Ionic Strength-dependent Isoforms of Sea Urchin Egg Dynein*

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Unfertilized sea urchin eggs provide a reservoir of molecules which later are involved in microtubule-mediated movements during embryonic development. Among these molecules is egg dynein, which has been isolated in two forms, 20 S and 12 S. Evidence obtained previously from our laboratory indicates that 20 S dynein is a latent activity precursor of ciliary dynein. In contrast, others have suggested that 12 S egg dynein functions in the mitotic apparatus. It is therefore important to determine the relationship between these egg dyneins. Here we demonstrate that the sedimentation velocity of the egg dynein is dependent on the ionic strength of the extraction conditions. The 20 S dynein is obtained with low ionic strength extraction, and the 12 S form is obtained in high salt (0.6 M KCl). The 20 S dynein, after collection from a sucrose gradient, can be converted quantitatively to the 12 S form by exposure to salt, and this conversion can be followed over time. Further, the 20 S dynein can be converted entirely to 12 S dynein and then partially reconstituted to a faster sedimenting species. During these conversions, the dynein high M, heavy chains are always coincident with the MgATPase activity, and antibodies show that the 12 S form comprises the 12 S species. These data suggest that 12 S dynein is an ionic strength-dependent isoform of 20 S dynein that results from a partial dissociation of the 20 S polypeptide complex, similar to the relationship between 12 and 21 S sperm flagellar dynein. If the 20 and 12 S enzymes are isoforms of the same dynein, then there is compelling evidence for only a single dynein in the unfertilized egg, and that dynein is probably a ciliary precursor.

Dynein is the best characterized translocator for microtubule-mediated movements (for reviews see Gibbons, 1981; Johnson, 1985). The axoneme has been shown to contain multiple dynein isoenzymes which act in concert to power active microtubule sliding which is converted to ciliary or flagellar bending (Gibbons et al., 1976; Ogawa and Gibbons, 1976; Tang et al., 1982; Satir, 1968; Summers and Gibbons, 1971; Brokaw, 1971, 1972; Brokaw et al., 1982). Important cytoplasmic movements also involve microtubules, although the structural bases for these movements are largely unknown. The sea urchin provides an extremely powerful system in which to characterize the translocator molecules driving various microtubule movements. Sea urchin spermatozoan flagellar 21 S dynein serves as a well understood model dynein (Gibbons and Fronk, 1979; Gibbons and Gibbons, 1979; Bell et al., 1979; Tang et al., 1982); additionally, events in the early embryo including pronuclear migration, mitosis, and blastula ciliation represent microtubule-mediated movements which can be resolved temporally and spatially.

Recently, several laboratories have reported the isolation of dynein-like activities from the unfertilized sea urchin egg (Pratt, 1980; Hisanaga and Sakai, 1980, 1983; Scholey et al., 1984; Hollenbeck et al., 1984; Asai and Wilson, 1985; Penningroth et al., 1985). Because the egg contains no polymerized microtubules, these dyneins are presumed to be stored precursors for later involvement in microtubule motility. An especially intriguing possibility is that one of the egg dyneins later functions in the mitotic spindle to drive anaphase separation (Pratt et al., 1980). Alternatively, an egg dynein may be a stored precursor for blastula cilia (Weisenberg and Taylor, 1968).

Generally, two species of sea urchin egg dynein have been described. Low ionic strength extraction produces a 20 S dynein that exhibits latent MgATPase activity and the ability to combine in vitro with microtubules in an ATP-sensitive fashion (Asai and Wilson, 1985; Asai et al., 1986). An anti-serum to 20 S dynein reveals that 20 S dynein is probably a ciliary dynein precursor and is not a component of the mitotic apparatus (Asai, 1986). A second dynein is obtained by high salt extraction of sea urchin eggs (Hisanaga and Sakai, 1980, 1983; Scholey et al., 1984). This 12-14 S activity has been proposed to be a mitotic spindle precursor (Pratt et al., 1980), although it remains to be shown that the same polypeptides that comprise the egg 12-14 S dynein can later be found in the mitotic apparatus.

We have been studying the possible relationship between these two species of egg dynein. In this paper, we provide evidence that the 20 S and the 12 S dyneins are isoforms of a single dynein. We show that the 20 S dynein can be converted quantitatively to 12 S dynein by changing the ionic strength of the dynein solution. Further, we have been able to reconstitute partially the 20 S dynein activity from the 12 S species. Our results suggest that the 12 S dynein is a partially dissociated subset of the 20 S dynein, analogous to the relationship between 12 and 21 S flagellar dynein (Tang et al., 1982). Therefore, there is persuasive evidence for only a single egg dynein, and that dynein is probably destined to be incorporated into cilia and not into cytoplasmic structures.

MATERIALS AND METHODS

Preparation of Egg Dyneins—Eggs were collected into cold seawater after intracoelomic injection of 0.55 M KCl of Strongylocentrotus purpuratus females. The eggs were passed through 100 µm Nitex to remove the jelly coat and then washed several times with cold seawater. 20 S dynein was obtained by homogenizing the washed eggs in

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PME buffer (0.1 M Pipes, 2 mM EGTA, 2 mM diithiothreitol, 1 mM MgSO₄, 0.2% (w/v) NaN₃ (pH 6.8)). 12 S dynein was obtained by homogenizing eggs in PME buffer that was supplemented with 0.6 M KCl (PME/KCl buffer) or by adding KCl to 20 S dynein. The proteolysis inhibitors p-tosyl-L-arginine methyl ester and phenylmethylsulfonyl fluoride, each at 1 mM, were used throughout the procedures. Microtubule monomers were centrifuged at 27,000 × g for 90 min at 4°C. The supernatants were then centrifuged in a Beckman 60 Ti rotor at 210,000 × g for 2 h at 4°C. The resulting high speed supernatant was collected and stored on ice and was the starting material for the experiments described here.

Preparation of Flagellar and Embryonic Ciliary Dyneines—Flagellar dynein was prepared from S. purpuratus spermatozoa by low ionic strength extraction (Ogawa et al., 1977). Embryonic cilia from freely swimming S. purpuratus gastrulae were prepared by the hypertonic seawater method of Stephens (1977). Ciliary dynein was obtained by resuspending the pelleted axonemes in 30 mM Tris-HCl (pH 8.0), 3 mM MgCl₂, 0.1 mM EDTA, 0.25% Nonidet P-40 for 15 min on ice, followed by centrifugation at 10,000 × g for 15 min at 4°C, and subsequent washing in this buffer without the detergent.

Sucrose Density Centrifugation—Sucrose density centrifugation (Tang et al., 1982) of dynein was carried out in 10.5 m linear 5–20% sucrose gradients as we have described previously (Asai and Wilson, 1985). 20 S dynein was recovered from sucrose gradients made with 0.15 M KCl. 12 S dynein was obtained from sucrose gradients made with 0.6 M KCl. One-ml samples were centrifuged through the gradients in a Beckman SW 41 rotor at 35,000 rpm for 15 h at 4°C. After centrifugation, the bottom of each tube was pierced with a hypodermic needle, and 20 fractions of equal volume were collected and placed on ice. Latent activity flagellar dynein (21 S, Gibbons and Fronk, 1979) and catalase (11.3 S) were used as sedimentation markers in identical gradients spun at the same time.

Conversion—Fractions of egg dynein collected off of the sucrose gradients were assayed for ATPase activity and the peak fractions pooled. The 20 S peak from the low salt extraction was pooled and dialyzed against 2 liters of PME/KCl buffer for 30 h at 4°C. The buffer was replaced every 5 h and was supplemented with the proteolysis inhibitors. Similarly, the 12 S peak from the high salt extraction was pooled and dialyzed against PME buffer in the same manner. The samples were then centrifuged through fresh sucrose gradients.

ATPase Assays and Determination of Protein Concentration—ATPase assays were performed at room temperature as described by Gibbons (1966) in 10 mM Tris-HCl (pH 8.2), 0.15 M KCl, 4 mM MgSO₄, 2 mM diithiothreitol, 1 mM ATP. The amount of phosphate released was determined by the malachite green method (Carter and Karl, 1982) and was quantified by reading the absorbance of the samples at 660 nm. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. ATPase activity is reported as absorbance at 660 nm where an absorbance of 1.0 represents 10 nmol of phosphate released/min.

Polyacrylamide Gel Electrophoresis—Discontinuous sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) was carried out by the procedure of Laemmli (1970) with 13% acrylamide and 0.11% bisacrylamide. Sodium dodecyl sulfate was from Sigma. Gels were stained with Coomassie Brilliant Blue.

Western Blotting—Immunoblotting was performed as described previously (Asai et al., 1982). After electrophoresis, the separated proteins were transferred to nitrocellulose (Schleicher and Schuell) and the blot was probed with 2 μl of anti-20 S antisera (Asai, 1986) diluted into 10 ml of gelatin-containing buffer. Antibody binding was detected with 125I-protein A (ICN) followed by autoradiography.

RESULTS

The 20 S and 12 S Dyneines from Unfertilized Eggs—The first goal was to isolate the dyneines from sea urchin eggs under different ionic conditions, thereby repeating the results that we and others have obtained. Two dyneins that differed in their sedimentation velocities through sucrose gradients were isolated from unfertilized S. purpuratus eggs. The 20 S dynein was obtained by low ionic strength extraction in PME buffer (Fig. 1a). Analysis of this dynein by SDS-PAGE revealed the coincidence of the two high M₅₅, polypeptides with the 20 S MgATPase activity (Fig. 1b). These are the egg dynein heavy chains that we have previously called bands 1 and 2 (Asai and Wilson, 1985). Bands 1 and 2 have been shown to contain the MgATPase activity and to bind to microtubules in vitro in an ATP-sensitive fashion (Asai et al., 1986). Typically, the 20 S egg dynein had an apparent specific activity of 30 nmol of P0₅, released/mg of protein/min.

The 12 S egg dynein was obtained by two different methods. Extraction of unfertilized eggs in PME that was made 0.6 M in KCl resulted in a 12 S MgATPase activity that was associated with bands 1 and 2 (Fig. 2). The specific activity of this 12 S dynein was generally about 12 nmol of P0₅, released/mg of protein/min. A second method was low ionic strength extraction in PME buffer followed by the addition of crystalline KCl to the 20 S dynein solution to a final concentration of 0.6 M KCl which also resulted in a 12 S MgATPase whose activity coincided with the high M₅₅, proteins by SDS-PAGE analysis of the sucrose gradient fractions (data not shown). The specific activity of this preparation was in the range of 15–19 nmol of P0₅, released/mg of protein/min. Western immunoblotting further demonstrated that the high M₅₅, pro-
FIG. 2. Sucrose gradient centrifugation of 12 S egg dynein. A 0.6 M KCl extract was prepared and sedimented through a 5–20% sucrose gradient. Twenty equal volume fractions were collected. a, MgATPase activity of 150–μl samples from each fraction, expressed as absorbance at 660 nm. Bottom of the gradient is fraction 1; arrows (left to right) indicate positions of 21 S and 11.3 S markers, respectively. The peak MgATPase activity (fraction 11) sedimented at approximately 12 S. b, electrophoretic analysis of the fractions shown in a. Equal volumes of each fraction were electrophoresed, and the gel was stained with Coomassie Brilliant Blue. The dynein heavy chains are indicated by arrows. Lanes containing fractions 10, 11, and 12, which contained the peak MgATPase activity, are indicated.

peptides corresponding to the MgATPase activity were the same for both the 20 and 12 S dyneins. As shown in Fig. 7, an antiserum to the egg 20 S MgATPase recognized the heavy chains of the 20 and 12 S dyneins.

Conversion of 20 S Dynein to 12 S Dynein—After establishing that two dyneins, which differed in their sedimentation velocities through sucrose gradients, could be isolated depending on the ionic strength of the extraction buffer, we next attempted to convert directly one dynein species to the other. The 20 S egg dynein could be converted to an approximately 12 S species after exposure to high ionic strength. The 20 S dynein peak from a sucrose gradient (e.g., fractions 7–9 from the gradient shown in Fig. 1) was pooled and dialyzed against PME, 0.6 M KCl for 36 h. The dialysate was then fractionated through a fresh sucrose gradient, and the fractions were assayed for MgATPase activity. This treatment resulted in the disappearance of the 20 S activity with the concomitant appearance of the 12 S species (Fig. 3). Again, high M$_r$ polypeptides were coincident with the MgATPase activity, appearing in the gradient in fractions 11 and 12 (data not shown). The specific activity of the 12 S conversion product was approximately 10 nmol of PO$_4$ released/mg of protein/min.

In the converse experiment, the 12 S species isolated in high ionic strength buffer was collected from sucrose gradients and resedimented after dialysis into low ionic strength buffer.

and resedimented after dialysis into low ionic strength buffer. In this case, the dynein did not shift in its sedimentation velocity, remaining at approximately 12 S (Fig. 4). SDS-PAGE analysis revealed that the high M$_r$ polypeptides remained coincident with the 12 S activity (data not shown). After this treatment, the specific activity decreased somewhat, to about 5 nmol of PO$_4$ released/mg of protein/min. The loss of MgATPase activity in both experiments was attributed to aging of the dynein which was necessary to accomplish conversion (36 h of dialysis). It was also possible to capture the 20 to 12 S conversion at a presumed intermediate stage. Exposure of the 20 S dynein to salt (by adding crystalline KCl) for a short period of time (30 min) resulted in a partial conversion in which the majority of the activity was shifted to 12 S, but with some remaining 20 S activity, represented as a shoulder of the MgATPase peak (Fig. 5). Again, the high M$_r$ dynein polypeptides exactly coincided with the MgATPase activity (data not shown).

Reconstitution of 20 S Dynein—Because the 20 S dynein could be converted to the 12 S species, but the 12 S dynein...
activity is expressed as absorbance at each fraction. Bottom of the gradient was being dissociated from the dynein and lost in the sucrose fraction. Twenty equal volume fractions were collected and 150 \mu l of each fraction was assayed for MgATPase activity. Bottom of the gradient is fraction 1; 21 S and 11.3 S markers are indicated with arrows; activity is expressed as absorbance at 660 nm. A bimodal distribution of MgATPase activity was obtained, suggesting that the conversion from 20 S to 12 S dynein was incomplete after the short incubation with salt.

![Graph](image)

**Fig. 5.** 20 S to 12 S conversion captured at an apparent intermediate stage. A low ionic strength extract (shown to contain only 20 S dynein, see Fig. 1) was made 0.6 M KCl by the addition of crystalline KCl. This material was incubated on ice for 30 min and then sedimented through a 5–20% sucrose gradient. Twenty equal volume fractions were collected and 150 \mu l of each fraction was assayed for MgATPase activity. Bottom of the gradient is fraction 1; 21 S and 11.3 S markers are indicated with arrows; activity is expressed as absorbance at 660 nm. A bimodal distribution of MgATPase activity was obtained, suggesting that the conversion from 20 S to 12 S dynein was incomplete after the short incubation with salt.

![Graph](image)

**Fig. 6.** Partial reconstitution of dynein. A low ionic strength extract (containing only 20 S dynein) was converted completely to 12 S dynein by the addition of crystalline KCl and by dialysis for 3 h versus PME, 0.6 M KCl (sufficient exposure to salt to convert completely the dynein to 12 S) and then dialyzed back into low ionic strength PME for an additional 30 h. The sample was then removed from the dialysis bag and sedimented through a 5–20% sucrose gradient. Twenty equal volume fractions were collected and 150 \mu l of each fraction was assayed for MgATPase activity. Bottom of the gradient is fraction 1; 21 S and 11.3 S markers are indicated with arrows; activity is expressed as absorbance at 660 nm. The 12 S dynein appeared to be partially reconstituted to a faster sedimenting species. In the experiment shown here, the MgATPase activity in fraction 11 sedimented at approximately 17.3 S.

collected from a sucrose gradient could not be converted to a faster sedimenting species, we reasoned that perhaps subunits were being dissociated from the dynein and lost in the sucrose gradient so that the 12 S enzyme no longer contained the full complement of proteins necessary to restore it to 20 S dynein. Therefore, a different reconstitution strategy was attempted. Eggs were extracted with low ionic strength buffer under conditions which would produce only 20 S dynein. A portion of the low ionic strength extract was sedimented through sucrose gradients to verify the existence of the 20 S dynein (results were similar to the gradients shown in Fig. 1). The rest of the low ionic strength 20 S dynein was converted by the addition of crystalline KCl and then dialyzed against PME, 0.6 M KCl for 3 h, a time more than sufficient to convert all of the dynein to 12 S. A portion of this sample was sedimented and shown to contain the 12 S MgATPase and no 20 S activity. The rest of the sample, still in the dialysis bag, was then dialyzed back into low ionic strength PME buffer for 30 h. After this reshift in ionic strength, the material was sedimented through a fresh sucrose gradient. A MgATPase activity that was heavier than 12 S was detected (Fig. 6). This activity contained high M, bands 1 and 2, although some of the heavy chain proteins remained in slower sedimenting fractions. The degree of reconstitution varied in different experiments. In the reconstitution shown in Fig. 6, the MgATPase sedimented at approximately 17.3 S.

In other experiments, the 20 S dynein was shown to be converted partially to a 12 S species with time and in the absence of added salt (data not shown). Thus, part of the difficulty encountered in the reconstitution experiment may have been due to this aging phenomenon, since the reconstitution attempts required 50 h or more to complete.

**Immunoblot Analysis—SDS-PAGE analysis followed by Western blotting of the MgATPase peaks for each experiment described above demonstrated that the high M, polypeptides corresponding to the 20 and 12 S dynein were indistinguishable antigenically (Fig. 7). An antiserum to the 20 S egg dynein (Asai, 1986) and a new antiserum to ciliary dynein heavy chains2 recognized the high M, proteins in the 20 and 12 S forms of egg dynein as well as the high M, proteins in samples that were converted from 20 to 12 S by exposure to salt (Fig. 7b).

**DISCUSSION**

The results presented here demonstrate that the dynein from unfertilized sea urchin eggs can be isolated as differently sedimenting species depending on the ionic strength of the extraction conditions. The 20 S species that we have described previously (Asai and Wilson, 1985) is isolated with low salt and a 12 S species is obtained under high salt conditions. Significantly, the 20 S dynein can be converted to the 12 S dynein by exposure to 0.6 M KCl buffer, but attempts to convert the 12 S species after its isolation from sucrose gradients to the 20 S species by removal of salt have failed. Attempts to reconstitute the 20 S by converting 20 S dynein to 12 S and then back again were partially successful in that

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a faster sedimenting activity was regained, corresponding to a shift in some, but not all, of the high M₁ dynein heavy chains. A recent report (Collins and Vallee, 1986b) indicated difficulty in the interconversion of 20 and 12 S dyneins. However, the extraction conditions employed by these investigators were performed under low salt, and the only exposure to the higher ionic strength was during sucrose density centrifugation. In light of our observation that conversion requires some time in high salt (see Fig. 5), it is possible that these workers did not observe conversion because they did not allow a sufficient length of time of exposure to high salt.

In all cases, the high M₁ bands 1 and 2 were found to be associated with the egg dynein MgATPase activity in the sucrose gradients. These high M₁ proteins have been shown to contain the important MgATPase and the microtubule binding activities (Asai et al., 1986). The shift in the position of the high M₁ proteins in the gradients is responsible for the shift in the MgATPase activity, similar to the differential sedimentation properties of sea urchin spermatozoan flagellar dynein (Tang et al., 1982).

An important conclusion from these data is that the 20 and 12 S activities appear to be isoforms of a single dynein, much the way in which 21 and 12 S sea urchin flagellar dynein appear to be different ionic strength-dependent subsets of the same enzyme (Tang et al., 1982). This conclusion is supported by two additional results: 1) the SDS-PAGE analysis shows that the high M₁ polypeptides are the same in both 20 and 12 S dyneins; and 2) the high M₁ proteins are immunologically indistinguishable. Although changes in conformation could lead to a difference in sedimentation velocities, it is probable that the difference is due to a salt-dependent dissociation of subunits (again, as in the case of flagellar 21 S dynein). Isolated fractions of 12 S dynein could not be converted back to the 20 S form by removal of salt, presumably because subunits were lost in the sucrose gradient. However, the dynein could be converted from the 20 S to the 12 S species and then partially reconstituted if the entire sample was retained in the dialysis bag (Fig. 6). This suggests that subunit associations account for the differences in apparent sedimentation velocities. We are currently improving the purification of 20 S dynein in order to define its entire protein composition and to refine the reconstitution experiment. We would expect to identify intermediate and light chains (Tang et al., 1982).

The 20 S dynein, first isolated by Asai and Wilson (1982, 1985), is comparable to flagellar 21 S latent activity dynein in its size, ability to rebend microtubules, and its enzymatic latency, in addition to its recognition by an antibody made against flagellar dynein heavy chains. By these criteria, it would appear that the 20 S egg MgATPase is an uncoupled dynein precursor, poised to combine with microtubules later in embryonic development. Indeed, our previous work indicates that the 20 S dynein is a precursor for blastula ciliary dynein (Asai, 1986).

The significantly smaller species that we describe here is comparable to the 12-14 S MgATPases characterized by other workers (Pratt, 1980; Hisanaga and Sakai, 1980, 1983; Scholey et al., 1984; Penningroth, 1985). This 12-14 S species has been suggested to be a "cytoplasmic dynein" which may function in the mitotic apparatus (Pratt et al., 1980), but it has not yet been demonstrated that the same proteins which constitute 12-14 S dynein later reside in the mitotic spindle. In fact, Leslie et al. (1985) have shown that very pure sea urchin mitotic spindle preparations do not appear to contain any dynein-like heavy chain proteins. While it is possible that a spindle dynein exists (e.g. McIntosh et al., 1969; Sakai et al., 1979; Cande, 1982, a and b), it remains to be established that the egg dynein functions in this role. Antisera made against flagellar dynein have been found to stain mitotic spindles (Itetsu et al., 1979; Yoshida et al., 1985; Hisanaga et al., 1987) but the antisera to 20 S dynein does not (Asai, 1986). It is also interesting to note that a kinesin-like molecule has been localized to the spindle (Scholey et al., 1985; Porter et al., 1987; Leslie et al., 1987) and, further, that a microtubule-activated 10 S ATPase that is distinct from both dynein and kinesin has been isolated from sea urchin eggs (Collins and Vallee, 1986b). Thus, while it has been tempting to assign the egg dynein a role in cytoplasmic microtubule-mediated events, the evidence for a spindle dynein is not conclusive.

In summary, our results indicate that the 12 S dynein is an isoform of 20 S dynein. Therefore, there is compelling evidence for only a single dynein in the unfertilized sea urchin egg, and this 20 S dynein has been shown previously to be a probable ciliary precursor. These data do not eliminate the possibility that dynein functions with non-axonemal microtubules. For example, perhaps the 20 S dynein can undergo a conformational change so that it can bind to the mitotic spindle microtubules and, at the same time, lose its reactivity with our anti-20 S antiserum. Alternatively, perhaps a new dynein is synthesized after fertilization and is then used in the mitotic apparatus (Piperno, 1984). We are producing additional antibodies to 20 S egg and ciliary dyneins for use as probes in localizing dynein in developing embryos. We are also developing molecular probes and labeling strategies to follow egg dynein during embryonic development and to study ciliogenesis in greater detail.

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