

# Isolated $\beta$ -heavy Chain Subunit of Dynein Translocates Microtubules In Vitro

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**Abstract.** Our goal was to assess the microtubule translocating ability of individual ATPase subunits of outer arm dynein. Solubilized outer arm dynein from sea urchin sperm (*Strongylocentrotus purpuratus*) was dissociated into subunits by low ionic strength buffer and fractionated by zonal centrifugation. Fractions were assessed by an in vitro functional assay wherein microtubules move across a glass surface to which isolated dynein fractions had been adsorbed. Microtubule gliding activity was coincident with the 12-S  $\beta$ -heavy chain-intermediate chain 1 ATPase fractions ( $\beta$ /IC1).

Neither the  $\alpha$ -heavy chain nor the intermediate chains 2 and 3 fractions coincided with microtubule gliding activity. The  $\beta$ /IC1 ATPase induced very rapid gliding velocities ( $9.7 \pm 0.88 \mu\text{m/s}$ , range 7–11.5  $\mu\text{m/s}$ ) in 1 mM ATP-containing motility buffers. In direct comparison, isolated intact 21-S outer arm dynein, from which the  $\beta$ /IC1 fraction was derived, induced slower microtubule gliding rates (21-S dynein,  $5.6 \pm 0.7 \mu\text{m/s}$ ;  $\beta$ /IC1,  $8.7 \pm 1.2 \mu\text{m/s}$ ). These results demonstrate that a single subdomain in dynein, the  $\beta$ /IC1 ATPase, is sufficient for microtubule sliding activity.

DYNEIN is a multiple subunit ATPase complex responsible for microtubule sliding in cilia and eukaryotic flagella and for certain forms of microtubule-based transport in the cytoplasm (reviewed in Gibbons, 1987). Numerous observations indicate that the sliding between microtubules results from a mechanochemical mechanism involving a cyclic detachment and reattachment of dynein to the adjacent microtubule concomitant with the binding and hydrolysis of ATP (reviewed in Johnson, 1985; Goodenough and Heuser, 1985). However, further appreciation of the mechanochemical mechanism of dynein will require detailed analysis of the molecular organization and function of its individual component parts.

One of the best studied dyneins is the outer arm dynein from sea urchin sperm tail axonemes. The outer arm can be solubilized as a functionally intact 21-S multimeric ATPase with a mass of 1,300 kD and at least nine different polypeptide subunits (Gibbons and Fronk, 1979). The 21-S outer arm ATPase appears to be the entire outer arm based upon functional reconstitution experiments (Gibbons and Gibbons, 1979), and is composed of the  $\alpha$ - and  $\beta$ -heavy chains ( $M_r > 400$  kD), three intermediate weight chains, and at least four light chains (Bell et al., 1979). Each of the heavy chain subunits possesses a nucleotide binding site and is capable of hydrolyzing ATP (Tang et al., 1982; Gibbons et al., 1987; Tang and Gibbons, 1987; Ow et al., 1987). EM has shown that each outer arm unit consists of two structurally distinct, large globular head domains joined by flexible stems and smaller globular subunits (Sale et al., 1985).

The isolated 21-S outer arm dynein can be dissociated and subfractionated into subunits by exposure to low ionic

strength buffers (Tang et al., 1982; Sale et al., 1985). The resulting subfractions include the following: the  $\beta$ -heavy chain-intermediate chain 1 ATPase subunit ( $\beta$ /IC1)<sup>1</sup>, the  $\alpha$ -heavy chain subfraction, and the intermediate chains 2 and 3 subfraction. Subunits can be separated by sucrose gradient centrifugation or by various forms of chromatography (Bell et al., 1982). Both the  $\beta$ /IC1 and  $\alpha$ -heavy chain subfractions contain ATPase activity, and EM has revealed that the  $\beta$ /IC1 subdomain comprises a pear-shaped globular head and attached stem-like domains, while the  $\alpha$ -heavy chain component comprises a distinct spherical head and possibly the associated stem domains (Sale et al., 1985). Although the  $\beta$ /IC1 and  $\alpha$ -heavy chain ATPases seem to have the requisite enzymatic activity and structural organization to drive microtubule translocation, the actual functional capacity of the subunits is not known.

Recently, an in vitro motility assay has been introduced by which microtubule-translocating activity was used in the discovery of kinesin (Vale et al., 1985a). This assay was subsequently adapted for study of the mechanochemical properties of isolated dynein obtained from ciliary or flagellar axonemes (Paschal et al., 1987a; Vale and Toyoshima, 1988). These assays consist of adsorption of isolated dynein to a glass slide followed by direct observation by video microscopy of microtubule gliding. These motility assays have directly led to the identification of functional forms of cytoplasmic dynein of a variety of cell types (Paschal et al., 1987b; Lye

1. Abbreviation used in this paper:  $\beta$ /IC1,  $\beta$ -heavy chain-intermediate chain 1 ATPase subunit of outer arm dynein.

et al., 1987; Paschal and Vallee, 1987; Vallee et al., 1988; Euteneuer et al., 1988). These dyneins may be responsible for vesicle transport (Pratt, 1986) or intermicrotubule sliding (Koonce et al., 1987). Such assays are also ideally suited for elucidation of the function of individual subunits of dynein. Therefore, we have used this assay to study the functional activity of isolated subunits of the outer dynein arm and report that the  $\beta$ /IC1 ATPase subunit is sufficient to induce rapid, linear translocations of purified microtubules.

## Materials and Methods

### Isolation of Dynein from Sperm Tail Axonemes

Axonemes were isolated from the sperm of the sea urchin *Strongylocentrotus purpuratus* by the method described by Piperno (1984). The outer arm dynein was then solubilized in a buffer containing 10 mM Tris, pH 7.3, 0.6 M NaCl, 4 mM MgSO<sub>4</sub>, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonic acid (PMSF), and 1 mM dithiothreitol (DTT) for 20 min at 4°C. To isolate the intact 21 S outer arm dynein, the extract was sedimented directly on 5–20% sucrose gradients made in 10 mM Tris, pH 7.3, 0.2 M NaCl, 4 mM MgSO<sub>4</sub>, 0.2 mM EDTA, 0.2 mM PMSF, and 1 mM DTT. For dissociation of outer arm dynein into subunits, the extract was dialyzed for 10 h at 4°C with two changes of a low ionic strength buffer containing 5 mM Tris, pH 7.3, 1 mM DTT, and 0.5 mM EDTA (Tang et al., 1982; Sale et al., 1985). The dialysate was sedimented through a 5–20% sucrose density gradient, made up in the same low ionic strength buffer, for 10 h at 39,000 rpm in a centrifuge rotor (model SW41; Beckman Instruments Inc., Palo Alto, CA). Twenty 0.6-ml fractions were collected from the bottom of the sucrose gradients and prepared for electrophoretic analysis, ATPase activity, and protein assay (Sale et al., 1985), or tested in the *in vitro* microtubule motility assay described below.

### Tubulin Purification and Microtubule Assembly

Bovine brain tubulin was purified by two cycles of temperature-dependent polymerization followed by DEAE-Sephacel ion exchange chromatography to separate tubulin from nontubulin proteins. Electrophoretic analysis revealed the DEAE-Sephacel tubulin fractions to be devoid of nontubulin proteins. Microtubules were assembled from purified tubulin at 37°C in 0.1 M Pipes, pH 6.8, 1 mM GTP, 1 mM EGTA, and 2 mM MgSO<sub>4</sub> at a tubulin concentration of  $\sim$ 9 mg/ml. After incubation for 30 min, the sample was mixed with an equal volume of the Pipes assembly buffer containing 20  $\mu$ M taxol and used directly in the motility assays following appropriate dilution.

### Motility Assay

For all motility assays, dynein-containing fractions were adjusted to  $\sim$ 0.2 mg/ml protein either by dilution in the motility buffer described below or by concentration in Centricon 30 cells (Amicon Corp., Danvers, MA). Motility assays were carried out in 5- $\mu$ l perfusion chambers constructed essentially as described in Vale and Toyoshima (1988). Dynein fractions were diluted 1:1 in motility buffer composed of 10 mM Tris, pH 7.3, 2 mM MgSO<sub>4</sub>, 1 mM EGTA, 0.1 M potassium acetate, 1 mM DTT, 1 mM ATP, and 10  $\mu$ M taxol to a final concentration of  $\sim$ 0.1 mg/ml. As described previously (Vale and Toyoshima, 1988), we found that there is a threshold of  $\sim$ 0.07 mg/ml for the induction of microtubule binding to and gliding on the glass surface. We discovered that, when present at subthreshold concentrations, dynein could be concentrated on the slide by incubations of successive 5- $\mu$ l aliquots in the perfusion chamber for 1 min each. Therefore, for all experiments described here, a standard system was followed: 5- $\mu$ l fractions were applied to the slide in two successive aliquots for 1-min incubation periods each. After incubation, the chamber was perfused with 50  $\mu$ l of motility buffer to rinse away nonadherent protein. 5  $\mu$ l of taxol-stabilized, purified bovine brain microtubules (0.25–0.35 mg/ml) were applied and nonadherent microtubules rinsed away by addition of 50  $\mu$ l of motility buffer. For microscopic observations, the ends of the perfusion chamber were usually sealed with VALAP (1:1:1 mixture of vaseline, lanoline, and paraffin).

Dark-field video microscopy used a 100-W mercury light source, heat reflecting and green interference filters, an oil-immersion dark-field condenser (NA 1.4) (Carl Zeiss Inc., Thornwood, NY), a Zeiss 40 $\times$  Plan Neofluar oil-immersion lens, and the SIT66 video camera (DAGE-MTI,

Inc., Wabash, MI). All assays were conducted at 21°C. Motility was recorded and analyzed using a half-inch video cassette recorder (model NV8950; Panasonic Co., Secaucus, NJ), and still images were obtained by directly photographing the monitor using Kodak technical pan film (4315). Gliding velocity was analyzed as described in Paschal et al. (1987a) using a stage micrometer, time generator, and single frame advances. ATP concentrations in motility buffers were verified by HPLC using a VIDAK 303 NT 405 nucleotide column (The Separation Group, Hesperia, CA). The criteria used in collecting data for microtubule velocity measurement included the following: microtubules moved in approximately straight paths for 20  $\mu$ m with no apparent hesitations, measurements were taken within the first 2 min after application of microtubules, and all gliding microtubules in a given field were analyzed.

### Materials

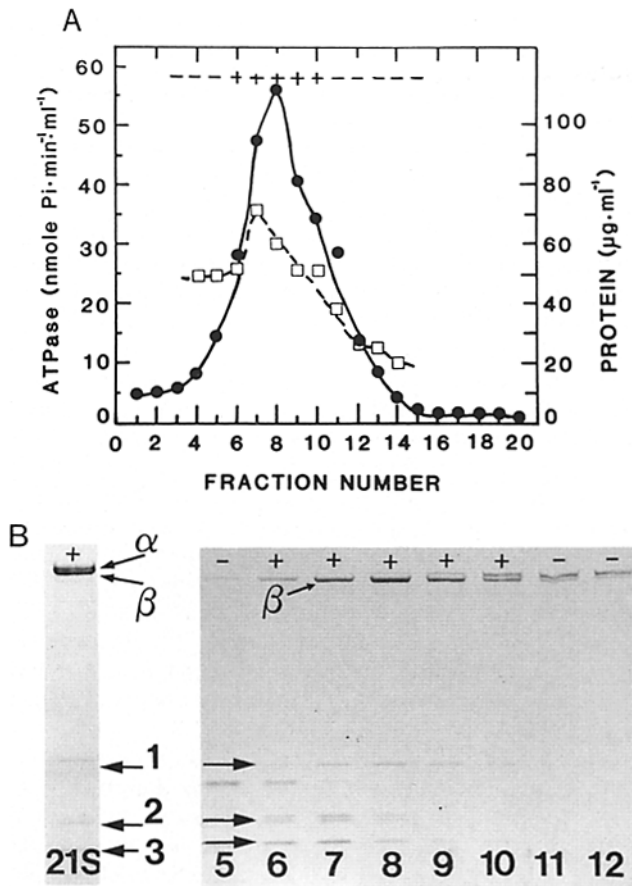
Deionized water was used throughout. ATP was from Boehringer Mannheim Diagnostics Inc. (Houston, TX), taxol was the generous gift of Dr. Matthew Suffness at the National Cancer Institute (Bethesda, MD), and bovine brain tubulin was generously supplied by Dr. Leah Haimo of the University of California at Riverside (Riverside, CA). DEAE-Sephacel was from Pharmacia Fine Chemicals (Piscataway, NJ). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

### Results

Outer arm dynein from isolated sea urchin sperm tail axonemes was solubilized in 0.6 M NaCl as previously described (Gibbons and Fronk, 1979; Bell et al., 1982; Sale et al., 1985). Peak ATPase activity sedimented at 21 S (Fig. 1 *B*, left) and contained the intact outer arm dynein composed of the  $\alpha$ - and  $\beta$ -heavy chains, intermediate chains 1, 2, and 3, and (not shown in low percentage acrylamide gels) at least four light chains (Gibbons and Fronk, 1979; Gibbons and Gibbons, 1979; Bell et al., 1979). Dialysis of the outer arm extract against 5 mM Tris-HCl, pH 7.3, 0.5 mM EDTA, and 1 mM DTT resulted in dissociation of the 21-S outer arm dynein into subfractions (Tang et al., 1982; Sale et al., 1985). After dissociation, the ATPase activity sedimented at  $\sim$ 12 S and was coincident with the  $\beta$ -heavy chain and intermediate chain 1 (Fig. 1, *A* and *B*, fractions 7 and 8). The ATPase peak contained only small amounts of the  $\alpha$ -heavy chain. The peak of the  $\alpha$ -heavy chain fraction sedimented toward the bottom of the gradient between 15 and 30 S as previously described (data not shown in entirety; see Tang et al., 1982).

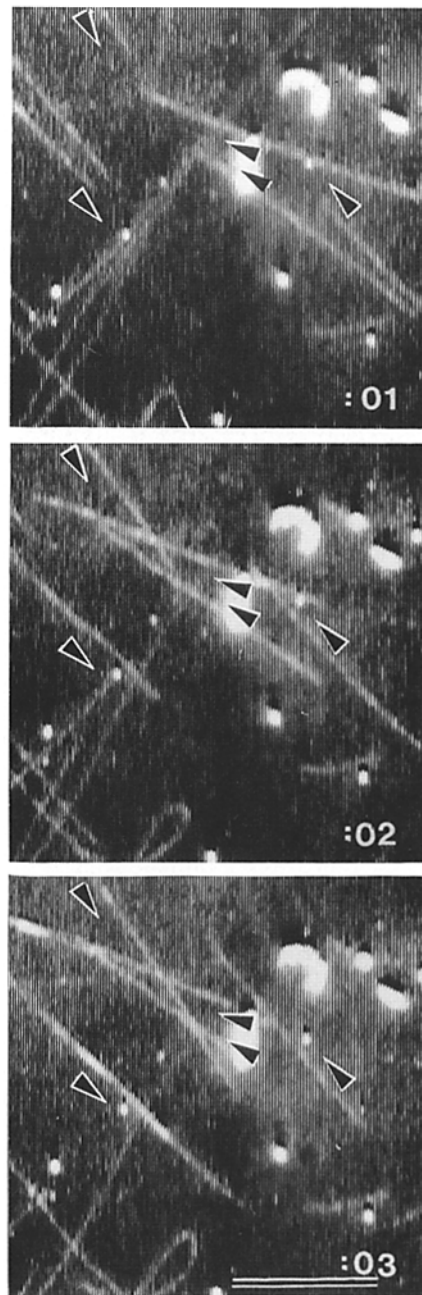
Using the methods described by Vale and Toyoshima (1988) and observing by dark-field video microscopy, we examined the ability of fractions to translocate microtubules (Fig. 2). As reported previously (Paschal et al., 1987a), the isolated 21-S outer arm dynein (Fig. 1 *B*, left) induced attachment and translocation of microtubules across the slide. Movement was rapid and unidirectional. Short microtubule fragments ( $<$ 5  $\mu$ m) frequently showed intermittent movement. Longer microtubules moved continuously. In the absence of dynein, microtubules never bound to the glass surface. Gliding velocity, in 1 mM Mg-ATP<sup>2-</sup>, was  $\sim$ 5.6  $\mu$ m/s (Fig. 3), which is faster than that reported by Paschal et al. (1987a).

The isolated  $\beta$ /IC1 subfraction also induced microtubule translocation *in vitro* (Figs. 1 and 2). The 12 S ATPase peak and microtubule translocating ability (Fig. 1 *A*, +) were coincident with the  $\beta$ /IC1 subfractions. Motility from the peak fraction (Fig. 1, *A* and *B*, fraction 8) is illustrated in Fig. 2 and was not qualitatively different from that observed in Fig. 1, *A* and *B*, fractions 6–10. Thus, the contaminating  $\alpha$ -chain in Fig. 1, *A* and *B*, fractions 7–10 did not appear to

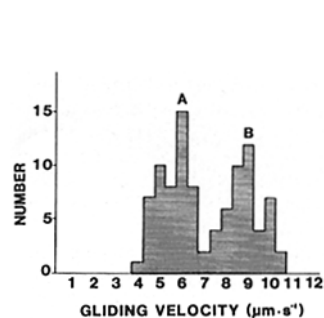


**Figure 1.** Analysis of density gradient of the low ionic strength dialysate of the dynein extract as described (Tang et al., 1982; Sale et al., 1985), illustrating the separation of  $\alpha$ - and  $\beta$ -heavy chain subunits. (A) The ATPase activity (●) and protein (□) from a sucrose gradient designed to isolate the 12-S  $\beta$ /IC1 ATPase subunit. Sedimentation direction is to the right, and microtubule translocating ability, as described in Fig. 2, is designated (+ and -). (B) The polypeptides from fractions 5-12 are shown on the bottom right. Notably, the peak ATPase activity is coincident with the microtubule gliding activity as well as the  $\beta$ -heavy chain and intermediate chain 1 fractions. The polypeptide composition of the peak fraction from another sucrose density gradient used to isolate the intact 21-S outer arm dynein is shown at the bottom left. The gels were stained with Coomassie Blue, and the separated  $\alpha$ - and  $\beta$ -heavy chains as well as intermediate chains 1, 2, and 3 are indicated.

contribute to motility in any obvious way. Essentially 100% of the microtubules glide in an uninterrupted fashion, usually in straight paths, and at extremely high velocities. The microtubules appeared to be more adherent to the substrate than comparable microtubules translocated by the intact 21-S outer arm dynein fraction. This description of the adherence of microtubules to the substrate is similar to that described for brain dynein (MAPIC) compared to outer arm dynein (Paschal et al., 1987a) and for 14-S dynein compared to 22-S dynein extracted from *Tetrahymena* cilia (Vale and Toyoshima, 1988). Also, short microtubules ( $\sim 5 \mu\text{m}$ ) showed less intermittent movement in comparison to assays using 21-S outer arm dynein. The average maximum velocity of the peak fraction was  $9.7 \pm 0.88 \mu\text{m/s}$  ( $n = 100$ ) with a narrow distribution and a range from 7.0 to 11.5  $\mu\text{m/s}$ . Velocity was



**Figure 2.** Successive fields from typical video views, separated by 1 s, of dynein-mediated microtubule gliding. These frames are taken from assays of the peak  $\beta$ /IC1 fraction (Fig. 1, fraction 8). Nearly every microtubule glides at  $\sim 9 \mu\text{m/s}$  in this field. The arrowheads are used as reference points in each field. Bar, 10  $\mu\text{m}$ .



**Figure 3.** Histograms of the velocity of microtubule gliding measured in motility buffer containing 1 mM Mg-ATP<sup>2-</sup> for both the purified 21-S outer arm dynein ( $5.6 \pm 0.7 \mu\text{m/s}$ ,  $n = 50$ ) and the 12-S  $\beta$ -heavy chain subunit fraction ( $8.7 \pm 1.2 \mu\text{m/s}$ ,  $n = 50$ ). Velocities are given as mean  $\pm$  SD and were measured as described in Paschal et al. (1987a).

independent of microtubule length. As described before (Tang et al., 1982; Sale et al., 1985), intermediate chains 2 and 3 sediment at  $\sim 9$ – $10$  S and were neither coincident with ATPase activity nor microtubule gliding activity. Similarly, the  $\alpha$ -heavy chain sedimented as an aggregate from  $\sim 15$  to  $30$  S and was not coincident with ATPase activity under these conditions, nor did it support microtubule gliding activity.

In other experiments, the microtubule gliding velocity induced by the 12-S  $\beta$ /IC1 subfractions was directly compared with the intact 21-S outer arm dynein in identical conditions (Fig. 3). The 21-S outer arm fraction generated an average maximal gliding velocity of  $5.6 \pm 0.7$   $\mu\text{m/s}$  (Fig. 3A), whereas the isolated  $\beta$ /IC1 subunit induced an average maximal gliding velocity of  $8.7 \pm 1.2$   $\mu\text{m/s}$  (Fig. 3B). This was a consistent result ( $n = 5$  entirely different experiments). Further, the isolated  $\beta$ /IC1 subfraction generated the highest velocity of microtubule gliding yet described (cf., Paschal et al., 1987a; Vale and Toyoshima, 1988). Maximum microtubule gliding velocity, for both 21-S outer arm dynein and its  $\beta$ /IC1 subunit, was independent of microtubule length, as previously reported (Paschal et al., 1987a).

## Discussion

In this work we have examined the ability of purified outer arm dynein and its isolated subunits to translocate microtubules along a glass surface to which dynein components had been adsorbed. The advantages of the perfusion system and assay have been discussed (Vale and Toyoshima, 1988). We confirm that the outer arm dynein by itself is capable of inducing microtubule gliding (Paschal et al., 1987a), and we demonstrate that the  $\beta$ -heavy chain–intermediate chain 1 ATPase subunit is sufficient for microtubule translocation.

Microtubules move continuously in a single direction for both 21-S outer arm dynein and its  $\beta$ /IC1 subunit. Presumably only those dynein molecules adsorbed with the proper orientation relative to the inherent structural polarity of the microtubule can bind to and generate force with that microtubule; misoriented dynein molecules evidently do not interact with microtubules. Similar conclusions have been drawn for the polarity of microtubule movement on kinesin-coated slides (Vale et al., 1985b) and for the polarity of thin filament movement on myosin-coated slides (Toyoshima et al., 1987). We predict that dynein fractions generate force such that the “+ end” of the moving microtubule leads (Sale and Satir, 1977; Fox and Sale, 1987; Paschal and Vallee, 1987; Vale and Toyoshima, 1988). This prediction can be tested experimentally.

One potential problem with our conclusion that the  $\beta$ /IC1 ATPase subunit is sufficient for microtubule translocation is that none of the active fractions (Fig. 1, A and B, fractions 6–10) is completely pure. Fig. 1, A and B, fractions 6–8, are contaminated with intermediate chains 2 and 3, and Fig. 1, A and B, fractions 8–10, are contaminated with the  $\alpha$ -heavy chain. However, the simplest interpretation of these data is that the  $\beta$ /IC1 subunit is sufficient for microtubule gliding activity, since activity only correlated with the  $\beta$ /IC1 subfractions and was not coincident with either the  $\alpha$ -heavy chain fractions or the intermediate chain 2 and 3 subfractions. These results do not eliminate the possibility that the  $\alpha$ -heavy chain subunit is also capable of microtubule translocation, since under these low ionic strength buffer conditions

$\alpha$ -heavy chain particles tend to aggregate and lose ATPase activity (Tang et al., 1982). It is possible that under different isolation conditions the  $\alpha$ -heavy chain subunit will also induce microtubule translocation.

There appears to be a threshold concentration of dynein required in the assay, as designed, for microtubules to bind and crawl across the surface. This observation may suggest that, as designed, the proteins (at  $\sim 70$   $\mu\text{g/ml}$ ) adsorb very quickly and completely to the glass surfaces. The idea that the proteins are efficiently adsorbed in the perfusion system is supported by experiments in which we were able to “concentrate” subthreshold amounts of force-generating proteins by successive 1-min additions to the glass surface. For example, we found that at  $\sim 35$   $\mu\text{g/ml}$  dynein, a 2-min incubation would not support microtubule binding or movement. However, two successive 1-min incubations of the same fraction resulted in microtubule gliding. If we assume that 100% of the molecules in a 5- $\mu\text{l}$  aliquot (at 0.1 mg/ml) adsorb to the surfaces of the perfusion chamber ( $\sim 70$   $\text{mm}^2$ ) and assume a mass of 1,300 kD, and that all molecules are properly aligned, then the density of aligned dyneins would be  $\sim 250$  per  $\mu\text{m}^2$ . However, it is unlikely that all adsorbed dyneins were aligned properly for movement, and it is therefore likely that there is a relatively large distance between aligned dyneins.

The isolated  $\beta$ /IC1 subfraction induced the fastest microtubule gliding velocities yet described (cf., Paschal et al., 1987a; Vale and Toyoshima, 1988). The maximum velocity ( $\sim 11$ – $12$   $\mu\text{m/s}$ ) approaches the maximum velocity of microtubule sliding between pairs of doublet microtubules in flagellar axonemes with both the outer and inner row of dynein arms ( $\sim 16$   $\mu\text{m/s}$ ; see Takahashi et al., 1982; Yano and Miki-Noumura, 1981; Fox and Sale, 1987) and is greater than the maximum velocity of microtubule sliding induced by the inner row of dynein arms alone ( $\sim 7$ – $8$   $\mu\text{m/s}$ ; Yano and Miki-Noumura, 1981; Fox and Sale, 1987; Sale et al., 1988; Sale, W. S., and L. A. Fox, unpublished data). Furthermore, the maximum gliding velocity was independent of the length of the microtubule (cf., Paschal et al., 1987a). Therefore, maximum velocity would appear to be independent of the number of force-generating cross-bridges, assuming that microtubules provide a negligible load. Within the axoneme, maximum microtubule sliding velocity is independent of the amount of overlap between adjacent doublet microtubules (Takahashi et al., 1982; Okagaki and Kamiya, 1986; Sale, W. S., and L. A. Fox, unpublished observations). Therefore, again assuming that microtubules provide a negligible load, the maximum velocity is likely to be limited by a rate-limiting kinetic step in the force-generating cycle of each individual force generator.

Of particular interest was the observation that the intact 21-S outer arm dynein generated a slower microtubule gliding velocity than its isolated 12-S  $\beta$ /IC1 subunit (Fig. 3). There are several possible explanations for this differential velocity between dynein and its  $\beta$ /IC1 subunit. The difference may have resulted from technical problems associated with the absorption of 21-S dynein to glass. It is also possible that the difference in maximal velocity was a property of each cross-bridge and that the intact 21-S dynein has the capability to regulate the gliding velocity generated by the  $\beta$ /IC1 subdomain. The isolated intact 21-S outer arm dynein has a relatively low ATPase activity, which can be activated by chemi-

cal procedures which cause dissociation of subunits (Gibbons and Fronk, 1979; Tang et al., 1982). The activation of total ATPase activity by separation of subunits may be related to the increase in gliding velocity of isolated  $\beta$ /IC1 subunit described here. Alternatively, the reduced microtubule gliding velocity may result from binding of microtubules at the ATP-insensitive binding site on the  $\alpha$ -heavy chain. There is evidence that the isolated  $\alpha$ -heavy chain is an ATPase (Tang et al., 1982; Gibbons et al., 1987) and that the  $\alpha$ -heavy chain either directly or indirectly mediates ATP-insensitive binding of dynein to the A-microtubules (Bell and Gibbons, 1982). Furthermore, both ATP-sensitive and -insensitive binding of axonemal dynein to microtubules has been demonstrated (Haimo et al., 1979; Haimo and Fenton, 1984, 1988). Therefore, in the motility assay, if we assume 21-S dynein adsorbs to the surface randomly, microtubules could bind to the ATP-insensitive "A-end" binding site. Such binding might provide an effective load which would resist sliding and reduce the maximum sliding velocity. Interestingly, using a similar assay system designed to study muscle proteins, Toyoshima et al. (1987) report that the isolated heavy meromyosin subdomain of myosin generates faster maximal gliding velocity of thin filaments than the comparable intact myosin from which it was derived.

In summary, the isolated 12-S  $\beta$ /IC1 subunit is sufficient for microtubule translocation, and the isolated  $\beta$ /IC1 subunit generates faster maximal microtubule gliding velocity than the intact 21-S outer arm dynein from which it was derived. The results support the hypothesis that the  $\beta$ /IC1 ATPase subunit of outer arm dynein functions in the axoneme by forming force-generating cyclic cross-bridges with the adjacent B-microtubule, with such activity resulting in microtubule sliding. The results do not eliminate the possibility that the  $\alpha$ -heavy chain ATPase also generates microtubule sliding in the axoneme. With the assays described in this report, we are now able to continue reconstitution experiments to investigate the functions, interactions, and regulation of the remaining subunits of the outer dynein arms.

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