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Polypeptide Subunits of Dynein 1 From Sea Urchin Sperm Flagella

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A high-resolution sodium dodecyl sulfate polyacrylamide gel electrophoresis system has been used to show the presence, in both whole sperm and isolated flagellar axonemes, of eight polypeptides migrating in the 300,000–350,000 molecular weight range characteristic of the heavy chains of dynein ATPase. Previously, only five such chains have been discernible. Extraction of isolated axonemes for 10 min at 4°C with a solution containing 0.6 M NaCl, pH 7, releases a mixture of particles that separate, in sucrose density gradient centrifugation, into a major peak, dynein 1 ATPase, sedimenting at 21S and a minor peak at 12–14S. The polypeptide compositions of these two peaks are different. The dynein 1 peak, which contains most of the protein on the gradient, contains approximately equal quantities of two closely migrating heavy chains, with a small amount of a third, more slowly migrating chain; no other heavy chains appear in this peak. Two groups of smaller polypeptides (three intermediate chains, within the apparent molecular weight range 76,000–122,000 and four newly discovered light chains, within the apparent molecular weight range 14,000–24,000) cosediment with the 21S peak. The heavy chain composition of the 12–14S peak is more complex, all eight heavy chains occurring in approximately the same ratios as occur in intact axonemes.

Key words: dynein, flagella, ATPase, sperm motility, sea urchin

Electrophoretic analysis of the flagellar ATPase, dynein, originally indicated the presence of two high molecular weight (HMW) polypeptides [1]. Subsequent work has shown that dynein exists in more than one isoenzymic form [2] and has distinguished a substantially greater number of distinct HMW polypeptides with electrophoretic mobilities corresponding to molecular weights of 300,000–360,000. Gibbons et al [2] reported the presence of five polypeptides in the characteristic HMW region of dynein from sea urchin sperm flagella, Warner et al [3] observed six polypeptides in the same molecular weight region in molluscan gill cilia, and Piperno and Luck distinguished ten HMW polypeptides in flagellar axonemes of Chlamydomonas [4]. In addition to these HMW polypeptides, Gibbons and Fronk [5] have reported that three intermediate molecular weight (75,000–125,000) polypeptides copurify with the HMW polypeptides in the 21S form of dynein 1 obtained by salt extraction of sea urchin sperm flagella.

Improved electrophoresis technique has now permitted us to discern eight HMW polypeptides in intact axonemes isolated from sea urchin sperm flagella, and to observe the presence of low molecular weight polypeptides (in addition to those of intermediate and high molecular weight) in the 21S form of extracted dynein 1.

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METHODS

Whole sperm from the sea urchin Tripneustes gratilla were prepared for electrophoresis by boiling in gel sample buffer containing 1% w/v sodium dodecyl sulfate for 1 min. The solution was then sonicated to break up the DNA gel and centrifuged at 100,000g for 15 min to sediment particulate matter. The supernatant was used for electrophoresis. Flagellar axonemes were isolated from sea urchin sperm, and the latent ATPase form of dynein 1 was extracted with 0.6 M NaCl, 4 mM $MgSO_4$, 1 mM $CaCl_2$, 0.1 mM ethylenediamine tetraacetic acid EDTA), 1 mM dithiothreitol, 7 mM 2-mercaptoethanol, 5 mM imidazole/NaOH buffer, pH 7.0 (0.6 M NaCl extraction buffer) as described by Gibbons and Fronk [5]. Sucrose density gradient centrifugation (5–20% w/v sucrose) was carried out as described earlier [5]. ATPase activities of fractions from gradients were assayed in a reaction buffer containing 0.2 M NaCl, 2 mM $MgSO_4$, 0.1 mM EDTA, 1 mM ATP, 0.03 M Tris/HCl, pH 8.1, the inorganic phosphate being determined by the method of Fiske and SubbaRow [6]. Discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out, on rod or slab gels, essentially as described by Laemmli [7], and stained with 0.05% w/v Coomassie brilliant blue R-250 in water/methanol/acetic acid (in the volume ratio 5/5/1, respectively). Optimum resolution of HMW bands was obtained with 3 mm thick slab gels employing 3–10% acrylamide gradients, although 5–20% gradients were required to observe low molecular weight bands. Apparent molecular weights of polypeptides were determined on acrylamide gradient slab gels calibrated with polypeptides of known molecular weight ranging from 14,300 (lysozyme) to 200,000 (myosin) [8]. Densitometry of strips cut from slab gels was performed at 600 nm with a Zeiss PMQII spectrophotometer and a Vicon V1050 linear drive, the output being recorded on a Sargent-Welch Model SRLG chart recorder. Peak areas were determined by weighing.

RESULTS

When intact, freshly shed sperm are prepared as described in Methods, electrophoresis reveals the HMW pattern shown in Figure 1a. The pattern is virtually identical to that found in this region after electrophoresis of isolated axonemes (Fig. 1b). The major polypeptides are two bands of approximately equal intensity migrating in the position assigned to the A band of dynein 1 [2]. The designations A_α and A_β are proposed for the upper and lower bands, respectively. The band above A_α and A_β migrates in the position of the C band, but the relationship of the four bands below A_α and A_β to the B and D HMW bands described previously [2] is uncertain. Densitometry indicates that three of these bands (indicated by lines) have equal absorbance, whereas the lowest band has twice this absorbance. On favorable gels, this lowest band is resolvable into two equal bands of equal absorbance. For this reason, we believe there are eight HMW bands; these bands will be referred to as the heavy chains of dynein. The dense, fuzzy band visible below the heavy chains in the intact sperm sample does not appear in isolated axonemes, indicating that it is probably a membrane or sperm head protein.

Extraction of isolated axonemes with 0.6 M NaCl extraction buffer releases the readily extractable fraction of dynein 1 [5]. This is sedimented in a sucrose density gradient, which is subsequently fractionated and analyzed electrophoretically as shown in Figure 2. Figure 2a, a 3–10% acrylamide gel separating polypeptides of molecular weight greater than about 30,000 shows the presence of two sedimenting peaks. The major one (peak 1) corresponds to the 21S peak of dynein 1 ATPase reported by Gibbons and Fronk [5]. The

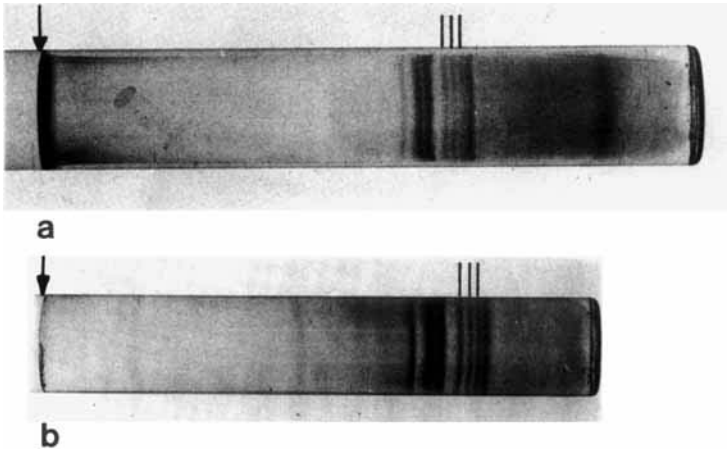

Fig. 1. a. Whole sperm; b. isolated axonemes. Both samples run on 4% acrylamide gels. Runs were continued for three times as long as was required for the dye front to pass off the end of the gel. Tops of the gels are shown on the left of the figure (arrows), and direction of migration was from left to right.

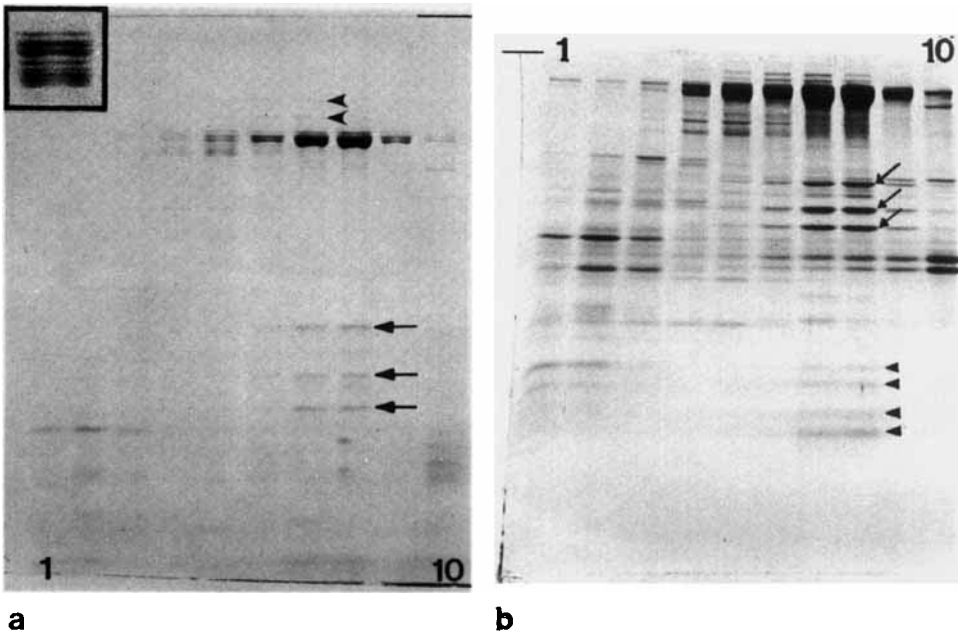

Fig. 2. Electrophoretic analysis of a sucrose density gradient separation of 0.6 M NaCl extract from flagellar axonemes. The 10 fractions represent a complete gradient, fractions 1 and 10 being, respectively, the top and bottom of the gradient. Most of peak 1 (21S) is found in fractions 7 and 8. Peak 2 is found primarily in fraction 5. a. 3–10% acrylamide gradient gel; b. 5–20% acrylamide gradient gel. In both gels, electrophoresis was stopped when the dye front reached the bottom. 20 μl of each fraction was applied to the 3–10% gel; 100 μl of each fraction was applied to the 5–20% gel. In the 3–10% gel, the A_α and A_β heavy chains are not resolved in peak 1 because of overloading, although they are resolved at the lower protein concentration on either side of the peak. The acrylamide concentration of the 5–20% gel is too high to resolve adequately A_α and A_β, regardless of load. Inset shows HMW bands of peak 2 (fraction 5) at higher magnification. Tops of gels are indicated by lines. Arrowheads in a indicate polypeptides with molecular weights greater than those of the dynein heavy chains; in b, arrowheads indicate light chains of dynein 1. Arrows indicate intermediate chains of dynein 1 in a and b.

second (peak 2) contains up to 20% of the protein found in peak 1, and sediments at 12–14S as judged by comparison with cosedimented catalase (11.3S). Peak 1 contains approximately equal amounts of the A_α and A_β heavy chains, as well as a small amount of the C heavy chain. Below the heavy chains lie three polypeptides of intermediate molecular weight (arrows), as observed by Gibbons and Fronk [5]; these three polypeptides will be referred to as intermediate chains. Peak 2 is distinguished mainly by the presence of all eight heavy chains in ratios similar to those found in whole axonemes (see inset, Fig. 2a). Between peaks 1 and 2 lie small amounts of two polypeptides (arrowheads) whose apparent molecular weights are substantially greater than those of the dynein heavy chains.

Figure 2b shows a 5–20% gel that separates polypeptides down to about 10,000 molecular weight. Once again, the three intermediate chains (arrows) can be seen below the heavy chains of peak 1, and these are accompanied by four polypeptides of low molecular weight (arrowheads). These low molecular weight polypeptides, which are reproducibly found in preparations of salt-extracted dynein 1, will be referred to as light chains. The absorbances of the bands corresponding both to the intermediate and the light chains appear to parallel those of the heavy chains of peak 1 (Figs. 2a and b).

The apparent molecular weights of the intermediate chains and light chains were determined, respectively, on 3–10% and 5–20% acrylamide gels in order to avoid the nonlinear mobility/log molecular weight relationships found at very low and very high mobilities. The apparent molecular weights for these polypeptides in peak 1 were: 122,000, 90,000, and 75,000 for the intermediate chains and 24,000, 21,000, 16,000, and 14,000 for the light chains, for the average of three preparations, with a variation of ±5% in all cases.

Densitometry of the bands corresponding to the heavy, intermediate, and light chains in gels of the peak 1 fraction from sucrose gradients was carried out to determine the relative amount of protein in each band. Heavy chains of A_α, A_β, and C and the three intermediate and four light chains together account for about 70% of the protein in peak 1, the rest being made up mainly of tubulin (which does not appear to be specifically related to the peak 1 polypeptides) and a low-density background of bands in the high to intermediate molecular weight range, which may be proteolytic fragments of the heavy chains. Table I presents a comparison of the relative amounts of the heavy, intermediate, and light chains in peak 1. The 14,000 molecular weight light chain displayed a wide variation in relative staining intensity, which could be due to anomalous staining behavior and/or variable losses of protein and stain from the gel after staining (often more noticeable with low molecular weight polypeptides).

The ATPase activity of the latent form of salt-extracted dynein after sedimentation in a sucrose gradient is shown in Figure 3. A single, relatively sharp peak of ATPase activity is present, coinciding with the appearance, in SDS gel electrophoresis, of the 21S peak 1 polypeptides. The ATPase activity drops less sharply on the trailing edge of the peak (toward the left in Fig. 3) than on the leading edge, but there does not appear to be any separate peak of activity associated with the 12–14S peak 2 polypeptides. The trailing skew of the peak of ATPase does not appear in all preparations.

DISCUSSION

The increasing resolution of gel electrophoresis techniques has led to the recognition that the dynein polypeptide composition of flagellar axonemes is relatively com-

TABLE I. Polypeptide Composition of 21S Dynein Peak*

	Polypeptide	Absorbance (percent)
Heavy chains	A_α	24 ± 4
	A_β	24 ± 4
	C	1.9 ± 0.5
Intermediate chains	122,000 mol wt	13 ± 1
	90,000 mol wt	10 ± 1
	76,000 mol wt	8.7 ± 1.0
Light chains	24,000 mol wt	1.9 ± 0.5
	21,000 mol wt	2.5 ± 0.2
	16,000 mol wt	3.8 ± 0.5
	14,000 mol wt	9.6 ± 3.0

*Gels were scanned as described. Absorbance of each polypeptide band is expressed as a percentage of the total absorbance of the bands corresponding to the heavy, intermediate, and light chains. Results represent average percent absorbance and standard deviation for three preparations.

plex. The work described here indicates the presence of eight different dynein heavy chains in sperm flagella of the sea urchin, Tripneustes. The constancy of this polypeptide pattern indicates that the multiplicity of bands is not caused by proteolytic activity following sperm shedding.

Extraction of isolated flagellar axonemes with 0.6 M NaCl extraction buffer releases the latent ATPase form of dynein 1. High-resolution electrophoresis in acrylamide gradient gels has revealed the presence, in the 21S peak from this preparation, of heavy chains A_α, A_β, and C, and of four light chains, in addition to the three intermediate chains reported by Gibbons and Fronk [5]. The apparent molecular weights of the intermediate chains are within experimental error of those found by Gibbons and Fronk. Piperno and Luck [4] have observed intermediate and light chains of similar molecular weight in preparations of dynein from Chlamydomonas flagella. The ratio of the intermediate to the heavy chains reported here (see Table I) is somewhat greater than that found by Gibbons and Fronk [5]; this may be caused by differences in the relative staining behavior of the polypeptides between the continuous electrophoresis system used by Gibbons and Fronk and the discontinuous system used here.

The intermediate and light chains appear to maintain a constant ratio to the A_α and A_β heavy chains as the 21S peak 1 polypeptides trail into peak 2 across the sucrose gradient. No relative increase in the absorbance of the intermediate and light chains is noted as the additional heavy chains appear in peak 2. This suggests that the intermediate and light chains are not associated with the five heavy chains below A_α and A_β.

The minor 12–14S peak (peak 2) in sucrose density gradients had a more complex HMW polypeptide composition, containing all eight heavy chains. This in turn suggests a more complex molecular composition for the 12–14S peak than for the 21S peak. If the molecular weights of the particles in the 12–14S peak are roughly half that of the 1,250,000 molecular weight of the 21S particles [5] (assuming similar hydrodynamic

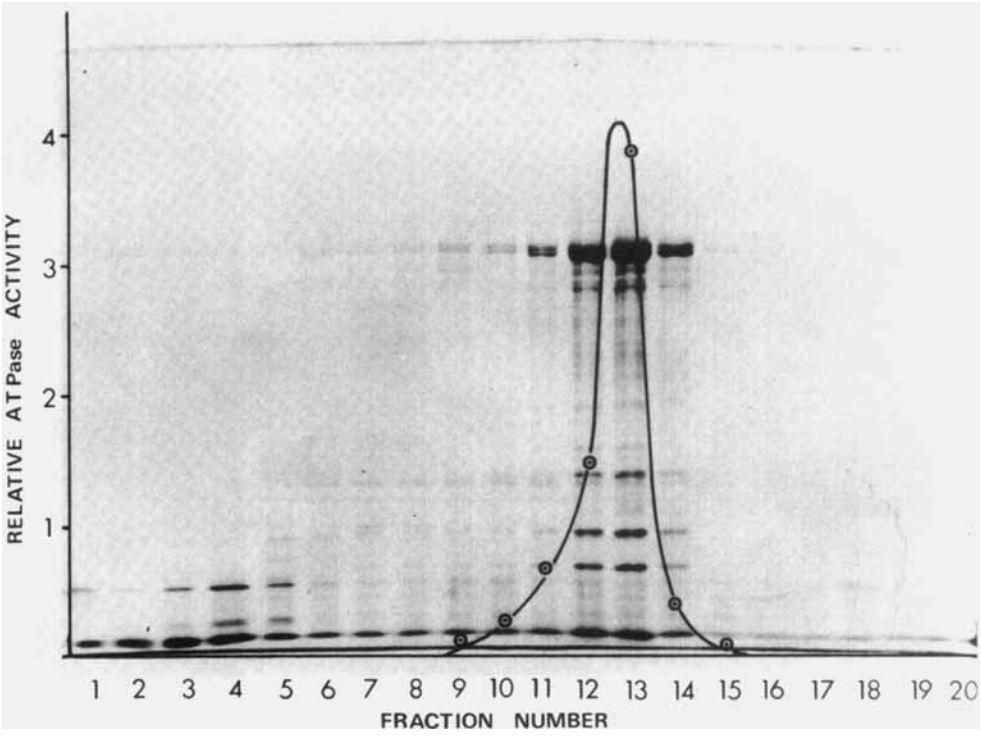

Fig. 3. ATPase activity superimposed on polypeptide content in fractions from a sucrose density gradient separation of a 0.6 M NaCl extract from flagellar axonemes. The top of the sucrose gradient is to the left. ATPase activity in each fraction was measured by adding 0.1 ml of the fraction to 2 ml of reaction buffer for 10 min at room temperature. ATPase activity is displayed in arbitrary units. 45 μl of each sample were electrophoresed on a 3–6% acrylamide gradient slab gel. Peak 1 is contained primarily in fractions 12 and 13; peak 2, in fraction 9.

properties), then the 12–14S peak, with eight different heavy chains of around 300,000–350,000 molecular weight, must contain at least four distinct forms of particles.

As reported by Gibbons and Fronk [5], sucrose gradients of salt-extracted dynein show a single peak of ATPase activity at about 21S coninciding exactly with the peak 1 polypeptides, thus confirming the identification of this peak with dynein 1 ATPase. Under the conditions of the assay, virtually no ATPase activity was found associated with the peak 2 polypeptides. However, rather than being indicative of complete lack of ATPase activity, this may be caused by the usually very small amount of protein in peak 2. The relationship of the particles found in peak 2 to the two known isoenzymic forms of dynein ATPase [2] remains to be determined.

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