Cell Motility and the Cytoskeleton 58:30–38 (2004)

Properties of the Full-Length Heavy Chains of *Tetrahymena* Ciliary Outer Arm Dynein Separated by Urea Treatment

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An important challenge is to understand the functional specialization of dynein heavy chains. The ciliary outer arm dynein from *Tetrahymena thermophila* is a heterotrimer of three heavy chains, called α, β and γ. In order to dissect the contributions of the individual heavy chains, we used controlled urea treatment to dissociate *Tetrahymena* outer arm dynein into a 19S β/γ dimer and a 14S α heavy chain. The three heavy chains remained full-length and retained MgATPase activity. The β/γ dimer bound microtubules in an ATP-sensitive fashion. The isolated α heavy chain also bound microtubules, but this binding was not reversed by ATP. The 19S β/γ dimer and the 14S α heavy chain could be reconstituted into 22S dynein. The intact 22S dynein, the 19S β/γ dimer, and the reconstituted dynein all produced microtubule gliding motility. In contrast, the separated α heavy chain did not produce movement under a variety of conditions. The intact 22S dynein produced movement that was discontinuous and slower than the movement produced by the 19S dimer. We conclude that the three heavy chains of *Tetrahymena* outer arm dynein are functionally specialized. The α heavy chain may be responsible for the structural binding of dynein to the outer doublet A-tubule and/or the positioning of the β/γ motor domains near the surface of the microtubule track. Cell Motil. Cytoskeleton 58:30–38, 2004.

Key words: motor protein; axoneme; microtubules; ATPase; motility

INTRODUCTION

Dynein is an important molecular motor that carries cellular cargoes along microtubule tracks [Holzbaur and Vallee, 1994]. In situ, dynein comprises multiple protein subunits. The light- and intermediate-sized subunits are responsible for the intracellular targeting and regulation of dynein [King, 2003; Vallee and Sheetz, 1996]. The motor activity, involving the transduction of chemical

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Contract grant sponsor: Ministry of Education, Science, Sport and Culture of Japan; Contract grant sponsor: National Science Foundation.

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Received 6 October 2003; accepted 25 November 2003

energy derived from ATP hydrolysis into mechanical force, resides in the heavy chains [Asai and Koonce, 2001]. Organisms with cilia or flagella express 14–15 different dynein heavy chain genes; each gene encodes a distinct protein isoform. Approximately 12 of these isoforms are axonemal dyneins, which produce the active sliding between adjacent outer doublet microtubules that underlies propagated bending of cilia and flagella.

The axonemal dyneins are an impressive example of functional specialization. Different inner and outer arm dyneins are precisely located within the axoneme where they generate distinct shear forces [Kamiya, 2002]. Functional specialization is observed among the different dyneins, and there is evidence for a division of labor within a single dynein particle [Yano-Toyoshima, 1985; Sale and Fox, 1988; Moss et al., 1992a,b]. An important challenge is to understand the structural basis for dynein heavy chain functional specialization.

The ciliated protozoan *Tetrahymena thermophila* presents an exceptional opportunity to dissect dynein functional specialization. *Tetrahymena* expresses 14 dynein heavy chain (DYH) genes [Xu et al., 1999; Asai, 2000]. Because any of its genes can be readily altered exclusively by homologous recombination [Cassidy-Hanley et al., 1997], *Tetrahymena* offers a powerful system for "reverse genetic" engineering of dynein heavy chains [Asai et al., 2001]. The high salt extraction of *Tetrahymena* ciliary axonemes yields 14S inner arm and 22S outer arm dyneins [Porter and Johnson, 1983]. The 22S outer arm dynein of *Tetrahymena* is a three-headed complex comprising three different heavy chains, two-three intermediate chains, and several light chains [Johnson and Wall, 1983]. The three heavy chain proteins are called α, β, and γ in the order of their increasing mobilities in urea-SDS-polyacrylamide gels [Toyoshima, 1987a], and are encoded by the *Tetrahymena* genes DYH3, 4, and 5, respectively. The three heavy chains are susceptible to vanadate-mediated photolysis [Marchese-Ragona et al., 1989].

Despite the experimental advantages of *Tetrahymena*, unraveling the contributions of individual heavy chain isoforms of *Tetrahymena* 22S dynein has been hampered by the difficulty of dissociating the complex into intact heavy chain proteins that retain their biological activity. Unlike the outer arm dyneins from other model organisms—including sea urchin, *Chlamydomonas*, and *Paramecium*—*Tetrahymena* 22S dynein is resistant to dissociation by changes in ionic strength. Heretofore, the only method that has successfully been applied to dissociate *Tetrahymena* dynein is by the limited proteolysis with chymotrypsin [Toyoshima, 1987a]. This treatment yielded a two-headed fragment comprising the proteolytically truncated β and γ heavy chains, and a single-headed species comprising the proteolytically truncated α heavy chain. The β/γ fragment bound microtubules and supported microtubule movement, but the truncated α chain did not produce movement. The three proteolyzed subunits could not be subsequently reconstituted.

A potential complication of the previous work is that the chymotrypsin may have destroyed parts of the heavy chains important for activities. Thus, in order to understand better the functional specialization of the different heavy chains, we explored new methods to dissociate the *Tetrahymena* ciliary outer arm dynein complex without proteolysis. In this article, we report that controlled urea treatment dissociated the 22S *Tetrahymena* dynein into two parts: a 14S species containing the α heavy chain, and a 19S species containing the β and γ heavy chains. The two separated species could be reconstituted into a 22S dynein. The α chain and the β/γ dimer were distinguishable in their microtubule-binding properties and in their ability to support microtubule movement in vitro.

EXPERIMENTAL PROCEDURES

Preparation of 22S Outer Arm Dynein

Tetrahymena thermophila ciliary outer arm dynein was prepared as described previously [Gibson and Asai, 2000; Shiroguchi and Toyoshima, 2001]. In the experiments described in this report, the starting material was 22S outer arm dynein purified on 5–20% sucrose gradients made in wash buffer (0.1 M NaCl, 10 mM HEPES [N-(hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)], 1 mM EDTA, 4 mM $MgCl_2$, 1 mM dithiothreitol [DTT], pH 7.4) supplemented with 0.1 mM ATP and 0.1 mM phenylmethylsulfonyl fluoride (PMSF).

Urea Treatment of Dynein

The sucrose gradient-purified 22S outer arm dynein (approximately 0.8–1.0 mg/mL) was dialyzed against 200 volumes of extraction buffer (wash buffer with 0.6 M NaCl) supplemented with 4.0 M urea for 60 min at 4°C. The samples were then immediately dialyzed against 1,000 volumes of extraction buffer for 15 min at 4°C in order to remove the urea. Control samples were dialyzed against 200 volumes of extraction buffer without urea for 60 min, and then for another 15 min against another 1,000 volumes of extraction buffer at 4°C. After dialysis, the samples were overlaid onto sucrose gradients. Dialysis tubing was Spectra/Por*4, molecular weight cutoff 12,000–14,000 (American Scientific Products). Electrophoresis grade urea (Bio-Rad, Richmond, CA, or Fischer Scientific, Fair Lawn, NJ) was used in these experiments. Partial reconstitution of 22S dynein was achieved by dialyzing the urea-treated dynein

against 1,000 volumes of urea-free extraction buffer for 30 min, followed by another 30 min against a fresh 1,000 volumes of extraction buffer.

Sucrose Density Gradient Centrifugation and Dynein MgATPase Assays

The urea- and mock-treated dynein preparations were sedimented through 4.2-mL linear 15–30% sucrose density gradients prepared in extraction buffer. Each gradient was overlaid with 0.6 mL dynein. The gradients were centrifuged in a Hitachi P50S2 swinging bucket rotor at 100,000*g* for 15 h at 2°C. After centrifugation, the gradients were divided into 24 equal volume fractions. Protein concentrations of the gradient fractions were determined by the method of Bradford [1976] as modified by Read and Northcote [1981]. MgATPase activities of sucrose gradient fractions were measured by standard methods [Carter and Karl, 1982; Kodama et al., 1986].

Vanadate-Mediated Photolysis of Dynein

Dynein samples were subjected to vanadate-mediated photolysis, as originally described by the Gibbons laboratory [Lee-Eiford et al., 1986] and modified [Gibson and Asai, 2000]. Under "V1" conditions, the dynein was in extraction buffer supplemented with 100 μM $MgATP^{2-}$ and 100 μM sodium orthovanadate and irradiated at 366 nm for 1–1.5 h on ice. After irradiation, the samples were diluted with 4 × Laemmli SDS-PAGE sample buffer, heated to 95°C for 5 min, and stored at −20°C.

SDS-Polyacrylamide Gel Electrophoresis and Western Blot Analysis

Protein samples were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [Laemmli, 1970]. In some experiments in order to resolve the three dynein heavy chains, the separating gels were made in 3.2% polyacrylamide, 6M urea, and 5% (v/v) glycerol. Gels were stained with Coomassie brilliant blue or by silver staining (Silver Stain "Daiichi" kit, Daiichi Pure Chemicals Co., Tokyo, Japan).

After electrophoresis, the proteins were transferred to nitrocellulose. The proteins transferred to the nitrocellulose were visualized by gold staining (Colloidal Gold Total Protein Stain™, Bio-Rad Laboratories). The blots were probed with isoform-specific antibodies raised against each of the *Tetrahymena* 22S dynein heavy chains [Toyoshima, 1987b], followed by goat anti-rabbit IgG conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The blots were developed using the BCIP/NBT phosphatase substrate system (Kirkegaard and Perry Laboratories). In the microtubule-binding experiment, dynein heavy chains were detected with the pan-reactive anti-axonemal rabbit antiserum [Asai et al., 1994]. These blots were then incubated with goat anti-rabbit IgG conjugated to peroxidase (Amersham, Arlington Heights, IL), and developed using the Super Signal™ chemiluminescence system (Pierce Chemicals, Rockford, IL) followed by exposure to X-ray film.

Microtubule-Binding Assays

Bovine brain microtubules were prepared by cycles of assembly and disassembly [Weingarten et al., 1975]. Tubulin was separated from the microtubule-associated proteins (MAPs) by chromatography through phosphocellulose [Sloboda and Rosenbaum, 1982]. Phosphocellulose P-11 was purchased from Whatman (Maidstone, UK). MAP-depleted tubulin (0.9 mg/mL) was assembled in 25 μM taxol (CalBiochem, Wako Pure Chemical Industries), then mixed with two volumes of dynein (1 mg/mL). The microtubules and dynein were incubated for 20 min at room temperature with occasional agitation. Immediately prior to centrifugation, some of the samples were treated with 5 mM ATP. After dynein binding, microtubules were collected by centrifugation in a microfuge at 14,000 rpm for 10 min at room temperature. Supernatants and pellets were separated and prepared for electrophoresis.

Motility Assays

In vitro microtubule gliding assays were performed as previously described [Shiroguchi and Toyoshima, 2001]. Dynein was applied to a flow chamber and absorbed onto the glass for 2 min. The following were then added in sequence: (1) three volumes of 0.5 mg/mL bovine serum albumin (BSA) to remove unabsorbed dynein and to block the surface of the glass; (2) three volumes of assay buffer (10 mM Pipes, 50 mM potassium acetate, 4 mM $MgSO_4$, 1 mM EGTA, and 1 mM dithiothreitol, pH 7.0) to wash away excess BSA; (3) three volumes of taxol-stabilized microtubules (30 μg/mL); (4) three volumes of assay buffer; and (5) 1 mM ATP with 10 μM taxol. The gliding of the microtubules was observed by dark-field illumination with a 40× objective and captured with an ICCD camera (Ikegami, Tokyo) and recorded on videotape. The recorded movies were digitized and analyzed in a personal computer. All observed microtubules in the visual field were identified and the displacement of microtubules during 15 sec was measured. If the displacement was less than 5 μm, the microtubule was classified as "immotile." If the displacement was greater than 5 μm in 15 sec, the microtubule was classified as "motile." The velocities of individual motile microtubules that traveled at least 10 μm were measured.

RESULTS

The starting material in these experiments was sucrose gradient-purified 22S dynein, which included the α-, β-, and γ- heavy chains, two or three intermediate chains, and several light chains. In contrast to dyneins from other experimental systems, *Tetrahymena* 22S outer arm dynein remained intact after several different treatments, including dialysis into lower or higher ionic strength buffers and gentle heating. We discovered that controlled dialysis against urea could be used to almost completely dissociate 22S dynein.

Dissociation of the 22S Outer Arm Dynein Into a 19S β/γ Dimer and 14S α-HC

The 22S outer arm dynein was treated with 4 M urea, then layered onto sucrose gradients made in extraction buffer (see Materials and Methods). As a control, mock-treated dynein—outer arm dynein dialyzed against extraction buffer with no urea—was prepared alongside the urea-treated samples. Equal amounts of urea-treated and mock-treated dynein samples were sedimented through 15–30% sucrose gradients (Fig. 1a). The control dynein sedimented at 22S. The urea-treated sample sedimented more slowly, with peaks of protein at 19S and 14S. Analysis of the gradient fractions by urea-SDS-PAGE revealed that the 19S species contained predominantly the β- and γ-HCs, and the 14S species contained α-HC and not β or γ (Fig. 1b). The identities of the heavy chains were confirmed in Western blotting experiments (Fig. 2).

The polypeptide compositions of the sucrose gradient fractions were evaluated by SDS-PAGE made in 15% polyacrylamide in order to identify dynein light chains (M_r 10–45K). Eight putative light chains were identified as part of the 22S dynein (Fig. 3). After urea-dissociation, seven of these remained with the 19S β/γ dimer, and one (approximately M_r 24K) sedimented with the 14S α-HC.

Shorter exposure to urea produced intermediate species (data not shown). After 20 min of urea treatment, the majority of the protein sedimented between 22S and 19S with a small amount of protein sedimenting at 14S. After 40 min, there were distinct protein peaks at 19S and 14S as well as faster sedimenting protein. After 60 min, nearly all of the dynein had been converted to species sedimenting at 19S and 14S.

To attempt the dissociation of the 19S species, extraction buffer without Mg^{2+} (0.6 M NaCl, 10 mM HEPES, 1 mM EGTA, 1 mM DTT, pH 7.4) was used for the urea treatment, removal of urea, and sucrose gradients. Under these magnesium-free conditions, the sedimentation profile was the same as in the presence of Mg^{2+}, and the 19S dimer was not further dissociated.

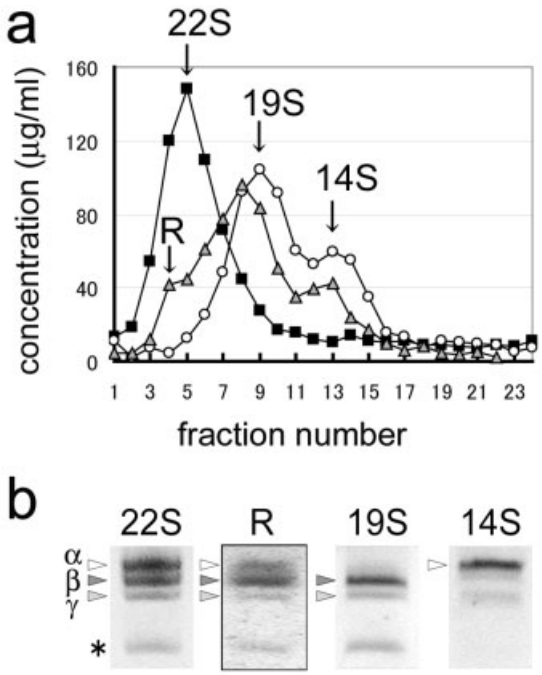

Fig. 1. Sucrose density gradient fractionation of urea-treated *Tetrahymena* ciliary outer arm dynein. **a:** Sedimentation profiles of mock-treated (*squares*), urea-treated (*circles*), and partially reconstituted (*triangles*) dyneins through 15–30% sucrose gradients. Protein profile of the reconstituted dynein revealed a shoulder sedimenting at around 22S (R). The bottom of the gradient is fraction 1. **b:** Urea-SDS-PAGE analysis of the dynein heavy chains in the different peak fractions. Under these conditions, *Tetrahymena* α-, β-, and γ- heavy chains can be resolved. 22S dynein contained all three heavy chains. The reconstituted 22S species (**lane R**) contained all three heavy chains. The 19S fraction contained mainly β and γ heavy chains. The 14S fraction contained mainly α heavy chain. The lowest band (*) is a degradation product of γ heavy chain.

MgATPase Activities of the Dynein Species

The specific activity of the intact 22S dynein was 0.3–0.4 μmol phosphate released per min per mg protein, which is in good agreement with previously published values [Omoto and Johnson, 1986]. The urea-dissociated 19S and 14S species retained significant MgATPase activities. The average specific activities (μmol Pi released mg^{-1} min^{-1}) from three experiments were as follows: 22S dynein, 0.39; 19S species, 0.51; 14S species, 0.98. These data indicate that the activity per molecule of each species increased about 1.6-fold after urea dissociation.

Vanadate-Mediated Photolysis of *Tetrahymena* Dynein Heavy Chains

When active dynein is combined with $MgATP^{2-}$ and micromolar amounts of vanadate, then exposed to

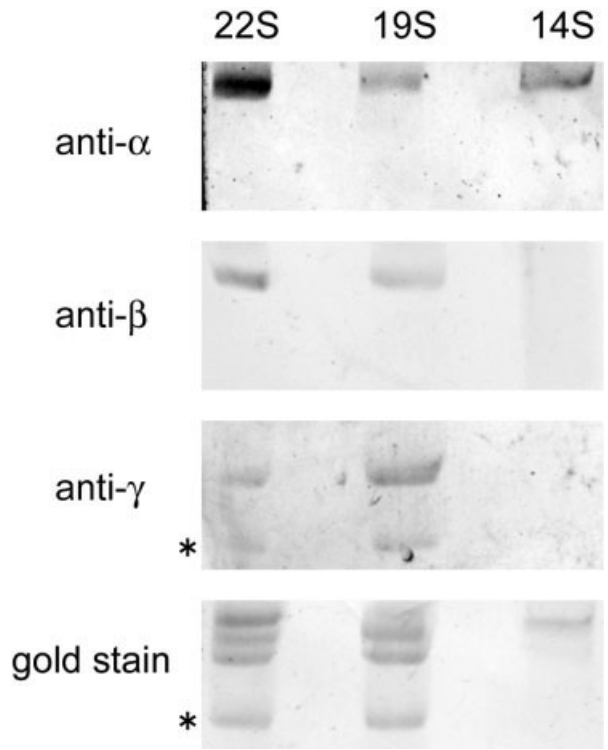

Fig. 2. Western blotting of dynein fractions. Intact outer arm dynein (22S), and the 19S and 14S species after urea treatment were electrophoresed in urea-SDS-PAGE, transferred to nitrocellulose, and probed with the isoform-specific antibodies. This method resolved the three heavy chains of 22S dynein, as shown in the gold-stained nitrocellulose blot (**bottom**). The corresponding Western blots with isoform-specific antibodies are shown in the **top panels**. The 14S fraction contained the α heavy chain, and not β- or γ- heavy chain. The 19S fraction contained β- and γ-heavy chains. The anti-α antibody is extremely sensitive and, in this experiment, detected some α-HC in the 19S species. The lowest band (*) is a degradation product of γ-HC.

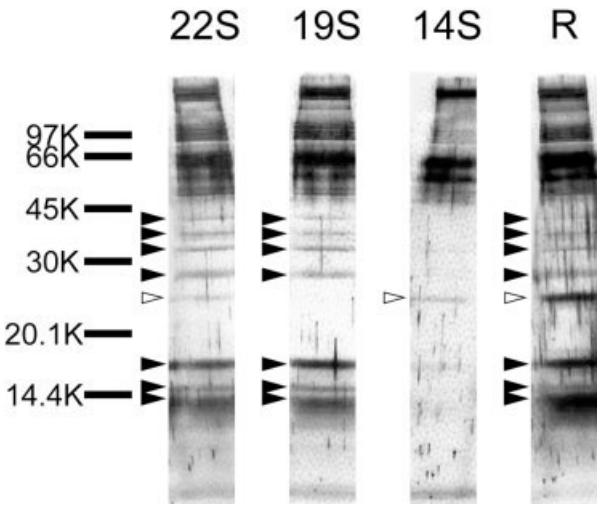

Fig. 3. Eight putative light chains. SDS-PAGE using 15% polyacrylamide followed by silver-staining of the gels was used to identify the proteins of M_r 10–45K. 22S dynein contained eight of these proteins, including a polypeptide of approximately M_r 24K (*open arrowheads*). After urea treatment and sucrose density centrifugation, the 19S fraction contained seven of the proteins (*closed arrowheads*) but was missing the M_r 24K polypeptide. The 14S fraction contained the M_r 24K polypeptide. The reconstituted 22S dynein (*lane R*) included all eight putative light chains.

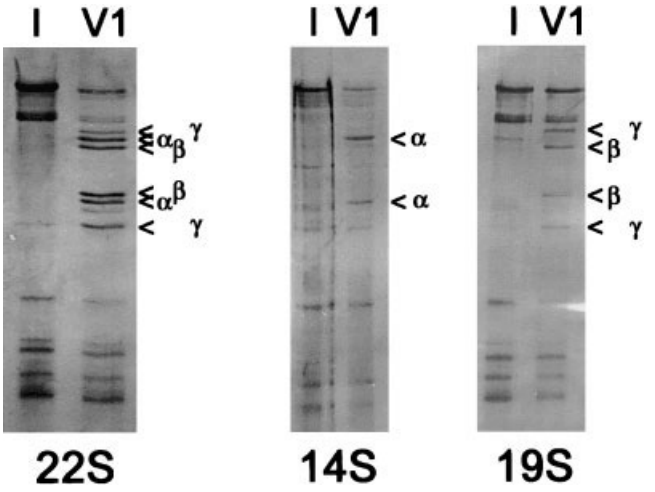

Fig. 4. Vanadate-dependent UV photolysis of dynein fractions. Intact outer arm dynein (22S), and the 14S and 19S species from the gradient of urea-treated dynein were analyzed by SDS-PAGE followed by silver staining. In each panel, two lanes of the gel are shown: **lane I**, intact sample; **lane V1**, after photolysis. The V1 photolysis of 22S dynein produced six fragments, two from each heavy chain. The identities of the heavy chains are indicated [Gibson and Asai, 2000]. The 14S species after urea treatment contained the α heavy chain; the 19S species contained the β and γ heavy chains.

near-UV irradiation, each dynein heavy chain undergoes a single scission at the catalytic ATP-binding site [Lee-Eiford et al., 1986]. The "V1" photolysis of a single heavy chain yields two unequally sized photolytic products, called HUV and LUV. The three heavy chains of *Tetrahymena* 22S outer arm dynein produce three pairs of photolytic products [Marchese-Ragona et al., 1989], and the electrophoretic pattern of the six peptides provides a "fingerprint" in which the derivation of the pair of photolytic fragments from each heavy chain has been established [Gibson and Asai, 2000].

The untreated 22S dynein and the 14S and 19S species obtained after urea treatment were subjected to vanadate-mediated photolysis, and the photolytic products were evaluated by SDS-PAGE (Fig. 4). The 14S fraction gave rise to two photolytic products corresponding to the α-HC. The 19S fraction gave rise to four photolytic products corresponding to the β- and γ- HCs. These data demonstrate that each of the dissociated heavy chains retained MgATPase activity and confirmed the identities of the individual heavy chains present in each fraction.

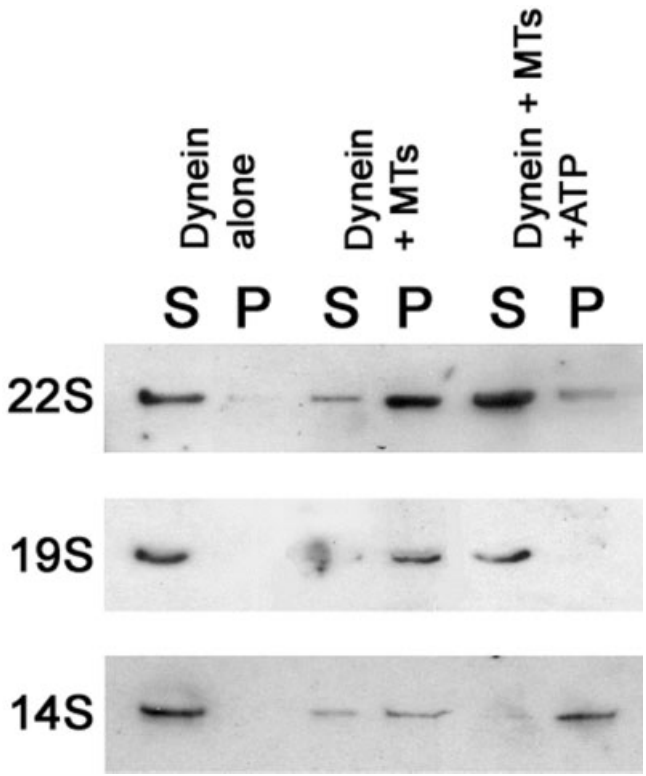

Fig. 5. Binding of dyneins to microtubules. Microtubules made from MAP-depleted bovine brain tubulin and stabilized with taxol were incubated with dynein fractions, sedimented, and the supernatants (S) and pellets (P) were analyzed by SDS-PAGE under conditions in which the three heavy chains were not resolved. Dynein heavy chains were detected with a pan-reactive antibody that reacts well with all three heavy chains. Three different dynein samples were used in this experiment: intact outer arm 22S dynein, 19S species, and 14S species. In the absence of added microtubules, the dyneins remained in the supernatant ("dynein alone"). In the presence of microtubules, all three dyneins bound microtubules and were present in the pellets ("dynein + MTs"). Upon the addition of 5 mM ATP, most of the 22S and 19S dyneins were found in the supernatant, but the 14S dynein remained bound ("dynein + MTs + ATP").

22S Dynein and the 19S β/γ Dimer, But Not the 14S α-HC, Bound Microtubules in an ATP-Sensitive Fashion

In vitro microtubule binding assays were performed (Fig. 5). In these experiments, microtubules were incubated with various dynein fractions, then collected by centrifugation. After sedimentation, the unbound (S) and bound (P) samples were examined by SDS-PAGE and Western blotting. All three dyneins bound microtubules in the absence of ATP (Dynein + MTs). The binding of 22S and 19S dyneins was reversed by the addition of 5 mM ATP (Dynein + MTs + ATP). However, the binding of the 14S fraction (α-HC) was not significantly reversed by ATP. The results demonstrate that the 14S dynein binds microtubules in an ATP-insensitive fashion that dominates over any ATP-sensitive binding.

TABLE I. MgATPase Activities and *in vitro* Microtubule Gliding Properties of Dynein Fractions

Dynein	% motility	(n)	Velocity ± s.d. (μm sec^{-1})
22S	64.5	(31)	1.47 ± 0.67
19S	92.3	(65)	4.13 ± 1.31
14S	0	(50)	n/a
reconstituted	47.5	(59)	1.82 ± 1.07

The dynein (100 μg/ml) was absorbed onto a glass slide, and microtubule gliding was effected in assay buffer. % motility: the number of gliding microtubules / total number of microtubules in the fields (n). Velocity: mean and standard deviation of the velocities of the moving microtubules. n/a: not applicable.

Partial Reconstitution of 22S Dynein From the 19S and 14S Fractions

After the 60 min dialysis vs. 4M urea, the dissociated proteins were dialyzed against 1,000 volumes of urea-free extraction buffer for 30 min twice. This material was then loaded onto a sucrose gradient and the protein profile is shown in Figure 1a. Under these conditions, approximately 27% of the dynein protein could be reconstituted into a 22S species (Fig. 1b). The reconstituted 22S species contained all eight light chains found in untreated 22S dynein (Fig. 3).

Microtubule Gliding Produced by the 19S β/γ Dimer but Not the 14S α-HC

In vitro microtubule-gliding experiments were performed with the dynein fractions (Table I). Intact 22S dynein produced motility with 64.5% of the microtubules; the motility was non-uniform and discontinuous. Some microtubules showed stop-and-go motility, some did not move, others moved continuously. The mock-treated 22S dynein produced microtubule motility indistinguishable from that produced by untreated 22S dynein. Unlike the three-headed dynein, the 19S species produced smooth microtubule translocation in which 92.3% of the microtubules were motile. The reconstituted dynein also supported microtubule gliding. The fraction of moving microtubules and gliding velocities were less than those of 19S species, but similar to those of 22S dynein.

In contrast, the isolated 14S α-HC did not support motility at all. The microtubules attached to the α-HC and remained tightly bound and did not move upon addition of ATP. No movement by the α-HC was observed under several conditions, including: (1) different concentrations of the α-HC; (2) cleaning the glass surface with detergent, or with 0.1M HCl, or with 1M KOH;

(3) siliconizing the glass surface; (4) adding 0.1% Nonidet P40 (NP40) to the motility buffer; (5) preincubating the dynein with tubulin [Sakakibara and Nakayama, 1998]; and (6) preincubating the dynein with 1 mM ATP [Shimizu et al., 1995].

DISCUSSION

Dissociation of the Outer Arm Dynein Complex

Outer arm dynein is organized around a heterodimer of two heavy chains, the β-HC and a "not β" chain. In metazoans, there are two heavy chains; in protists, there is a third heavy chain that arose from a relatively recent duplication of the β-HC gene [Mitchell and Brown, 1994]. In *Chlamydomonas*, the duplicated heavy chain is the α-HC; in *Tetrahymena*, it is the γ-HC. The outer arm dyneins from some species can be partially dissociated into two parts, the β-HC (in protists, attached to its recently duplicated partner) and the "not β" chain: from sea urchin, β and α [Tang et al., 1982]; from *Chlamydomonas*, β/α dimer and γ [Sakakibara and Nakayama, 1998]; and from *Tetrahymena*, β/γ dimer and α (this study). The isolated *Chlamydomonas* γ-HC produced microtubule motility whereas the isolated α-HCs from sea urchin and *Tetrahymena* did not [Yano-Toyoshima, 1985; Sale and Fox, 1988] (this study).

Controlled urea treatment can be used to unfold in a stepwise manner a multi-domain protein such as dynein. In the experiments reported here, the urea treatment loosened the association between the α-HC and the rest of the dynein complex, but did not unfold the functional domains of the heavy chains, including the ATP- and microtubule-binding sites. The dissociation of the dynein by urea was reversible, a further indication that the urea treatment under these conditions did not significantly damage the proteins. The reconstitution of active 22S dynein also demonstrated that no other factors are required to assemble the outer arm dynein complex. Eight putative light chains (M_r 10–45K) were identified, which is consistent with other reports [Chilcote and Johnson, 1990; Christensen et al., 2001]. All but one of the light chains remained with the 19S species after urea treatment. Our data support a model in which the *Tetrahymena* 22S outer arm dynein includes a core of the β/γ dimer upon which is added the α-heavy chain. The previous study in which chymotrypsin digestion was used to free a fragment of the α-HC from the dynein complex [Toyoshima, 1987a] indicates that the approximately M_r 120K N-terminal portion of the α-HC plays an important role in binding this heavy chain to the dimer core.

Increased exposure time (90–120 min) further dissociated the dynein complex, as judged by SDS-PAGE. However, increasing the exposure time and/or increasing the urea concentration resulted in the irreversible inactivation of the MgATPase of the dynein, and we did not further pursue these other conditions.

Motility Produced by the Dissociated Dynein Species

In the present study, the *Tetrahymena* 19S β/γ dimer produced a significantly greater fraction of moving microtubules compared to that produced by the intact 22S dynein (92 vs. 65%); the 19S species also produced faster velocities (4.1 vs. 1.5 μm sec^{-1}). Thus, the presence of the α-HC resulted in microtubule gliding that was non-uniform, discontinuous, and slower than when the α-HC was removed. Even though the 14S α-HC retained significant MgATPase activity, its binding to microtubules was insensitive to ATP and it failed to support microtubule gliding under a variety of conditions.

Evidence for Heavy Chain Specialization in the Outer Arm Dynein Complex

In situ, axonemal outer arm dynein complex binds outer doublet microtubules in two ways: (1) ATP-sensitive binding of one or more of the globular heads with the B-tubule of the adjacent outer doublet microtubule, which is part of the force-producing cross-bridge cycle; and (2) ATP-insensitive structural binding of the base of the dynein with the A-tubule. In the present experiments, the dissociated species and the intact 22S dynein each bound microtubules in vitro. However, the α-HC binding to microtubules was ATP-insensitive whereas the binding by the other species was reversed by ATP. In the previous study, the chymotryptic fragment of the α-HC, which lacked the N-terminal region of the α-HC, did not bind microtubules in an ATP-insensitive manner [Toyoshima, 1987a]. Thus, one interpretation of these results is that the N-terminal region of the α-HC forms the base of the dynein complex and is required for the structural binding of dynein to the A-tubule.

A second interpretation of the results is that the ATP-insensitive microtubule binding by the isolated α-HC is through the B-link and reveals a specialized contribution of the α-HC to the cross-bridge cycle. Compared to the movement produced by the β/γ dimer, there was a significant impairment of movement with the intact and reconstituted dyneins (Table I, this study), suggesting that α-HC inhibited the free translocation of microtubules at the high dynein concentrations used in the experiment. Although the architecture of the axoneme ensures that the dyneins remain physically near the adjacent B-tubule, it may be important to tether the outer arm dynein to the microtubule track in order to appropriately position the motile β/γ heavy chains; the α-HC

may serve as the tether. Electron microscopy of axonemes suggested that a B-link maintains contact with the microtubule track even when the axonemes were fixed in the presence of ATP [Burgess, 1995].

Our results are consistent with the hypothesis that most of the force-producing activity resides in the β-HC or β/γ dimer, and that the α-HC serves to hold the dynein complex very near the surface of the microtubule track. A passive tethering function of the α-HC B-link would be very different from the force transferring function envisioned for the B-link of the force-producing heavy chain, in this case the α-HC [Asai and Koonce, 2001; Burgess et al., 2003]. Tethering the dynein to the microtubule track in this way would lead to the processivity of *Tetrahymena* outer dynein observed in low ATP concentrations [Hirakawa et al., 2000]. Future experiments will be aimed at understanding the orientation of the isolated α-HC when it binds microtubules, and how the α-HC contributes to the cross-bridge cycle of intact outer arm dynein.

ACKNOWLEDGMENTS

We thank K. Furuta for his help in analyzing the in vitro motility data. We thank Charles Brokaw for helpful comments, and we are grateful to the two reviewers who encouraged us to consider more completely the unique contributions of the α heavy chain to the outer arm dynein cross-bridge cycle. This work was supported by a grant from the Ministry of Education, Science, Sport and Culture of Japan to Y.Y.T. and grants from the National Science Foundation to D.J.A. We thank the Japan Society for the Promotion of Science, which enabled us to complete this study.

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