polypeptide (data not shown). The significance of other polypeptides in the peak fraction is under current investigation.

The ATP or NaCl extracts of AMPPNP-treated microtubules were also fractionated by sucrose density gradient centrifugation (see Fig. 3B). As with kinesin prepared directly from crude extracts (Fig. 2), the microtubule translocating activity present in high salt extracts of microtubules copurified with the 130-kDa polypeptide, sedimenting at 9.5 S (Fig. 3B). While this procedure was less efficient than gel filtration at separating the 130-kDa polypeptide from other polypeptides, it demonstrated that the microtubule translocating activity could be completely separated from the HMR-3 polypeptide sedimenting at 20 S (see Fig. 3B, fractions 8-10). The peak of activity also coincided with the peak of the antigen recognized by the antibody to the 110-kDa subunit of squid kinesin (see Fig. 3B, fractions 14-17). These results agree with previous observations on the copurification of kinesin polypeptides and microtubule translocating activity on Bio-Gel A-5m columns (Scholey et al., 1985; Vale et al., 1985c).

Characteristics of Sea Urchin Egg Kinesin Activity—Egg

³ J. M. Scholey and M. E. Porter, unpublished observations.
Egg Kinesin Induced Microtubule Motility

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ATP AMPPNP A. ATP
P 'S 12 14 16 18 20
s

FIG. 2. Cosedimentation of p130 and kinesin activity in crude extracts of sea urchin eggs. The following fractions were run on a 5-20% polyacrylamide gel (bottom), blotted to nitrocellulose (top), and probed with a blot affinity purified antibody to p130; ATP-P, pellet of microtubules following addition of 10 mM MgATP to the cell extract. S, ATP supernatant containing motility activity which was loaded onto a 5-15% sucrose density gradient. 12-20, even-numbered fractions from the sucrose gradient of the ATP supernatant. Motility activity peaked at 9.5 S in fractions 13-17, which also contained the p130 antigen. AMPPNP-S, supernatant from cell extract which was treated with 10 mM MgAMPPNP and centrifuged to remove microtubules. P, pellet of AMPPNP-treated microtubules containing the p130 antigen. Identical results were obtained with the antibody to the squid 110-kDa kinesin polypeptide.

Sea urchin egg kinesin will induce three types of microtubule mediated motility in vitro: movement of microtubules relative to other microtubules in solution, movement of carboxylated latex beads along microtubules, and gliding of microtubules over a glass surface (Scholey et al., 1985; this report). Since microtubule gliding was the easiest movement to observe and measure quantitatively, we primarily used this assay to characterize kinesin-induced motility. We have also examined kinesin activity at all stages of purification and, except where

FIG. 3. Copurification of the 130-kDa kinesin polypeptide and microtubule translocating activity following extraction of AMPPNP-treated microtubules. A, the polypeptides present in a typical ATP extract of AMPPNP-treated microtubules (ATP MAPS) and the corresponding peak motility-inducing fraction from a Bio-Gel A-5m column (PEAK) were visualized on 5-15% polyacrylamide SDS gels stained with Coomassie Blue. Similar data were published in Scholey et al., 1985. B, A high salt extract (HS) of AMPPNP-treated microtubules was fractionated by sucrose density gradient centrifugation, and the resulting fractions (2-20) were then tested for motility in the microtubule gliding assay (+ or -) and analyzed by SDS-polyacrylamide gel electrophoresis (top) and immunoblotting (bottom) with a 1:1000 dilution of the rabbit antiserum to the 110-kDa polypeptide of squid kinesin. The direction of sedimentation was from left to right, with fraction 20 on the far left from the top of the gradient. Note that the peak of the 130-kDa sea urchin kinesin polypeptide in frations 15 and 16 corresponded with the peak of the antigen recognized by the antiserum to the squid 110-kDa polypeptide and the peak of motility-inducing activity. These fractions were active at a 4-fold dilution.
noted, have detected no difference as a result of purification. We have therefore focused our characterization on column purified material (e.g. Fig. 3A).

Characteristics of Microtubule Gliding—Kinesin-induced microtubule gliding was routinely assayed by incubating the test sample with taxol-stabilized microtubules and MgATP. Microtubules moved across the glass surface on linear paths parallel to their long axes (see Fig. 4). As the active kinesin concentration was decreased by aging or by addition of inhibitors, microtubule movement became progressively more irregular. Linear paths became more serpentine, and microtubule activity gradually became limited to bending and flexing movements. Successive dilutions of active kinesin eventually reduced the binding of the microtubules to the coverslip surface, and very few microtubules were bound to glass in the absence of kinesin.

Kinesin also induced the gliding of flagellar axonemes that had been stripped of their dynein arms (see Fig. 5). Axoneme gliding was less consistent than singlet microtubule gliding, in that fewer axonemes were moving in a given field than in a similar field of singlet microtubules. However, the axonemes that did glide moved at rates comparable to those of singlet microtubules.

Polarity of Egg Kinesin Motility—Earlier work indicated that neuronal kinesin induces bead movement toward the plus ends of microtubules nucleated and assembled from centrosomes in vitro and induces microtubule gliding toward their minus ends (Vale et al., 1985d). We tested the polarity of sea urchin egg kinesin motility as shown in Fig. 6. Carboxylated latex beads coated with sea urchin egg kinesin purified by gel filtration translocated away from the centrosome toward the plus ends of the microtubules. In addition, axonemes which were seeded with brain tubulin were also observed to glide toward their minus ends (data not shown).

MgATP Dependence of Motility—Motility mediated by sea urchin egg kinesin showed a strict requirement for Mg\(^{2+}\) and nucleoside triphosphates (see Table I). Incubation with apyrase to deplete nucleotides inhibited microtubule movement, although many microtubules were still attached to the glass surface. The removal of Mg\(^{2+}\) ions by chelation with 5 mM EDTA or high concentrations of other nucleotides (ADP, AMP, etc.) also inhibited microtubule gliding.

The rate of microtubule gliding increased with ATP concentration, saturating at a V\(_{max}\) of \(~0.54 \mu m/s\) in the preparation shown in Fig. 7. No movement was observed at \(~1 \mu m\)

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**FIG. 4. Microtubule gliding.** Column purified sea urchin egg kinesin was allowed to adhere to a glass coverslip, mixed with taxol-stabilized brain microtubules, and 50 \(\mu M\) ATP, and then sealed to a glass slide. Microtubule gliding on the coverslip surface was viewed by video-enhanced DIC microscopy. Shown here are four frames taken from the video monitor at 14-s intervals. The ends of three numbered microtubules are indicated (*), and their direction of movement is shown throughout the sequence (→). These microtubules are gliding at a rate of 0.39 ± 0.08 \(\mu m/s\). Scale bar = 5 \(\mu m\).
Fig. 5. Axoneme gliding. Sea urchin egg kinesin was allowed to adhere to a glass coverslip and then mixed with 1 mM ATP and sea urchin flagellar axonemes which were stripped of their dynein arms. Axoneme gliding on the glass surface was viewed by video-enhanced DIC microscopy. Shown here are three frames taken at 20-s intervals from the video monitor. Scale bar = 5 μm.

ATP, but at 10 μM ATP, microtubules translocated at a mean rate of ~0.2 μm/s. Half-maximal velocity was reached between 10 and 20 μM ATP.

Other nucleotide triphosphates also supported motility by column purified kinesin (see Table I). GTP was the best substitute for ATP; at 1–5 mM GTP, kinesin-induced MT gliding was indistinguishable from movement in the presence of ATP. 1–5 mM ITP also substituted for ATP, but the rate of microtubule gliding was approximately one-half the rate observed in ATP (0.25 μm/s at 1 mM ITP). Finally, CTP and UTP were poor substrates for kinesin activity. Motility in the presence of 1–5 mM CTP or UTP was marginal and might be due to the presence of contaminating ATP in commercial preparations of these nucleotides.

Inhibition of Motility by ATP Analogs—Kinesin-induced motility probably requires ATP turnover since addition of nonhydrolyzable nucleotide analogs (ATPγS and AMPPNP) immediately inhibited microtubule gliding in the presence of equimolar concentrations of ATP. In the presence of these analogs, microtubules were bound to the glass surface but did not move. Lower concentrations of these analogs slowed the rate of movement in a dose-dependent fashion and induced...
Twenty PM vanadate was without obvious effect, but 50 WM
control experiments with 10 mM ATP indicated that UV irradia-
tion to activate the ATPase activity of soluble sea urchin flagellar
dynein but inhibit its ability to stimulate flagellar beat fre-
quency (Gibbons and Gibbons, 1979). However, low (0.05–
0.16%) concentrations of Triton X-100 and Tween 20 did not
affect kinesin’s ability to induce microtubule gliding (see Table III).

Egg kinesin activity is also relatively insensitive to modifi-
cation of —SH groups by preincubation with N-ethylmaleimide,
since high concentrations (>3–5 mM) were required to
block motility. Furthermore, at such concentrations of N-
ethylmaleimide, microtubules were not frozen in place, but
were released from the glass surface, unlike the effects of
AMPPNP or vanadate.

Several agents that inhibit energy metabolism have been
reported to block microtubule-based motility in vivo (Spurck
et al., 1986). None of these agents, however, had any direct
effect on kinesin activity in the presence of ATP in vitro (see
Table IV).

We also tested a variety of other agents and treatments on
kinesin activity. As expected, treatments that inactivate pro-
teins (e.g. trypsin digestion or boiling) irreversibly inactivated
egg kinesin. Agents that coat the glass surface (e.g. poly-L-
lysine or high concentrations of nonspecific proteins) also
interfere with kinesin binding and blocked motility.

High concentrations of calcium (10 mM) had no effect on
purified kinesin activity, but occasionally blocked motility in
crude extracts, an effect which may be due in part to calcium-
activated proteolysis.

**DISCUSSION**

**Egg Kinesin Is Associated with the Microtubule Translocat-
ing Activity in Crude Egg Extracts—** Sea urchin egg extracts
contain a complex mixture of MAPs and ATPase activities
(Vallee and Bloom, 1983; Schol ey et al., 1984; Pratt, 1984).
These include several molecules that might serve as micro-
tubule-associated motors in vivo: cytoplasmic dynein (Wei-
senberg and Taylor, 1968; Pratt, 1980; Pratt et al., 1980;
Hisinaga and Sakai, 1980, 1983; Schol ey et al., 1984; Piperno,
1984; Hollenbeck et al., 1984; Asai and Wilson, 1985; Porter
et al., 1985), HMR-3 (Schol ey et al., 1984), kinesin (Schol ey

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**FIG. 7. The rate of microtubule gliding is a function of the
ATP concentration.** Column purified kinesin was dialyzed, diluted,
and reconstituted to reduce the [ATP] to less than 1 µM. This
material was then recombined with taxol-stabilized microtubules
containing less than 50 µM GTP and assayed for microtubule gliding
in the presence of various concentrations of ATP. No movement was
observed in the absence of added ATP. The rate of microtubule
gliding between 0 and 10 µM ATP is represented by a dashed line
because of uncertainty concerning the threshold concentration of
ATP required for microtubule gliding.

**TABLE II**

<table>
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<th>Nucleotide</th>
<th>Concentration [Mg]</th>
<th>[ATP]</th>
<th>Motility</th>
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<td>ATPγS</td>
<td>1.0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3</td>
<td>1</td>
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<tr>
<td>AMPPNP</td>
<td>0.5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5</td>
<td>1</td>
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<td>1</td>
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<td>8-azido-ATP</td>
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<td>10 mM</td>
<td>0.2 mM</td>
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<td></td>
<td>+UV, 10 mM</td>
<td>10</td>
<td>0.2</td>
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<tr>
<td></td>
<td>–UV, 10 mM</td>
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<tr>
<td></td>
<td>+UV, 10 mM</td>
<td>10</td>
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**TABLE III**

<table>
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<th>Inhibitor</th>
<th>Concentration/condition</th>
<th>Motility</th>
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<td>Vanadate</td>
<td>20 µM</td>
<td>+</td>
</tr>
<tr>
<td></td>
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<td>±</td>
</tr>
<tr>
<td></td>
<td>100 µM</td>
<td>–</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>0.16%</td>
<td>+</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.05%</td>
<td>+</td>
</tr>
<tr>
<td>NEM⁺</td>
<td>2 mM</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>–</td>
</tr>
</tbody>
</table>

*The effect of N-ethylmaleimide (NEM) on kinesin activity was
tested by preincubation of test sample with N-ethylmaleimide at the
concentrations indicated at room temperature (°C) for 15 min. These
samples were then treated with 10 mM dithiothreitol on ice for 15
min before mixing with microtubules. Control experiments in which
10 mM dithiothreitol was added prior to N-ethylmaleimide treatment
indicated that kinesin activity was normal.

**TABLE IV**

<table>
<thead>
<tr>
<th>Inhibitors of energy metabolism</th>
<th>Concentration</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain</td>
<td>50 µM</td>
<td>+</td>
</tr>
<tr>
<td>Azide</td>
<td>5 mM</td>
<td>+</td>
</tr>
<tr>
<td>Dinitrophenol (DNP)</td>
<td>1 mM</td>
<td>+</td>
</tr>
<tr>
<td>Decyglycose (DEOG)</td>
<td>1 mM</td>
<td>+</td>
</tr>
<tr>
<td>DNP + DEOG</td>
<td>1 mM + 1 mM</td>
<td>+</td>
</tr>
</tbody>
</table>

---

**more irregular and serpentine microtubule movement (see Table II).**

The photoactivatable analog, 8-azido-ATP (Czarnecki et al., 1979), inhibited kinesin-induced motility following UV
irradiation of kinesin and 8-azido-ATP mixtures. As shown in Table II, motility was normal in the presence of 10 mM 8-
azido-ATP. One-minute exposure of kinesin to UV and 10 mM 8-azido-ATP, however, completely blocked motility. Control
experiments with 10 mM ATP indicated that UV irradiation itself did not inhibit motility. Furthermore, addition of
10 mM ATP to the mixture of kinesin and 10 mM 8-azido-ATP prevented the UV-induced inactivation. These results
suggest that UV irradiation cross-links the 8-azido-ATP to an ATP-binding site essential for kinesin activity.

**Inhibition of Motility by Other Reagents—** We have also
compared the effects of other ATPase inhibitors on kinesin-
induced microtubule motility. Addition of the phosphate an-
alog, sodium vanadate (Na₃VO₄), interfered with microtubule
gliding at concentrations greater than 50 µM (see Table III).
Twenty µM vanadate was without obvious effect, but 50 µM
vanadate caused microtubule gliding to become more irregular
and serpentine, and 100 µM vanadate completely blocked
microtubule movement.

Nonionic detergents such as Triton X-100 have been shown
to activate the ATPase activity of soluble sea urchin flagellar
dynein but inhibit its ability to stimulate flagellar beat fre-
tency (Gibbons and Gibbons, 1979). However, low (0.05–
0.16%) concentrations of Triton X-100 and Tween 20 did not
affect kinesin’s ability to induce microtubule gliding (see Table III).

Egg kinesin activity is also relatively insensitive to modifi-
cation of —SH groups by preincubation with N-ethylmaleimide,
since high concentrations (>3–5 mM) were required to
block motility. Furthermore, at such concentrations of N-
ethylmaleimide, microtubules were not frozen in place, but
were released from the glass surface, unlike the effects of
AMPPNP or vanadate.

Several agents that inhibit energy metabolism have been
reported to block microtubule-based motility in vivo (Spurck
et al., 1986). None of these agents, however, had any direct
effect on kinesin activity in the presence of ATP in vitro (see
Table IV).

**DISCUSSION**

**Egg Kinesin Is Associated with the Microtubule Translocat-
ing Activity in Crude Egg Extracts—** Sea urchin egg extracts
contain a complex mixture of MAPs and ATPase activities
(Vallee and Bloom, 1983; Schol ey et al., 1984; Pratt, 1984).
These include several molecules that might serve as micro-
tubule-associated motors in vivo: cytoplasmic dynein (Wei-
senberg and Taylor, 1968; Pratt, 1980; Pratt et al., 1980;
Hisinaga and Sakai, 1980, 1983; Schol ey et al., 1984; Piperno,
1984; Hollenbeck et al., 1984; Asai and Wilson, 1985; Porter
et al., 1985), HMR-3 (Schol ey et al., 1984), kinesin (Schol ey
Egg Kinesin Induced Microtubule Motility

M. E. Porter and J. M. Scholey, unpublished observations.
the McIntosh Laboratory for lively discussion. Finally, we greatly appreciate the excellent secretarial skills provided by Cathy Inouye and Karen Brown and the photographic help of Larry Harwood, Andrea Olaheskie, and Maureen Gaines.

REFERENCES

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