# A DYNEIN-LIKE PROTEIN FROM BRAIN

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## 1. Introduction

Dynein forms the arms on the microtubule doublets of cilia and flagella [1–3], has a monomeric sedimentation coefficient of approximately 14 S [1–5], and separates on SDS-polyacrylamide gel electrophoresis into two high molecular weight components [5, 6]. In the presence of $Mg^{2+}$ or $Ca^{2+}$, dynein catalyzes the hydrolysis of ATP [3–5, 7]. The bending of cilia and flagella is thought to result from the sliding of adjacent microtubule doublets [8, 9], affected by the dynein-dependent hydrolysis of ATP [10, 11].

We have detected a brain protein with physical properties similar to those of one of the *Spisula* sperm tail dyneins, although the isolated brain protein has little or no ATPase activity. This dynein-like protein is associated with microtubules reassembled in vitro.

## 2. Methods

### 2.1. *Brain extracts*

Dogfish or guinea pig brains were homogenized at 0°C in an equal volume of 100 mM KCl–1 mM EDTA–10 mM imidazole chloride (pH 7.0) and centrifuged at 4°C for 1 hr at 25 000 *g* or 2 hr at 100 000 *g*.

### 2.2. *Microtubule reassembly*

Dogfish or guinea pig brains were extracted and microtubules reassembled in vitro by warming to 25°C for 1 hr using the procedure of Olmstead and Borisy [12]. The assembled microtubules and associated material were pelleted by centrifugation at 25 000 *g* for 1 hr at 25°C. For gel filtration, the pellet was suspended in 100 mM KCl–10 mM imidazole chloride (pH 7.0) at 0°C to permit the dissociation of the microtubules and then clarified by centrifugation at 60 000 *g* for 2 hr at 4°C.

### 2.3. *Sperm axonemes*

*Spisula solidissima* sperm tail axonemes were prepared [13] and a crude preparation of dynein extracted by dialyzing Triton X-100 demembranated axonemes against 1 mM EDTA–5 mM Tris chloride (pH 8.0) for 24 hr at 4°C and centrifuging at 15 000 *g* for 30 min at 4°C [5].

### 2.4. *Gel filtration chromatography*

Samples were brought to 10% sucrose, loaded onto a 2.5 × 50 cm column of 4% agarose (Biogel A-15m, 200–400 mesh) and eluted at 4°C with 100 mM KCl–10 mM imidazole chloride (pH 7.0), with or without the addition of 1 mM EDTA. The eluate was monitored at 280 nm using a Chromatronix absorbance monitor. The column was calibrated with DNA, muscle myosin, bovine thyroglobulin, catalase, human gamma-globulin, bovine serum albumin and ATP, and the Stokes radii of unknowns calculated by the method of Ackers [14].

### 2.5. *Biochemical assays*

ATPase activity was measured in 100 mM KCl–10 mM imidazole chloride (pH 7.0)–2 mM ATP with 4 mM $MgCl_2$ (or sometimes 4 mM $CaCl_2$ or 2 mM

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EDTA) at 25°C and inorganic phosphate determined colorimetrically [15]. Protein concentration was estimated by Lowry's method [16] with bovine serum albumin as a standard, or in very dilute column factions by absorbance at 280 nm using an arbitrarily chosen extinction coefficient of 6 for a 1% solution.

### 2.6 *Gel electrophoresis*

The method of Fairbanks, et al. [17] was modified by making the acrylamide concentration 3.5 or 4% and 'buffer B' was used at pH 9.2 rather than 7.4. Coomassie Blue stained gels were scanned at 550 nm and bands quantitated by cutting and weighing.

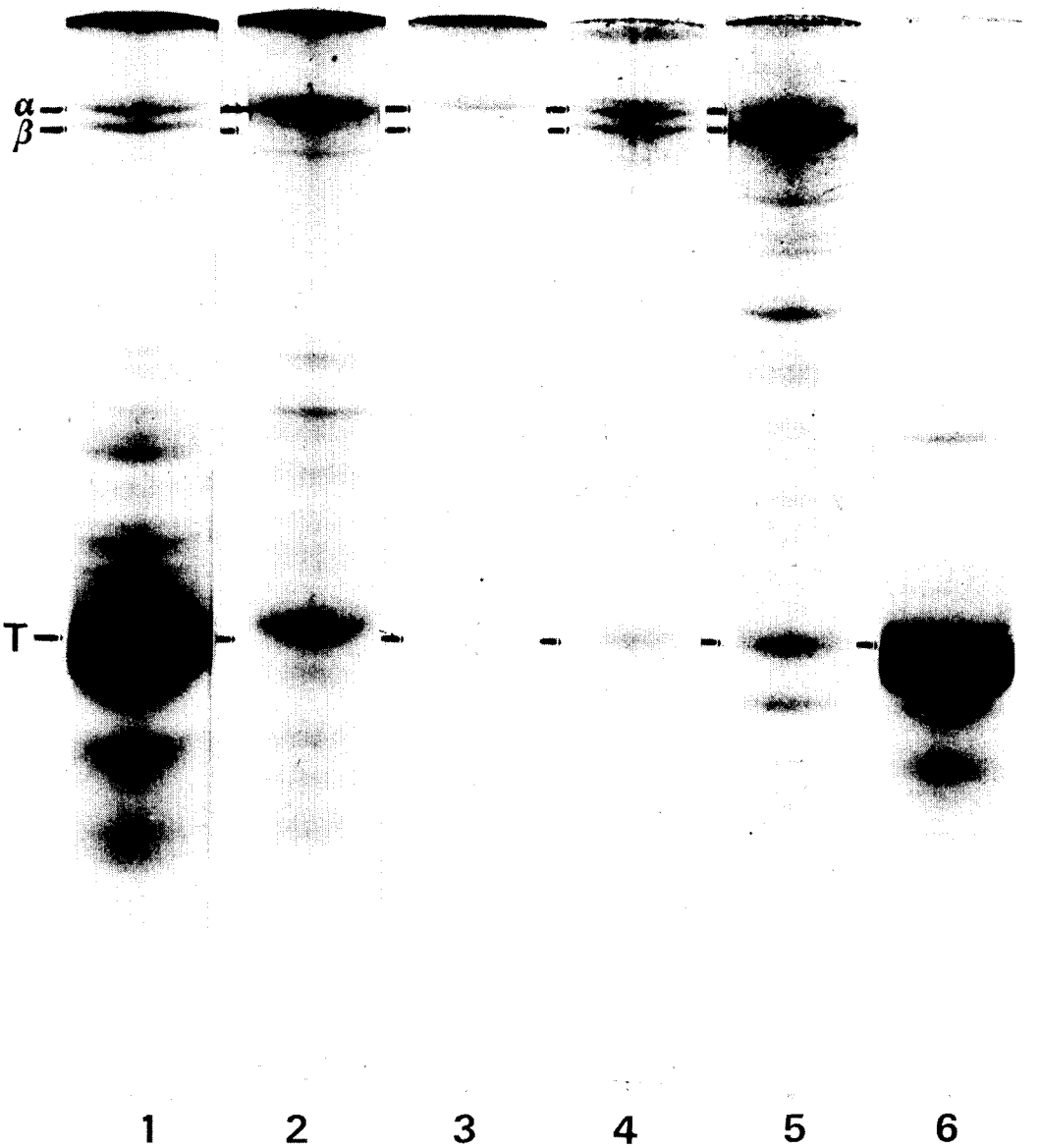


Fig. 1. Electrophoretic gels of *Spisula* sperm fractions from fig. 2a. (1) Axonemes. (2) Column fraction $K_d$ 0. (3) Column fraction $K_d$ 0.13. (4) Column fraction $K_d$ 0.30. (5) Column fraction 0.45. (6) Column fraction $K_d$ 0.75. Symbols: α-dynein (α); β-dynein (β); tubulin (T).

## 3. Results

The slower migrating sperm dynein band on SDS-gel electrophoresis will be termed α-dynein and the faster band β-dynein (fig. 1). Fractionation of the crude sperm dynein on 4% agarose (fig. 2a) results in some of the $Mg^{2+}$ ATPase activity eluting in the void volume, however the majority of the activity elutes as a broad peak ($K_d$ 0.1–0.5). Analysis of the protein composition of individual fractions by SDS-gel electrophoresis (fig. 1) indicates that the two dyneins are incompletely separated, but that the α-dynein is eluted in the void volume and as a broad peak ($K_d$ 0.1–0.3), while the β-dynein is found in a few fractions ($K_d$ 0.3–0.4). Similar results were obtained using 0.5 M KCl column buffer.

Both guinea pig and dogfish brains contain a polypeptide which comigrates with sperm α-dynein on SDS-gel electrophoresis (fig. 3a) and accounts for about 0.18% of the total brain protein. The guinea

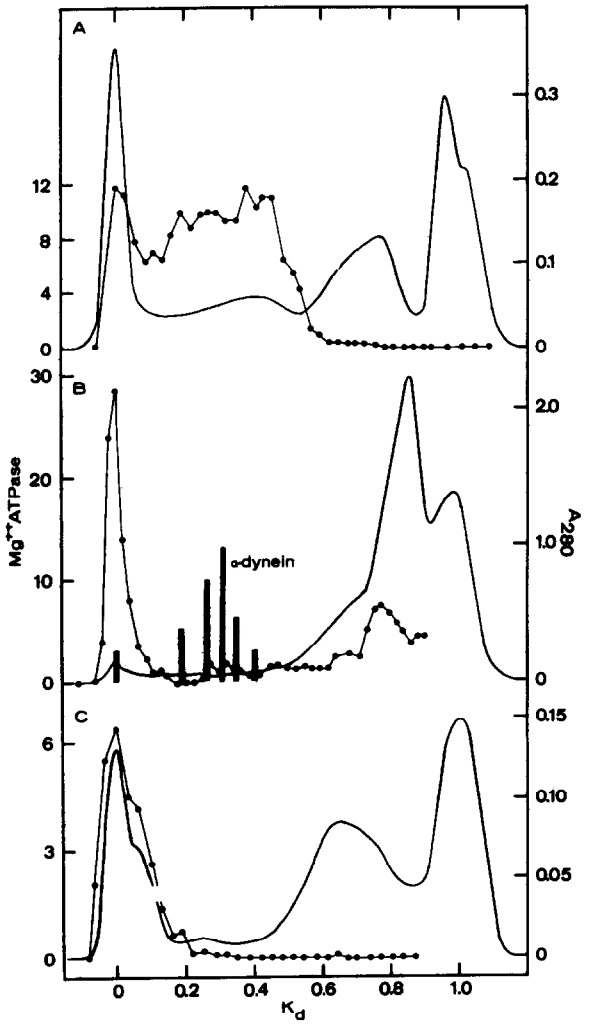


Fig. 2. Gel filtration on 4% agarose. (A) Crude *Spisula* sperm tail dynein. (B) High speed extract of guinea pig brain. (C) Reassembled dogfish brain microtubules. $Mg^{2+}$ ATPase activity is given in nmoles/min/ml. The α-dynein content of several fractions in (B) was determined by quantitative gel electrophoresis and is given in arbitrary units. ATPase (●---●---●); $A_{280}$ (-----).

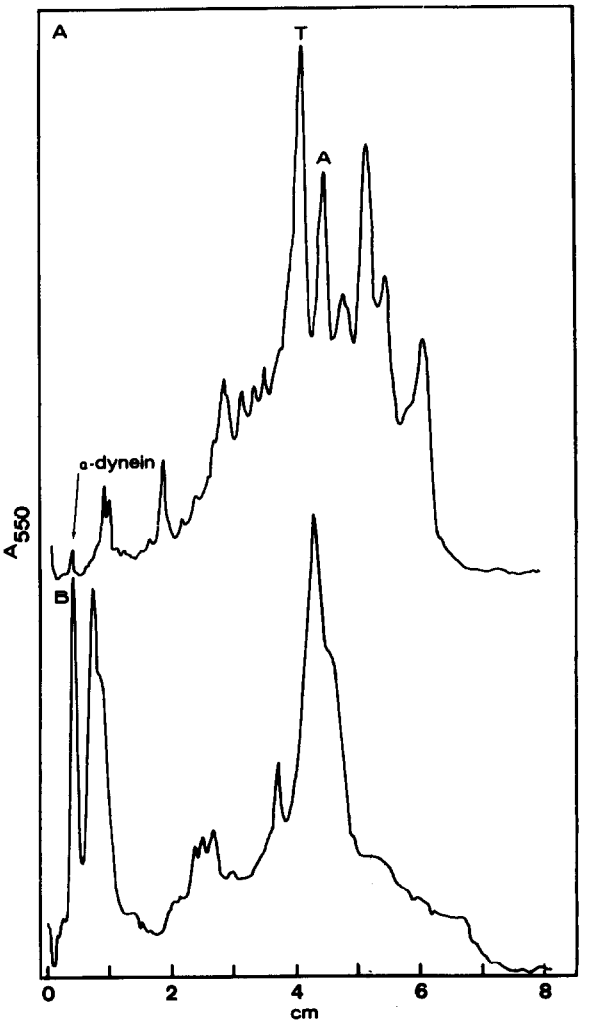


Fig. 3. Densitometer scans of electrophoretic gels. (A) Whole guinea pig brain. (B) Column fraction $K_d$ 0.30 from fig. 2b.

pig brain α-dynein is extracted into low and high speed supernatants, and can be further purified by gel filtration (fig. 2b). While some of the α-dynein elutes in the void volume, most is found in a peak at $K_d$ 0.30 (fig. 2b), where it comprises 6.1% of the total protein (fig. 3b), representing a 34-fold purification. Most of the ATPase activity is voided and the peak α-dynein fraction has a specific activity of less than 20 nmoles/min/mg. Similar results were obtained with dogfish brains.

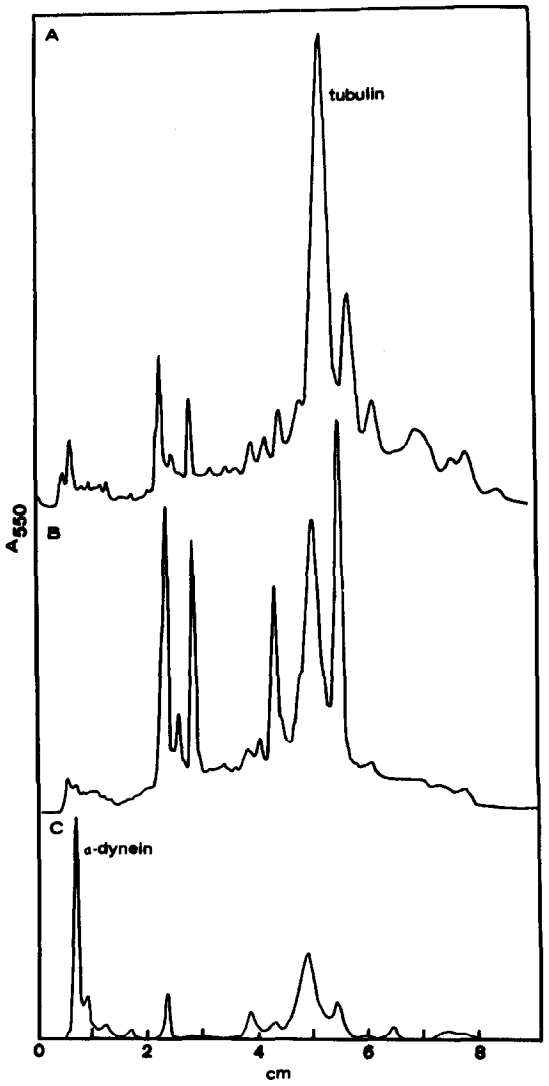


Fig. 4. Densitometer scans of electrophoretic gels. (A) Reassembled dogfish brain microtubule pellet. (B) Column fraction $K_d$ 0 from fig. 2c. (C) Column fraction $K_d$ 0.26 from fig. 2c.

When microtubules assembled in vitro are sedimented, the pellet (fig. 4a) is enriched in α-dynein (1.4%) as well as tubulin (32%) and has high $Mg^{2+}$ ATPase activity (about 2 μmoles/min/mg for dogfish). Part of this ATPase activity if very labile; in preliminary experiments, we found that conditions which favor microtubule disassembly (cold or high salt) also favor the loss of this labile ATPase activity. Inhibition of microtubule assembly with 10 mM colchicine or 2 mM $CaCl_2$ inhibits the pelleting of α-dynein, tubulin and part of the ATPase activity. Gel filtration of the microtubule pellet, after dissociation at 0°C, separates the residual ATPase activity from the α-dynein and tubulin (fig. 2c). Over 90% of the applied ATPase activity was recovered in the void volume, which was enriched in polypeptides with molecular weights of 80 000, 140 000 and 170 000 daltons (fig. 4b). The minor peak eluting at $K_d$ 0.26

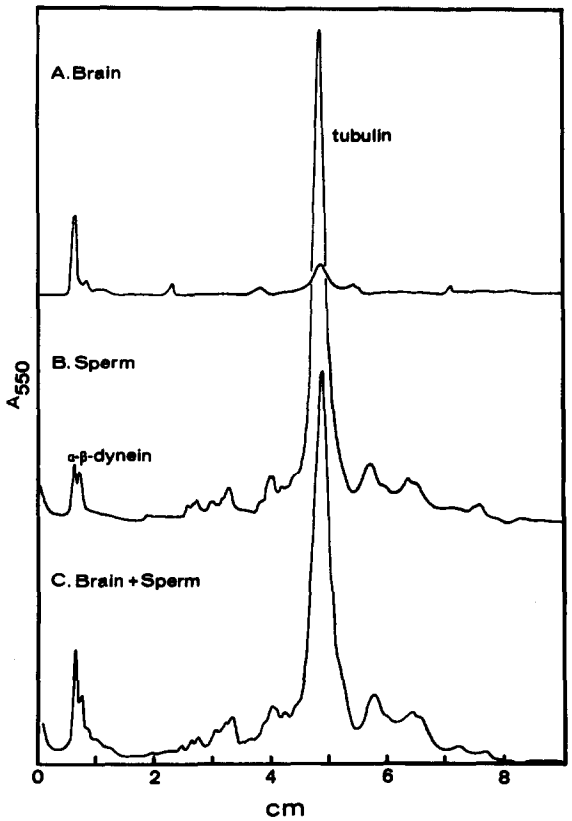


Fig. 5. Densitometer scans of electrophoretic gels. (A) Column fraction $K_d$ 0.26 from fig. 2c, dogfish brain α-dynein. (B) *Spisula* sperm axonemes. (C) A + B.

has ATPase activity of less than 5 nmoles/min/mg, but consists of 27% α-dynein (representing a 150-fold purification from the homogenate) contaminated with some tubulin and other proteins (fig. 4c). This purified brain α-dynein comigrates precisely with the α-dynein from *Spisula* (fig. 5).

There are serious difficulties in calibrating SDS-gels for molecular weight determinations in the range above 200 000 daltons where the dyneins are found, because (a) there are no well characterized polypeptides with molecular weights greater than the muscle myosin heavy chain (200 000 daltons) and (b) unreduced polymers of standard proteins such as myosin, gamma globulin and BSA do not fall on the same curve as reduced (and/or carboxymethylated) standard proteins (fig. 6). Given these uncertainties, which deserve further detailed investigation, we estimate that molecular weights of α- and β-dynein are 380 000 and 360 000 daltons (fig. 6), which are lower than earlier measurements [5].

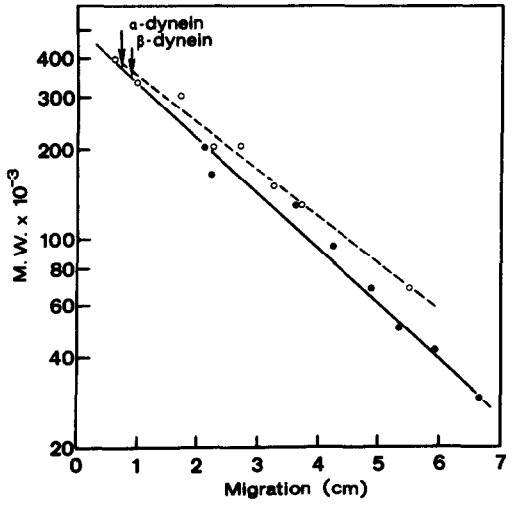


Fig. 6. Calibration of 3.5% polyacrylamide gels for molecular weight determination. Open circles are unreduced proteins; from the left they are rabbit muscle myosin heavy chain dimer; bovine thyroglobulin dimer; human gamma-globulin dimer; myosin heavy chain monomer; bovine serum albumin (BSA) trimer; human gammaglobulin; BSA dimer; BSA monomer. Closed circles are reduced and alkylated (15) standards; from the left they are myosin heavy chain, thyroglobulin, β-galactosidase, phosphorylase-A, BSA, gamma-globulin heavy chain, actin and carbonic anhydrase. The vertical arrows mark the mobility of reduced and alkylated *Spisula* sperm α- and β-dynein.

## 4. Discussion

Brains of dogfish and guinea pigs contain a small amount (0.18%) of a very high molecular weight polypeptide which we have tentatively named brain α-dynein, because it (a) shares with sperm α-dynein two important physical properties (subunit molecular weight and Stokes radius) and (b) like sperm α-dynein, appears to be associated with microtubules. In contrast to the isolated sperm α-dynein, the brain α-dynein has little or no ATPase activity. Similar observations have been made independently by Gaskin, et al. [18].

### 4.1. *Physical properties*

Brain α-dynein has an apparent subunit molecular weight of 380 000 daltons, and from its partition coefficient of 0.26–0.30 on 4% agarose, one can calculate that its Stokes radius is 13.7–14.8 nm. Together these values indicate that the brain α-dynein must have considerable asymmetry if it is a monomer or small oligomer under the conditions of the chromatography. A spherical protein molecule with a Stokes radius of 14 nm would have a molecular weight of about $10 \times 10^6$ daltons.

Sperm α-dynein has the same subunit molecular weight, but does not elute from the gel filtration column as a discrete peak whose Stokes radius can be determined unambiguously, rather it appears to aggregate, perhaps with itself or with tubulin, and much of it appears in the void volume. The included fraction of α-dynein peaks at about $K_d$ 0.3 corresponding to a Stokes radius of 14 nm. Other investigators have found that dyneins from other flagella have similar partition coefficients on 4% agarose columns [7, 19].

### 4.2. *Association with microtubules*

The association of brain α-dynein with microtubules has not been proven directly by studying the binding of the isolated protein to microtubules, but the following circumstantial evidence supports the idea that brain α-dynein binds to microtubules. Microtubules partially purified by reassembly in vitro (fig. 4; ref. [18, 20, 21]) are enriched in α-dynein. For example, in whole brain (fig. 3a) the weight ratio of α-dynein to tubulin is about 1 : 50, whereas in reassembled microtubules (fig. 4a) the ratio is about

1 : 23. In addition, inhibition of in vitro microtubule assembly with $Ca^{2+}$ or colchicine concomitantly inhibits the pelleting of α-dynein. Finally, we have detected a protein with the same electrophoretic mobility as α-dynein in preparations of intact microtubules isolated directly from both dogfish and guinea pig brains with hexylene glycol [22] and from guinea pig brain with DMSO–glycerol buffer [23].

### 4.3. *ATPase activity*

Isolated *Spisula* dynein (fig. 2a) has $Mg^{2+}$ ATPase activity of about 0.1 μmoles/min/mg under our assay conditions, while the ATPase activity in column fractions enriched in isolated brain α-dynein is less than 1/5 (fig. 2b) or 1/20 (fig. 2c) as high. Although assay conditions were varied in terms of pH (7.0, 8.0) and the concentration of KCl or NaCl (0–0.6 M), $MgCl_2$ or $CaCl_2$ (0–10 mM) and ATP (1–4 mM), none of these conditions give higher activity.

There are a number of possible reasons for this apparent difference in ATPase activity between brain and sperm α-dyneins. The brain α-dynein may have been denatured during its isolation, or assay conditions favorable to its ATPase activity may not have been tested, but a more intriguing possibility is that the brain α-dynein may be active only when attached to intact microtubules. Indeed, this idea is consistent with our preliminary experiments suggesting that the lability of the ATPase activity in pellets of reassembled microtubules is associated with the dissociation of the microtubules. This sort of influence of a structural protein upon the activity of an energy transducing enzyme is not without precedent, as sperm tail dynein which is free or bound to microtubules have significant differences in ionic optima [4], the ATPase activity of bound sperm tail dynein is tightly coupled to microtubule movement [24], and myosin ATPase is activated by interacting with actin filaments [25].

### 4.4. *Source of brain α-dynein*

It is unlikely that the α-dynein found in brain originates from ciliated brain cells, since it represents about 0.2% of the total brain protein, and because β-dynein, which has been found in all cilia, is not present in significant quantities in these brains.

### 4.5. *Function of brain α-dynein*

Since the brain α-dynein is a large asymmetrical molecule which appears to bind to microtubules, it is reasonable to suggest that it forms the side arms which have been observed on axonal microtubules [26]. These side arms are comparable to those born by the A-microtubules of cilia and flagella [1] which contain both α- and β-dynein [5] and by the microtubules of the axostyle of *Cryptococcus* which appear to contain only α-dynein [6]. If the brain α-dynein bound to microtubules can be shown to have significant ATPase activity, one might then postulate a role for this protein in axoplasmic transport.

Regardless of its actual function this protein may of interest in its own right because its extraordinary size makes it the largest polypeptide in vertebrate brain.

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