Tryptic Digestion of Dynein 1 in Low Salt Medium

ORIGIN AND PROPERTIES OF FRAGMENT A*

(Received for publication, April 30, 1986)

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Dynein 1 was extracted from sperm flagella of the sea urchin *Tripneustes gratilla* with 0.6 M NaCl and dialyzed against 0.5 mM EDTA, 14 mM 2-mercaptoethanol, 5 mM imidazole/HCl buffer, pH 7.0, for 24–48 h. In some cases, fractions containing the α heavy chain and the β/intermediate chain 1 complex (β/IC1) were separated by density gradient centrifugation in the same solution. Treatment of the samples at a trypsin:protein ratio of 1:10 w/w for 32 min at room temperature yields a crude digest from which Fragment A is purified by density gradient centrifugation. The purified Fragment A consists of two principal peptides (M, 195,000 and 130,000) that cosediment with the peak of ATPase activity at 12.5 S, which is slightly faster than the 11 S of the original β/IC1 complex. When digests of the separated α chain and of the β/IC1 complex are followed as a function of time, the early cleavages of the two heavy chains proceed to form Fragment A. The remainder of the β chain, termed Fragment B, occurs as an M, 110,000 peptide sedimenting at 5.7 S with no associated ATPase activity. Fragment A has a specific ATPase activity of 4.3 μmol P, min⁻¹·mg⁻¹, with a K₅₀ of 29 μM in 0.1 M NaCl medium, and an apparent K₅₀ for inhibition by vanadate of 1.2 μM in the absence of salt, and 22 μM in 0.6 M NaCl. Photoaffinity labeling with [α-³²P]8-azidoadenosine 5’-triphosphate indicates that the ATP binding site on the β chain of dynein 1 is located on the M, 195,000 peptide of Fragment A. The possibility that Fragments A and B of the β/IC1 complex may correspond to the head and tail regions of the tadpole-shaped particle seen by electron microscopy is discussed.

Dynein 1 ATPase constitutes the outer arms on the doublet tubules of sea urchin sperm flagella, where it plays a major role in converting the chemical energy provided by ATP hydrolysis into the sliding movements between adjacent doublets that underlie normal flagellar movement (1). When demembranated flagella are extracted with 0.6 M NaCl, the dynein 1 is solubilized as a 21 S particle of M, 1,250,000 with a low latent ATPase activity (2). Such preparations retain the functional capability of rebind and restoring the beat frequency of dynein-depleted sperm flagella (3).

The 21 S dynein 1 is a large multisubunit complex consisting of two heavy polypeptide chains, α and β, each of M, 428,000, three intermediate chains, IC1, IC2, and IC3 of M, 122,000, 87,000, and 78,000, as well as 4–6 subunits of M, 15,000–25,000 (4, 5). Exposure of the 21 S dynein to low salt causes it to dissociate into the α heavy chain, a complex of the β heavy chain with IC1 (β/IC1) and a complex of IC2 and IC3. There is ATPase activity associated with both the α and the β/IC1 fractions (6, 7). The vanadate anion (V₅⁰) is a potent inhibitor of dynein ATPase and of flagellar motility (8, 9), acting by replacing the P, in the dynein-ADP·P₅ product complex to form a more stable dynein-ADP·V₅ complex that acts as a dead-end kinetic block (10–12). UV irradiation of the dynein 1 in the presence of ATP and V₅ cleaves the α and β heavy chains at specific sites near their middle, suggesting that the ATP and V₅ binding sites involve the midregions of the α and β chains; the ATP binding site is located in the slightly heavier of the two cleavage peptides from each chain (5, 32).

Limited proteolysis is a powerful method for investigating the domain structure of complex proteins, and earlier work from this laboratory (13) demonstrated that limited tryptic digestion of 21 S dynein 1 in 0.6 M NaCl produced well-defined peptides of the α and β heavy chains. On this basis a two-headed model for the dynein outer arm, attached at its base by salt-sensitive bonds to the A tubule of one doublet and attached through one of its heads by ATP-sensitive bonds to the B tubule of the next doublet, was proposed (13, 14).

The detailed electron microscopic fine structure of soluble dynein was first observed by Johnson and Wall (15), who showed that the 22 S dynein from *Tetrahymena* cilia consists of three globular heads (~15 nm diameter), joined by slender flexible stems (25–30 nm long) to a common base. Similar more recent study of the comparable 21 S dynein 1 from sea urchin sperm flagella has shown that it consists of just two globular heads joined by slender stems, and in this case it was possible to say that each of the globular heads and its stem is formed from a single heavy chain, with the head containing the α chain being spherical and that containing the β/IC1 complex being pear-shaped (16). IC2 and IC3 possibly constitute smaller globular units associated with the stem overlap region. Other studies of outer arm dynein from *Chlamydomonas* flagella have shown that it is similar to that from *Tetrahymena*, with each outer arm consisting of three head/stem units and containing three electrophoretically distinct heavy chains (17, 18). In all cases, the number of head/stem units observed in a dynein or dynein subfraction by electron

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*This work was supported in part by Grant GM 30401 from the National Institute of General Medical Sciences. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡The abbreviations used are: IC1, IC2, and IC3, intermediate chains 1, 2, and 3; 8-N-ATP, 8-azidoadenosine 5’-triphosphate; DI digestion index; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; iEM solution, imidazole-EDTA-mercaptoethanol solution (see "Materials and Methods"); V₅⁰, inorganic vanadate.
Basis of this correlation and of the molecular weight balance, the sucrose procedure (22). The crude dynein 1 was either used photoaffinity labeling with 8-azidoadenosine 5'-triphosphate (8-N-ATP) to locate the ATP binding site. Tryptic digestion of dynein at low salt generates a well-defined particle, termed Fragment A, that has a native M, of 370,000 and retains much of the ATPase activity of the original dynein. It consists principally of two peptides of M, = 195,000 and 130,000 and is relatively resistant to further proteolysis. Fragment A is reported to have little affinity for rebinding to extracted flagellar doublet tubules, although its ATPase activity is stimulated up to 70% by crude flagellar tubulin (20).

In this paper, we describe the main digestion pathway leading to formation of Fragment A from dynein 1 and have used photoaffinity labeling with 8-azidoadenosine 5'-triphosphate (8-N-ATP) to locate the ATP binding site. Tryptic digestion of dynein 1 under these low salt conditions attacks localized regions of the β heavy chain relatively rapidly and selectively so that a simple pattern of tryptic cleavage leads to formation of two subparticles that are separable by density gradient centrifugation. This opens the possibility for future study of the properties of the different individual functional regions of the β heavy chain in the dynein outer arm.

**MATERIALS AND METHODS**

**Preparation of Dynein 1 and Separated Subunit Fractions**—Dynein 1 was prepared from sperm flagella of the sea urchin *T. gratilla* by the sucrose procedure (22). The crude dynein 1 was either used immediately or was stored as an (NH₄)₂SO₄ precipitate at 0 °C until required. In all cases, the dynein 1 was dialyzed for 24–48 h against a buffer containing 5 mM imidazole/HCl, pH 7.0, 0.5 mM EDTA, and 14 mM 2-mercaptoethanol (IEM buffer) prior to digestion. Zonal centrifugation of this digested preparation on a 5–20% sucrose gradient in IEM buffer yields two fractions, one containing principally the α heavy chain and the other containing the β/IIC1 complex as well as some of IC2 and IC3 (6, 14, 22).

**Digestions**—The extent of digestion is expressed by the digestion index parameter (DI) introduced by Bell and Gibbons (13), where DI = (protease concentration in mg/ml per protein concentration in mg/ml) × (digestion time in min) × 10⁴.

Digestions were routinely conducted in IEM buffer at a trypsin-protein weight ratio of 1:10 for 32 min at 22–24 °C (DI = 32,000) and were stopped by adding a 10-fold weight excess of soybean trypsin inhibitor. Samples for ATPase determination were stored on ice until assay. Samples for electrophoresis were heated in a boiling water bath for 2 min prior to addition of 5 × concentrated sample dye buffer containing 5% sodium dodecyl sulfate and were then rapidly cooled on ice.

Stock trypsin solutions were prepared freshly in 1 mM HCl at a concentration of 2 mg/ml. The activity of the stock trypsin solutions was monitored with the artificial substrate p-toluene-sulfonyl-L-arginine methyl ester. Solutions of soybean trypsin inhibitor were prepared freshly in 0.1 M Tris/HCl buffer, pH 8.0, at concentrations of 4–10 mg/ml.

**Preparation and Purification of Fragment A**—Routine preparations of Fragment A were made either from crude dynein 1 or from the separated β/IIC1 fraction by digestion to a DI of 32,000. Fragment A was purified from this crude digest by centrifugation through a 5–20% sucrose density gradient in IEM buffer for 20 h at 37,000 rpm or as noted in the figure legends.

**Electrophoresis**—Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed on 3–6% gradient gels by the method of Laemmli (23) or on 8% gels by the method of Dreyfuss et al. (24) followed by staining in Coomassie Brilliant Blue R-250. The quantitation of material in gel bands was performed by extraction of the dye from the excised band, and molecular weights of peptide bands were determined as described previously (5). Quantitation of ³²P was performed by heating the excised gel band overnight at 60 °C in 1 ml of 19 parts 30% H₂O₂, 1 part 30% NH₄OH (v/v), before adding to scintillation fluid. Appropriate areas of gel for background compensation were also excised and dissolved.

**RESULTS**

**Origin of Fragment A**

Sucrose density gradient centrifugation of dynein 1 that has been digested by trypsin in IEM buffer separates two major fragments derived from the heavy chains of the original dynein (Fig. 1). Fragment A contains ATPase activity, sediments at approximately 12.5 S, and consists of two principal tryptic peptides of M, = 195,000 and 130,000, often accompanied by small amounts of lower M, peptides that appear to be the result of more extensive digestion. Fragment B is a particle with no ATPase activity that sediments at approximately 5.7 S. Depending upon the exact extent of digestion, it appears as either a single or a closely spaced pair of electrophoretic bands of mean M, 110,000 (Fig. 1). This paper describes the origin and properties of Fragment A; the isolation and properties of Fragment B are under investigation and will be published later.

**Digestion Pathways of Separated α and β/IIC1 Fractions**—The high molecular weight of the main peptides in Fragment A indicates that they are derived from either one or both of the original α and β heavy chains in intact dynein. We examined the origin of Fragment A peptides by comparing tryptic digests of the separated α and β heavy chain fractions with those of the unseparated mixture (Fig. 2). Gel electro-

**Fig. 1.** ATPase activity and polyacrylamide gel analysis of the fractions from a sucrose gradient separation of digested dynein 1. 13.6 ml of freshly prepared dynein 1 (0.7 mg/ml) were dialyzed for 72 h against 2 liters of 0.5 mM EDTA, 14 mM 2-mercaptoethanol, 5 mM imidazole/HCl buffer, pH 7.0, at 4 °C, with one change. The dialyzed dynein was then digested at a trypsin-protein ratio of 1:10 for 32 min at room temperature (DI = 32,000), before the digestion was stopped by adding a 10-fold weight excess of soybean trypsin inhibitor and chilling to 0 °C. 6 ml of the digested sample were loaded onto 5–20% sucrose gradients prepared in dialysis buffer and were centrifuged in a SW-41 (Beckman) centrifugation rotor at 31,400 rpm for 20 h at 4 °C. After separation of the gradients into 17fractions, 50 µl-aliquots of the fractions were assayed for ATPase activity and 100-µl aliquots were loaded onto the lanes of an electrophoresis gel (4–8% w/v polyacrylamide gradient of Laemmli composition (23)). The top of gradient is fraction 1 and the bottom is fraction 17. The distribution of ATPase activity in the fractions is shown by the dotted line. Fragment A peaks in fractions 10–11 corresponding to a sedimentation coefficient of 12.5 S; Fragment B peaks in fractions 4–5, corresponding to 5.7 S. The dots on the right side of the figure indicate the positions of the α and β heavy chains, IIC1, IIC1, IC2, and IC3 (from top to bottom) in an adjoining M, standard lane that is not shown.
phoretic analysis shows that at a DI of 32,000, the digest of the β/IC1 fraction contains the two tryptic peptides of M$_r$ = 195,000 and 130,000 that are characteristic of Fragment A, as well as a peptide of M$_r$ = 110,000 that corresponds to Fragment B. At the same stage of digestion, the separated α chain fraction shows a small amount of what might be the M$_r$ 195,000 peptide of Fragment A, but no trace of the 130,000 peptide is apparent. This comparison indicates that all of the M$_r$ 130,000 and most of the M$_r$ 195,000 components characteristic of Fragment A are peptides derived from the original β heavy chain, with a peptide derived from the α heavy chain possibly making a minor contribution to the 195,000 component.

The progressive changes in peptide band pattern with increasing DI up to 32,000 provide information regarding the pathway of formation of Fragment A. This is seen most clearly in digests of the β/IC1 fraction, in which formation of Fragment A proceeds through a single intermediate of M$_r$ 296,000 (Fig. 2b), with its apparent complement being a peptide of M$_r$ 132,000 that migrates just ahead of the position of the intermediate chain 1' in undigested preparations. The subsequent growth in intensity of the 195,000 and 130,000 components of Fragment A appears to occur in parallel, suggesting that these components are complementary peptides formed by cleavage of the M$_r$ 296,000 intermediate, with the approximate 8% discrepancy in molecular weight being due to anomalous binding of sodium dodecyl sulfate by one peptide (25). The 132,000 peptide from the first cleavage of the β chain is the probable source of the 110,000 component of Fragment B, after having passed through one or more transient intermediates; however, the numerous peptides in the 135,000–110,000 range make the details of this transition uncertain. As reported previously (13), the original IC1 polypeptide of the β/IC1 complex is digested very early, and on gels it appears essentially all gone by a DI of 200. However, it is possible that some of the peptides formed by cleavage of IC1 may remain physically associated with the Fragment A particle.

The digestion pattern of the separated α chain fraction shows a prominent early intermediate of M$_r$ 316,000 (Fig. 2b), but longer digestion yields many very faint bands with little in the way of semi-stable peptides, except for the low intensity band of M$_r$ 195,000 at a DI of 32,000 that was mentioned above.

The pathways of the α and β heavy chains resemble each other in that both give rise to a moderately stable intermediate of M$_r$ 296,000–316,000, but there is no evidence of similarity in the subsequent steps. In particular, they differ greatly in quality, with the β chain giving a small number of well-defined peptides and the α chain giving mostly a multitude of faint bands (Fig. 2, b and c). On the basis of these data, we propose the scheme shown in Fig. 3 for the principal pathway of tryptic digestion of the β heavy chain of dynein 1 in low salt medium. The major early site of tryptic cleavage of the β chain is termed the T1 site, and the major site at which the relatively slow cleavage of the M$_r$ 296,000 intermediate occurs is termed the T2 site.

Effect of Digestion on ATPase Activity—In order to obtain additional evidence on the origin of Fragment A, we compared the effects of digestion on the ATPase activity of the whole dialyzed dynein with its effects on the ATPase activity of the separated heavy chain fractions (Fig. 4). The ATPase activity of the whole dynein increases rapidly with small amounts of digestion up to DI of about 50 and then increases more slowly, peaking with an approximate 3-fold activation over its initial

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In many preparations of dynein, a portion of the intermediate chain 1 occurs in an alternate form (IC1') with an apparent M$_r$ of 135,000, but the exact relationship between these two forms is not yet clear. (I. R. Gibbons and G. Klein, unpublished data.)
appear as closely spaced doublets, suggesting the possible presence of closely related parallel pathways which involve early nicking of a small portion (~3,000) from the products are shown and some small peptides may be lost at certain stages. At certain stages of the digestion, some of the major bands appear as closely spaced doublets, suggesting the possible presence of closely related parallel pathways which involve early nicking of a small portion (~3,000) from the β chain. Numbers denote $M_r$ in thousands.

![Fig. 3](image3.png)

**Fig. 3.** Principal pathway of tryptic cleavage of the dynein $\beta$ heavy chain in low salt medium. $T_1$ indicates the major early site of cleavage in the $\beta$ chain and $T_2$ the major site of subsequent cleavage of the M, 296,000 intermediate. Only the major cleavage products are shown and some small peptides may be lost at certain stages. At certain stages of the digestion, some of the major bands appear as closely spaced doublets, suggesting the possible presence of closely related parallel pathways which involve early nicking of a small portion (~3,000) from the $\beta$ chain. Numbers denote $M_r$ in thousands.

level at a DI of 15,000, and then declining slowly. The effect of digestion on the ATPase activity of the $\beta$/IC1 complex is very similar, with the level of its specific activity being 2-3-fold greater than that of whole dynein at all stages of digestion. The specific ATPase activity of the $\alpha$ chain fraction is initially about 25% of that of the $\beta$/IC1 complex, and although it increases about 2-fold over the course of the digestion, it remains a minor contribution to the total ATPase activity of whole dynein. These data indicate that 80-90% of the ATPase activity in samples of Fragment A prepared from whole dynein is associated with tryptic peptides deriving from the $\beta$/IC1 fraction.

**Fig. 4.** Effect of trypsin digestion on the ATPase activity of dialyzed dynein 1 and of the separated $\alpha$ and $\beta$/IC1 fractions. Samples digested to the indices shown were assayed for ATPase activity in medium containing 0.1 M NaCl, 2 mM MgSO$_4$, 1 mM ATP, 0.1 mM EDTA, 10 mM Tris/HCl buffer, pH 8.0.

**Fig. 5.** Electrophoresis gel showing sucrose gradient sedimentation of mixed sample containing undigested $\alpha$ and $\beta$ heavy chains, $M_r$ 316,000 and 296,000 intermediates, and the $M_r$ 195,000 and 130,000 peptides of Fragment A. A standard preparation of low salt-dialyzed dynein 1 was divided into two. One half was digested with trypsin at a ratio of 1:10 (w/w) for 32 min (DI = 32,000) and the digestion stopped with a 10-fold excess of soybean trypsin inhibitor. 0.5 ml of the digested preparation was mixed with 0.5 ml of the undigested preparation and loaded onto a 5-20% sucrose density gradient prepared in IEM solution and centrifuged as in Fig. 1. The M, 316,000 and 296,000 intermediates were formed from residual tryptic activity after mixing. The intact $\beta$/IC1 complex, which cosediments with the M, 296,000 intermediate and peaks in fraction 8, is taken as a sedimentation standard of 11 S for this gradient (see Refs. 6 and 14). The M, 195,000 and 130,000 peptides of Fragment A peak in fractions 8-9, corresponding to approximately 12.5 S, and Fragment B peaks in fractions 4-5 corresponding to approximately 5.7 S. The M, 316,000 intermediate from the $\alpha$ chain sediments partially with the aggregated intact $\alpha$ chain in fractions 10-17 and part of it trailing toward fraction 10.

**Enzymatic Properties of Fragment A**

Hydrolysis of ATP and 8-N$_3$ATP—In standard assay medium containing 0.1 M NaCl and 1 mM MgATP at pH 8.0, preparations of Fragment A hydrolyzed ATP at an average rate of 4.3 ± 0.7 μmol Pi·mg$^{-1}$·min$^{-1}$. In the same medium, the $K_m$ of Fragment A averaged 29 ± 9 μM, substantially greater than the value of 1.3 μM for latent, undigested dynein 1 determined at the same time as a control. The $K_m$ of Fragment A in assay medium containing 0.1 M sodium acetate averaged 19 ± 1 μM.

At a concentration of 1 mM, 8-N$_3$ATP was hydrolyzed by Fragment A at about 10% of the rate of ATP. The accuracy of the data at lower substrate concentrations did not permit a valid determination of the $K_m$.

**Sensitivity to Vanadate Inhibition**—Since the sensitivity of dynein ATPase to vanadate inhibition depends markedly upon the conditions used for the ATPase assay (8, 9, 12), we examined the vanadate sensitivity of Fragment A ATPase in standard assay media containing no added salt, 0.45 M sodium acetate, or 0.6 M NaCl. The apparent values of $K_v$ in these media, defined as the $V_i$ concentration giving 50% inhibition, were found to be 1.2, 2.1, and 22 μM, respectively.

**Localization of ATP-binding Site**—Previous photoaffinity labeling studies on sea urchin dynein with [α-32P]8-N$_3$ATP (5) have shown that the 8-N$_3$ATP binding is primarily localized in the heavy chains of native 21 S dynein 1. In the present work, we have quantitated the $^{32}$P in the heavy chain band and found an average level of 4.1 nmol $^{32}$P/mol of polypeptide, with this being reduced 89% by competition with 50 μM ATP (Table I). Lesser amounts of $^{32}$P are found in IC1 and IC2, as
TABLE I
Quantitation of [α-32P]8-N3-ATP incorporated into polypeptides of dynein 1 and of Fragment A

The dynein 1 and Fragment A were both purified by centrifugation through 5–20% sucrose gradients in 0.45 M sodium acetate, 2.5 mM magnesium acetate, 0.5 mM EDTA, and 20 mM HEPES buffer, pH 7.5, prior to use. Photoincorporation was performed by irradiating 12 µg of dynein 1 or 1 µg of Fragment A in 120 µl of the same medium supplemented with 0.8 µM [α-32P]8-N3-ATP (14 Ci/mmol) and 4 µM 2-mercaptoethanol for 68 s with a 254-nm low pressure mercury lamp (Ultra-Violet Products Inc., San Gabriel, CA, Model UVS 12). All samples were subjected to electrophoresis on an 8% polyacrylamide gel by the Dreyfuss procedure (24) and stained with Coomassie Blue.

The specificity of photoincorporation was determined with parallel samples of dynein 1 and Fragment A in a medium containing 50 µM ATP as well as 0.8 µM [α-32P]8-N3-ATP.

| Polypeptide | 32P in polypeptide | Competition by 50 µM ATP |
|-------------|-------------------|-------------------------|
| α + β       | 4.1               | 89                      |
| IC1         | 0.06              | 11                      |
| IC2         | 0.15              | 40                      |
| IC3         | 0.06              | 17                      |
| 195,000     | 1.0               | 80                      |
| 130,000     | 0.40*             | 0                       |

well as in a trace polypeptide of apparent M, 48,000 which is not visible on Coomassie-stained gels, but which coelectrophoreses with sea urchin actin (data not shown). Competition with 50 µM ATP substantially reduces the amount of 32P in IC1 (Table I and data not shown).

In order to localize the ATP binding site in Fragment A, dynein that had been subjected to photoaffinity labeling with [α-32P]8-N3-ATP was digested to a DI of 40,000, and fractionated by sucrose gradient centrifugation. Autoradiography of the resultant electrophoresis gel shows that essentially all the 32P is located in the M, 195,000 peptide of Fragment A, with almost none in the M, 130,000 peptide of Fragment A or in the M, 110,000 peptide of Fragment B (Fig. 6). Similar digestion and autoradiography of a control sample of dynein labeled with [α-32P]8-N3-ATP in the presence of 50 µM ATP shows that this incorporation of 32P into the M, 195,000 peptide is blocked by the competition with ATP (data not shown).

The amount of sample yielded by the above protocol was too small for reliable quantitation of 32P, and therefore this was performed by a different procedure in which the Fragment A was prepared and purified prior to photoaffinity labeling. The resultant quantitation confirmed that specific 8-N3-ATP binding occurs in the M, 195,000 peptide of Fragment A, with 1.0 mmol of 32P being incorporated per mol of polypeptide and 80% of this being competed out by the presence of 50 µM ATP during photoincorporation (Table I). A minor amount of 32P is found in the M, 130,000 peptide, but this is due to nonspecific binding for it is unaffected by the presence or absence of ATP.

**DISCUSSION**

The present data show that tryptic digestion of outer arm dynein from sea urchin sperm in a low salt medium leads to rapid formation of a peptide of M, 296,000 from the β heavy chain, with the complementary peptide of M, 132,000 being preserved at least partially intact. More extended digestion to a DI of 32,000 divides the β/IC1 heavy chain complex into two smaller units, Fragment A and Fragment B, that can be separated by density gradient centrifugation. Fragment A is derived principally from the β heavy chain via the M, 296,000 intermediate and it possesses the V1-sensitive ATPase properties of the intact β chain. Fragment B, which has not been observed previously, is derived from the early M, 132,000 peptide of the β chain and it has no ATPase activity.

An estimate of the shape and hydration of the dynein fragments can be obtained by calculating their frictional ratios (f1/f0) (26). The β/IC1 complex has a total molecular weight of about 550,000 and sediments at approximately 11 S (6, 14), giving a frictional ratio of 1.9 that is approximately the same as that of intact dynein 1, which has an M, of 1,250,000 and sediments at 21 S (2). In contrast, Fragment A, which has a native molecular weight determined by equilibrium centrifugation of 370,000 (21) and sediments at 12.5 S, has a frictional ratio of only about 1.3. The lower frictional ratio of Fragment A indicates that it behaves hydrodynamically as a significantly more compact, less asymmetrical particle than the β/IC1 complex from which it was formed. The aggregation state of Fragment B and therefore its native molecular weight is not yet known, but its sedimentation coefficient of 5.7 S is smaller than that of any dynein fragment reported previously.

As a basis for discussion, we propose that the early tryptic cleavage of the dynein heavy chains in low salt medium occurs between the head and stem of the structure seen by electron microscopy, with Fragment A corresponding to the globular head of the β/IC1 complex and Fragment B to its flexible stem. This hypothesis is supported by the fact that the early tryptic cleavage appears to occur at nearly the same location on both the α and β chains, which have similar electron microscopic substructures (15), as well as by the fact that Fragment A sediments as a more compact, less asymmetric particle than the whole tadpole-shaped β/IC1 complex, although it obviously needs to be confirmed by direct electron microscopic study of the fragments.

Although the early tryptic cleavages of the α and β chains resemble each other in that both lead to formation of similarly sized peptides of M, 316,000 and 296,000, the subsequent digestion differs greatly and the limited cleavage pattern that leads to Fragment A occurs only with the β chain. In the light of the evidence from UV cleavage, suggesting that the general
the principal ATP-binding sites in dynein 1 are located in the heavy chain of dynein 1. The separation of Fragments A and B of the 1 heavy chain of dynein 1 provides a basis for the determination of their conformations by physical chemical procedures and this information should help clarify the functional role of the dynein arms in producing microtubule sliding.

Acknowledgment—We thank Dr. Barbara Gibbons for helpful discussions and assistance with the preparation of the manuscript.

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