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Isolation and characterization of a novel dynein that contains C and A heavy chains from sea urchin sperm flagellar axonemes

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SUMMARY

A novel dynein (C/A dynein), which is composed of C and A heavy chains, two intermediate chains and several light chains, was isolated from sea urchin sperm flagella. The C/A dynein was released by the treatment with 0.7 M NaCl plus 5 mM ATP from the axonemes depleted of outer arm 21 S dynein. Sedimentation coefficient of this dynein was estimated by sucrose density gradient centrifugation to be 22-23 S. The C/A dynein particle appeared to be composed of three distinct domains; two globular head domains and one rod domain as seen by negative staining electron microscopy. The mobility of 'A' heavy chain of C/A dynein on SDS-gel electrophoresis was similar to that of A heavy chains (Aα and Aβ) of 21 S dynein. However, UV-cleavage patterns of C and A heavy chains of C/A dynein were different from those of A heavy chains of 21 S dynein. Furthermore, an antiserum raised against A heavy chain of C/A dynein did not crossreact with A heavy chains of 21 S dynein. Under the conditions in which the C/A dynein was released, some of inner arms were removed concomitantly from axonemes as observed by electron microscopy. These results suggested that C/A dynein is a component of the inner arms.

Key words: sea urchin, sperm, flagellum, inner arm, dynein

INTRODUCTION

The bending waves of cilia and flagella are formed by the sliding movement between the outer doublet microtubules of the axonemes (Satir, 1968; Summers and Gibbons, 1971). There is abundant evidence that this active sliding occurs through interaction of dynein arms on the A-tubules with adjacent B-tubules of the outer doublet microtubules (Gibbons, 1981; Johnson, 1985).

Two rows of arms, which are referred to as outer and inner arms, respectively, are attached to the outer doublet microtubules. The outer arms repeat at a 24 nm periodicity along the length of the outer doublet microtubules and seem to be uniform in shape (Goodenough and Heuser, 1989). Each outer arm consists of outer arm dynein, which is a large complex protein containing heavy, intermediate and light chains (Johnson, 1985; Witman, 1989). In sea urchin sperm flagella, the outer arm dynein is selectively extracted by a high salt solution. It sediments as a 21 S particle (Gibbons and Fronk, 1979) and appears as a two-headed structure (Sale et al., 1985).

In contrast to the outer arms, population of the inner arms is considered to be heterogeneous: at least two kinds of arms are present in *Chlamydomonas* flagella, which are called the triad and dyad. These arms are arranged to form a triad-dyad-dyad triplet. The triplets repeat every 96 nm along each outer doublet microtubules (Goodenough and Heuser, 1985). The dynein molecules which compose the triad, dyad and dyad arms were called I1, I2 and I3, respectively (Piperno et al., 1990), and heavy chain components of these dyneins have been studied by the use of mutant cells missing subsets of the inner arms (Piperno et al., 1990; Kamiya et al., 1991). Recently, the arrangement and molecular composition of the *Chlamydomonas* inner arms have been reported to be more complicated; the heavy chain compositions of I2 and I3, respectively, are different in proximal and distal regions of the flagella, although that of I1 does not vary along the entire length (Piperno and Ramanis, 1991). Furthermore, it has been observed that inner arms are arranged in a couple of rows on the surface of the outer doublet microtubules (Kamiya et al., 1991; Muto et al., 1991).

In sea urchin sperm flagella, several kinds of heavy chains, which are considered to be components of the arms, are observed on SDS-gel electrophoresis and were disignated as C, Aα, Aβ, D and B heavy chains (Gibbons et al., 1976; Gibbons and Gibbons, 1987a) or I, II, III, IV, V and VI (Sale et al., 1989) in order of increasing rate of migration. In this study, the nomenclature by Gibbons et al. is used. Parts of Aα and Aβ heavy chains, respectively, have been shown to be components of 21 S dynein constituting the outer arms (Bell et al., 1979). However, the molecular composition of the inner arms has not been clarified. B and D heavy chains have been purified (Inaba et al., 1988; Ogawa and Gibbons, 1976), but both the function and localization of these proteins in the axonemes are not known.

In the present study, we isolated a novel dynein that is composed of C and A heavy chains from flagellar axonemes of sea urchin sperm.

MATERIALS AND METHODS

Materials

ATP, CTP, GTP, ITP and TTP were purchased from Yamasa Shoyu Co., Tokyo.

Preparation of 21 S dynein and C/A dyenin

Spermatozoa were obtained from sea urchin *Anthocidaris crassispina* by injection of 2 mM acetylcholine chloride into the body cavity. Axonemes were isolated from sperm tails without detergent treatment as described previously (Yokota et al., 1987). The axonemes were suspended in MEM solution (4 mM $MgSO_4$, 0.2 mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, 0.2 mM PMSF and 10 mM MOPS-NaOH, pH 7.2) containing 0.6 M NaCl and kept on ice for 1 hour. The suspension was then centrifuged at 12,000 ***g*** for 10 minutes. The pellet was further extracted with a solution of 0.75 M NaCl, 4 mM $MgSO_4$, 0.2 mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, 0.2 mM PMSF and 10 mM Tris-HCl (pH 8.0) for 1 hour on ice and then centrifuged at 12,000 ***g*** for 10 minutes. This extraction was repeated once more. The pellet fraction was called S-axonemes. S-axonemes were resuspended in the MEM solution containing 0.7 M NaCl and 5 mM ATP and kept on ice for 20 minutes. The suspension was then centrifuged at 12,000 ***g*** for 10 minutes. The 0.6 M NaCl extract and 0.7 M NaCl-ATP extract were further centrifuged at 100,000 ***g*** for 30 minutes and concentrated by ultrafiltration using Amicon PM 10 membrane (Amicon Co., Lexington, MA), respectively. The extracts were dialyzed against MEM solution containig 0.1 M NaCl, and were centrifuged on a 5% to 20% (w/v) sucrose linear density gradient made up in the dialysis solution at 94,000 ***g*** (Hitachi 70P ultracentrifuge, RPS27-2 rotor, Hitachi Koki Co., Ltd., Tokyo) for 23 hours. After fractionation, the peak fraction of ATPase activity obtained from the 0.6 M NaCl extract was used as purified 21 S dynein. The first peak fractions of ATPase activity from the 0.7 M NaCl-ATP extract was pooled and further chromatographed on a hydroxylapatite column or a DEAE-Sephacel (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) column. The peak fraction of ATPase activity obtained from the DEAE-Sephacel column was used as purified C/A dynein.

Measurement of ATPase activity

The ATPase activity was assayed in 0.15 M KCl, 4 mM $MgSO_4$, 0.5 mM EDTA, 1 mM ATP and 20 mM Tris-HCl (pH 8.0). The amount of P_i liberated was determined by the method of Fiske and SubbaRow (1925).

Determination of protein concentration

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Gel electrophoresis

Polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) was performed according to the method of Laemmli (1970). Proteins were stained with 0.2% Coomassie Brilliant Blue. Gels were scanned with a dual wavelength chromatoscanner CS-910 (Shimadzu Seisakusho, Ltd., Kyoto).

Preparation of antibody

The purified C/A dynein was subjected to SDS-PAGE using a 3% to 6% (w/v) acrylamide gradient slab gel. The A and C heavy chain bands were cut out and homogenized in Freund's complete adjuvant. The A band protein was injected into male ICR mice. The C band protein was injected into male rabbits. The injection was performed for five times with two-week intervals. The animals were bled two weeks after the final injection. The sera were incubated at 57°C for 30 minutes and stored frozen at −80°C.

Immunoblotting

After SDS-PAGE, proteins were electrophoretically transferred to a nitrocellulose membrane (0.45 μm pore size; Schleicher and Schuell, Inc., Dassel, FRG) at 200 mA for 3 hours according to Towbin et al. (1979). The nitrocellulose membrane was first incubated with the antiserum diluted 200- or 300-fold with PBS (0.15 M NaCl and 10 mM sodium phosphate buffer, pH 7.5). Antigens were detected using Vectastain ABC kit (Vector Labs., Inc., Burlingame, CA, USA).

Immunoprecipitation of C/A dynein using the antiserum against C heavy chain

C/A dynein fraction (60 μg protein/ml) was dialyzed against 0.1 M NaCl, 4 mM $MgSO_4$, 0.2 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 10 μg/ml leupeptin and 10 mM MOPS-NaOH (pH 7.5). The dialyzed sample was first mixed with the antiserum against C-band and kept on ice for 1 hour. As a control, the dialyzed sample was mixed with a pre-immune serum. Then 1/100 vol. of cell wall fraction of *Staphylococcus aureus* was added to the mixture and was kept standing on ice for 50 minutes. After centrifugation at 14,000 ***g*** for 20 minutes, both the supernatant and the pellet were analyzed by SDS-PAGE.

Thin-section electron microscopy

The samples were fixed with 8% (w/v) tannic acid and 2.5% glutaraldehyde dissolved in 50 mM sodium phosphate buffer (pH 6.8) for 1 hour at room temperature and then postfixed with 1% (v/v) OsO_4 dissolved in 0.1 M sodium cacodylate buffer (pH 7.4) for 1 hour at room temperature. After rinsing in distilled water, the samples were dehydrated in a graded concentration series of ethanol and embedded in Spurr (Polyscience Inc., Warrington, PA, USA) for electron microscopy.

Negative staining electron microscopy

The DEAE-Sephacel fraction was applied to a carbon-coated copper grid and stained with 2% (w/v) uranyl acetate. Specimens were examined with a JEM 100B electron microscope (JEOL Ltd., Tokyo) operating at 80 kV.

UV cleavage of dynein heavy chains

Dynein fractions were dialyzed against a solution of 0.45 M sodium acetate, 2 mM $MgSO_4$, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF and 10 mM MOPS-NaOH (pH 7.2) and then irradiated with UV light at 365 nm using a Manaslu UV-light (Manaslu Corp., Tokyo) in the presence of 0.2 mM ATP and 0.05 mM vanadate on ice for 1 hour.

RESULTS

Preparation of C/A dynein

When S-axonemes were extracted with 0.7 M NaCl plus 5 mM ATP, C, A and D heavy chains were released specifically as shown in Fig. 1. Approximately 60 to 70% of the C heavy chain in the axoneme was consistently extracted. These bands were also seen in NaCl extracts in the absence or presence of other nucleotides (CTP, GTP, ITP or TTP). However, the amounts of these bands were much smaller than those in the NaCl-ATP extract. Furthermore, no detectable C heavy chain was extracted with the first 0.6 M NaCl treatment to remove outer arms, although some D heavy chains were extracted. Consequently, most of the C heavy chain and parts of the A chains were released specifically by ATP.

The NaCl-ATP extract was centrifuged on a 5% to 20% sucrose density gradient. Fig. 2 shows a sedimentation pattern of protein and ATPase activity. Three major peaks of ATPase activity were detected. The heaviest peak (fraction 5 in Fig. 2a) coincided with a protein peak. The sedimentation coefficient of this peak was estimated to be 22-23 S. Upon SDS-PAGE, C and A heavy chains were found to be main components in

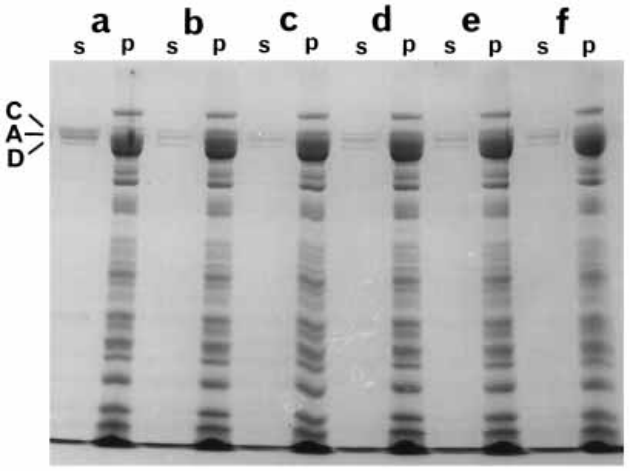


Fig. 1. Extraction of C and A heavy chains from S-axonemes. S-axonemes were extracted with a 0.7 M NaCl solution containing ATP (a), CTP (b), GTP (c), ITP (d) or TTP (e) at a concentration of 5 mM or no nucleotide (f). After centrifugation, the supernatant (s) and pellet (p) were analyzed by SDS-PAGE using a 5% (w/v) acrylamide gel. The complete set of dynein bands (A-D) is shown in Figs 4, 7 and 9.

this peak (Fig. 2b). Two intermediate sized chains of 120 kDa and 103 kDa co-purified in the same fraction. The second ATPase peak (fraction 7 in Fig. 2a) coincided with the position of outer arm 21 S dynein (arrowhead in Fig. 2a). Two polypeptide chains having same molecular masses as those of intermediate chains of 21 S dynein (94 kDa and 73 kDa, respectively) were observed in this fraction (arrowheads in Fig. 2b). Therefore, it is likely that this dynein was contained in this fraction. The third peak (fraction 15 in Fig. 2a) corresponded to the position of 10 to 14 S. The D heavy chain was the main component in this peak (Fig. 2b).

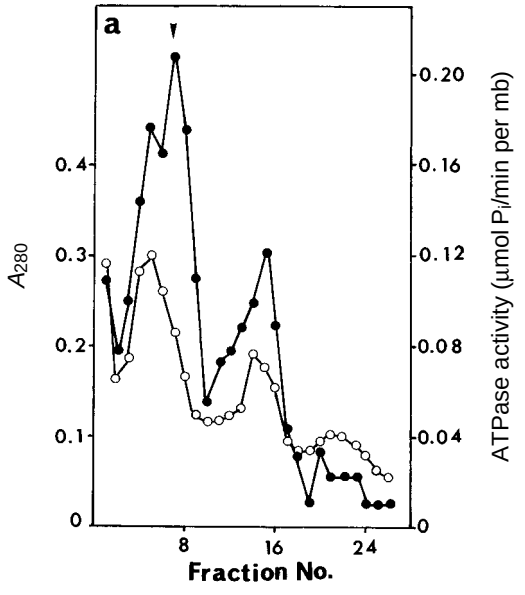
The first peak of ATPase activity (fractions 3-6 in Fig. 2a) was pooled and further chromatographed on a hydroxylapatite column or a DEAE-Sephacel column. Fig. 3 shows a result of the DEAE-Sephacel column chromatography. Protein and ATPase peaks coincided with each other. Four polypeptides, namely C and A heavy chains, and 120 kDa and 103 kDa polypeptides, were co-eluted in this fraction (Fig. 3). These four polypeptides co-eluted from the hydroxylapatite column at 0.25 M sodium phosphate (pH 7.2) (data not shown). Assuming that Coomassie Brilliant Blue stained these polypeptides equally, the weight ratios of C heavy chain, A heavy chain, 120 kDa polypeptide, 103 kDa polypeptide were 1:0.96:0.20:0.16 from densitometry of fraction 16 in Fig. 3b. The molar ratio of these chains will be 1:1:1:1, since the molecular masses of C and A heavy chains were 445 kDa and 425 kDa, respectively (see Discussion).

Immunoprecipitation using the antiserum against C heavy chain

From the above results, it was strongly suggested that the four polypeptides form a complex. To confirm this possibility, immunoprecipitation using an antiserum against C heavy chain was performed. This antiserum specifically recognized the C heavy chain in the DEAE-Sephacel column fraction 16 or in the isolated axonemes (Fig. 4). When the antiserum was mixed with fraction 16, not only C but also A heavy chain co-precipitated with antibodies. With increasing amounts of antiserum, the amount of both chains in the pellet fraction increased in a similar manner (Fig. 5). Thus, it was concluded that the C and A heavy chains form a complex. We call this complex C/A dynein.

Molecular shape of C/A dynein

The structure of C/A dynein was examined by negative staining electron microscopy. As shown in Fig. 6, the molecule of C/A dynein appeared to be composed of three domains: two globular 'head' domains and a rod domain. The diameter of the head domains was about 14 nm. Both the width and length of the rod domain were variable and they were 6 to 10 nm and 25 to 32 nm, respectively.



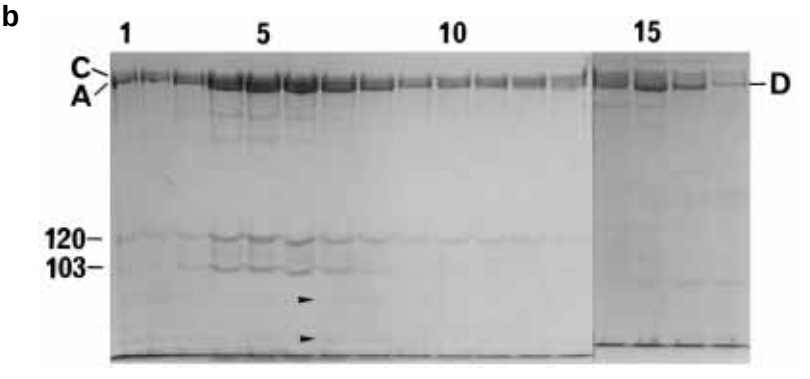


Fig. 2. Sucrose density gradient centrifugation of the 0.7 M NaCl-ATP extract. Proteins sedimented from right to left (a). (●) ATPase activity; (○) absorbance at 280 nm. Arrowhead indicates the position of 21 S dynein. Fractions 1-17 were electrophoresed on a 5% (w/v) acrylamide gel (b). In (b) C, A and D heavy chains and intermediate chains of 120 kDa and 103 kDa are indicated. Arrowheads indicate intermediate chains IC-2 and IC-3 of 21 S dynein.

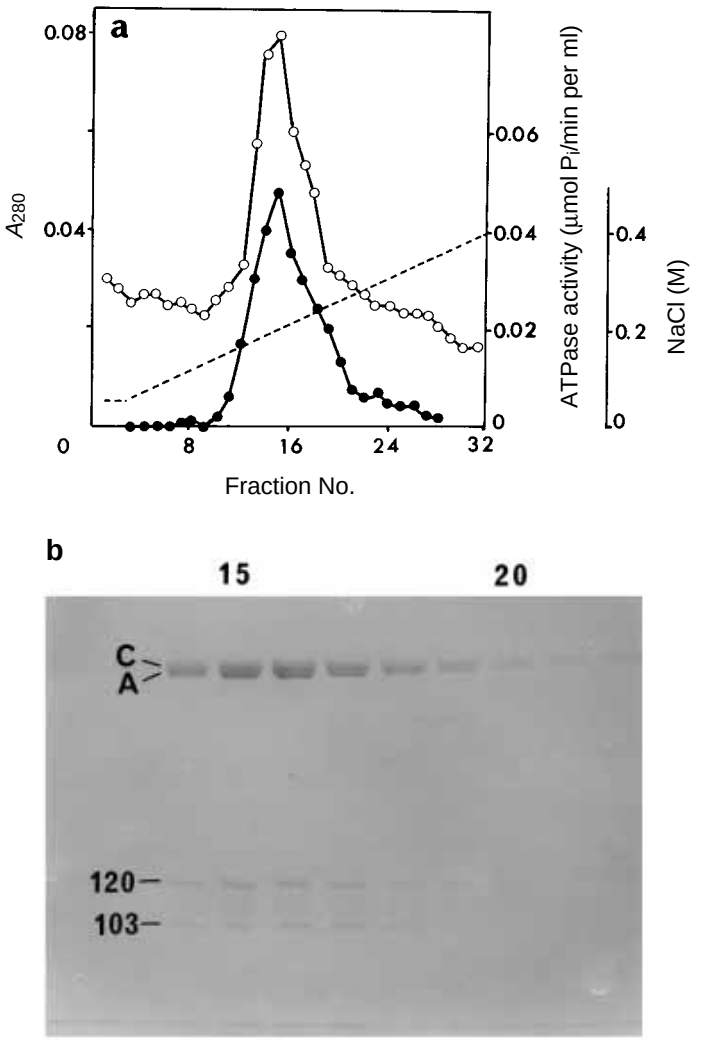


Fig. 3. DEAE-Sephacel column chromatography of sucrose density gradient centrifugation fractions (fractions 2-6 shown in Fig. 2). (a) (●) ATPase activity; (○) absorbance at 280 nm. Broken line shows NaCl concentration. (b) Fractions 12-22 were electrophoresed on a 5% (w/v) acrylamide gel. C and A heavy chains and intermediate chains are indicated.

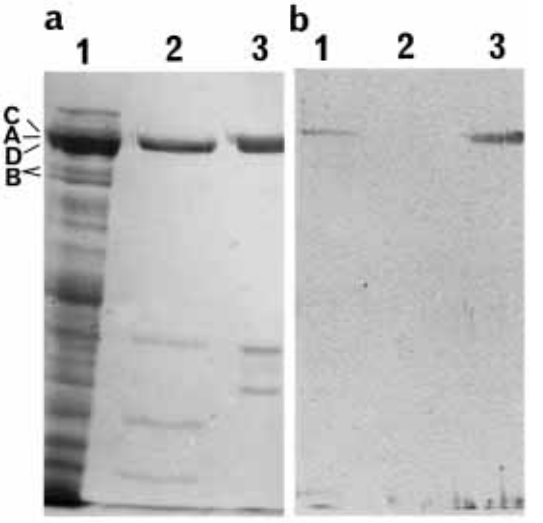


Fig. 4. Immunobloting of axonemal proteins by antiserum against C heavy chain. SDS-PAGE was performed on a 5% (w/v) acrylamide gel. (a) Coomassie Brilliant Blue staining. (b) Immunoblot. Lane 1, isolated axonemes. Lane 2, 21 S dynein fraction. Lane 3, fraction 16 obtained by DEAE-Sephacel column chromatography shown in Fig. 3. Positions of dynein heavy chains are indicated.

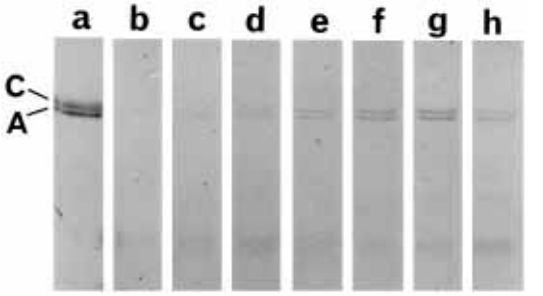


Fig. 5. Immuno-precipitation using the antiserum against C heavy chain. Fraction 16 shown in Fig. 3 was mixed with pre-immune serum diluted 20-fold (c) or with the antiserum diluted 200- (d), 100- (e), 50- (f) or 20- (g) fold, respectively. Then cell wall fraction of *Staphylococcus aureus* was added. After incubation on ice for 50 minutes, samples were centrifuged and pellets were analyzed by SDS-PAGE on a 5% (w/v) acrylamide gel. A mixture of the antiserum and cell wall fraction of *S. aureus*, which did not contain fraction 16, was processed in the same manner (b). The supernatant of lane g was further incubated with the 20-fold diluted antiserum and then processed as described above (h). Lane a shows fraction 16 alone. Dynein heavy chain region of the pellet fractions is shown.

Comparison of C/A dynein with 21 S dynein

SDS-PAGE of purified 21 S dynein showed an A heavy chain, three intermediate chains called IC-1, IC-2 and IC-3, respectively, and several light chains on a 3% to 15% acrylamide gradient gel (Fig. 7a, lane 1). Trace amounts of tubulin subunits were seen in the gel (arrowheads in Fig. 7a, lane 1). The A heavy chain was further split into two bands, Aα and Aβ heavy chains, on a 3 to 6% acrylamide gradient gel (Fig. 7b, lane 1). On the other hand, purified C/A dynein fraction contained C and A heavy chains, two intermediate chains (120 kDa and 103 kDa) and at least four light chains (Fig. 7a, lane 2). The heavy chains did not split further under the conditions where the A heavy chain of 21 S dynein was split into two (Aα and Aβ) chains (Fig. 7b, lane 2). The intermediate chains of C/A dynein did not co-migrate with those of 21 S dynein. At least two of the four light chains in C/A dynein did not co-migrate with those of 21 S dynein. The mobility of A heavy chain of C/A dynein was similar to that of Aα heavy chain of 21 S dynein (Fig. 7b, lane 2). However, the antiserum raised against A heavy chain of C/A dynein did not crossreact with the A heavy chains of 21 S dynein (Fig. 7c). Furthermore, the antiserum raised against Aα heavy chain of 21 S dynein did not recognize A heavy chain of C/A dynein (data not shown).

Next, the pattern of UV-cleavage of C/A dynein was compared with that of 21 S dynein. The A heavy chains of 21 S dynein were cleaved into two fragments called HUV (228 kDa) and LUV (200 kDa) by the irradiation of UV light in the presence of both ATP and vanadate (Fig. 8a, lane 2) as reported previously (Lee-Eiford et al., 1986). Both C and A heavy

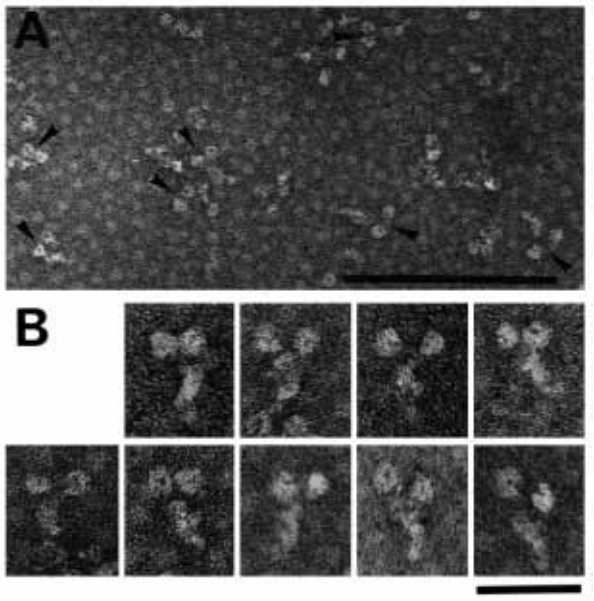


Fig. 6. Electron micrographs of C/A dynein. Fraction 16 shown in Fig. 3 was applied to a carbon-coated copper grid and stained negatively with uranyl acetate. (A) Low-power image. Arrowheads indicate the globular-head domains. Bar, 0.2 μm. (B) High-power images of C/A dynein. Bar, 50 nm.

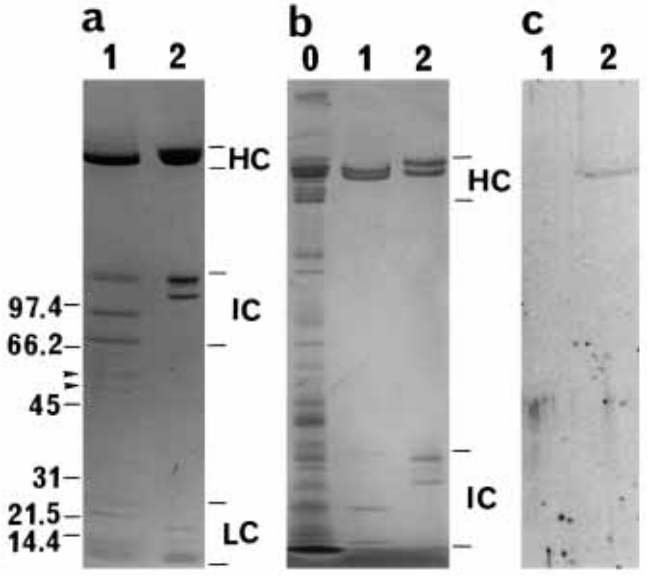


Fig. 7. SDS-PAGE of 21 S dynein and C/A dynein (a,b) and immunoblotting by the antiserum against A heavy chain of C/A dynein (c). 21 S dynein (lane 1), C/A dynein (lane 2) and isolated axonemes (lane 0) were electrophoresed on a 3% to 15% (w/v) (a) or 3% to 6% (w/v) acrylamide gradient gel (b,c). Arrowheads indicate tubulin bands. Heavy chain regions (HC), intermediate chain regions (IC) and light chain regions (LC) are indicated by pairs of bars, respectively. The apparent molecular masses ($\times 10^{-3}$) of the standard proteins are indicated on the left of (a).

chains of C/A dynein were also cleaved by the UV irradiation and generated three fragments (Fig. 8a, lane 4). The molecular masses of these fragments were estimated to be 235 kDa, 210 kDa and 190 kDa, respectively. Upon immunoblotting, intact

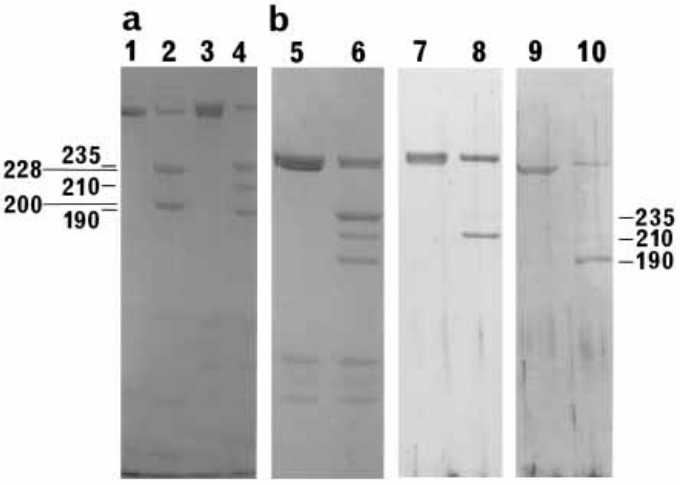


Fig. 8. UV-cleavage patterns of 21 S and C/A dyneins. (a) Coomassie Brilliant Blue staining of 5% acrylamide gel. Lanes 1, intact 21 S dynein; 2, UV-cleaved 21 S dynein; 3, intact C/A dynein; 4, UV-cleaved C/A dynein; b, Coomassie Brilliant Blue staining (5, 6) and immunoblots (7-10) of C/A dynein on a 5% acrylamide gel; 5, intact C/A dynein; 6, UV-cleaved C/A dynein; 7, intact C/A dynein reacted with anti-C heavy chain; 8, UV-cleaved C/A dynein reacted with anti-C heavy chain; 9, intact C/A dynein reacted with anti-A heavy chain; 10, UV-cleaved C/A dynein reacted with anti-A heavy chain. Molecular masses ($\times 10^{-3}$) of the cleavage products are indicated.

A heavy chain and 190 kDa-cleavage fragment crossreacted with the antiserum against A heavy chain, while intact C heavy chain and 210 kDa-cleavage fragment crossreacted with the antiserum against C heavy chain (Fig. 8b). The amounts of C and A heavy chains that underwent UV cleavage were 41% and 60%, respectively, from densitometric estimation. On the other hand, the weight ratio of the 235 kDa:210 kDa:190 kDa cleavage fragments was 1:0.48:0.63.

Localization of C/A dynein in the axonemes

From the fact that C/A dynein was extracted from the S-axonemes from which 21 S outer arm dynein had been removed, it is likely that C/A dynein is a component of the axoneme other than the outer arm. To elucidate the localization of C/A dynein in the axoneme, the electron microscopic appearance of the axonemes was examined before and after extraction of C/A dynein.

Before NaCl extraction, the outer doublet microtubules, the outer arms, the inner arms, the radial spokes and other associated structures remained intact (Fig. 9a) as seen in the cross-sectional view. Upon NaCl extraction, almost all outer arms were removed from the outer doublets (Fig. 9b and Table 1), and Aα and Aβ heavy chains were concomitantly reduced in the axonemes (S-axonemes) (Fig. 9, lane 2). On the other hand, about 21.6% of inner arms were lost from the outer doublets by the NaCl-ATP extraction (Fig. 9c and Table 1). Furthermore the density of some of the remaining inner arms was decreased by this treatment. These inner arms were counted as those remaining after the extraction in Table 1. Therefore, extracted inner arms may be more than 22%. Concomitantly, almost all the C and the remaining Aα heavy chains were removed from the S-axonemes (Fig. 9, lane 3). Other structures

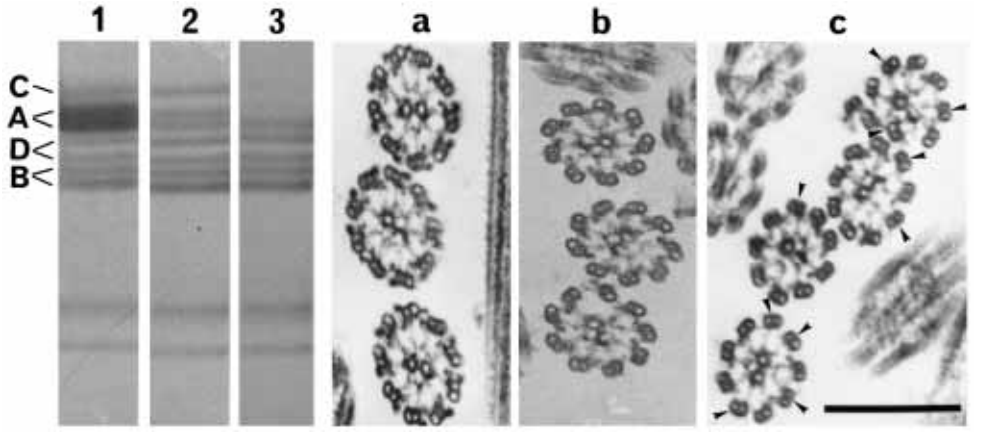


Fig. 9. SDS-PAGE of heavy chains (lanes 1,2 and 3) of axonemes and electron micrographs of the thin sections (a,b and c). Lane 1 and (a) isolated axonemes. Lane 2 and (b) S-axonemes. Lane 3 and (c) 0.7 M NaCl-ATP-extracted axonemes. SDS-PAGE was performed on a 3% to 6% acrylamide gradient gel. The high molecular mass region is shown here. The heavy chains are indicated on the left of lane 1. Bar, 0.25 μm. Arrowheads in (c) indicate outer doublet microtubules lacking inner arms.

Table 1. Thin-section electron microscopic analysis of arms

	Number of arms (%)		Number of outer doublets counted
	Outer arms	Inner arms	
Axonemes	98.9	99.4	851
S-axonemes	2.5	97.0	976
ATP-S-axonemes	2.0	75.4	1004

S-axonemes: axonemes that were extracted with 0.6 M NaCl.
ATP-S-axonemes: S-axonemes that were extracted with 5 mM ATP and 0.7 M NaCl.

appeared to be unchanged before and after the NaCl-ATP extraction (Fig. 9).

DISCUSSION

This is the first report on the structural and functional studies of the C heavy chain in the axoneme of sea urchin sperm flagella. It is demonstrated that the C heavy chain is a major component of a novel dynein tentatively called C/A dynein. C/A dynein appeared to be a 22-23 S particle composed of C and A heavy chains, two intermediate chains of 120 kDa and 103 kDa and at least four light chains. Negative staining electron microscopy demonstrated that C/A dynein has two globular head domains and a rod-like domain. The diameter of the globular head domains was about 14 nm, which is almost the same size as that of the isolated outer arm dynein of sea urchin sperm flagella (Sale et al., 1985), *Tetrahymena* cilia (Johnson and Wall, 1983; Toyoshima, 1987) or *Chlamydomonas* flagella (Goodenough and Heuser, 1984), or that of some inner arm dyneins of *Chlamydomonas* flagella (Goodenough et al., 1987). The structural analyses of axonemal and cytoplasmic dyneins have demonstrated that the number of globular heads per molecule is equal to the number of heavy chain species (Johnson and Wall, 1983; Sale et al., 1985; Vallee et al., 1988; Goodenough and Heuser, 1989; Smith and Sale, 1991). Since the molar ratio of C and A heavy chains of C/A dynein was 1:1 (see below), each globular head domain of C/A dynein may be constituted from C or A heavy chain, respectively. The heavy chains seemed to be connected at the rod-like domain to form a bouquet-like configuration of the molecule. The rod-like domain may be the site for binding of the intermediate chains as proposed for outer arm dyneins of sea urchin sperm (Sale et al., 1985) or *Chlamydomonas* (King and Witman, 1990) flagella. Densitometry of the SDS-gel also suggested that a molecule contains each one of 120 kDa and 103 kDa intermediate chains.

The C/A dynein heavy chains underwent UV cleavage producing three fragments of 235 kDa, 210 kDa and 190 kDa. Gibbons and Gibbons (1987a) also reported that C heavy chain in the axonemes was cleaved by the UV irradiation in the presence of ATP and vanadate. These results suggest that both C and A heavy chains are ATP-binding or ATPase peptides like heavy chains of other dyneins. The immunoblotting using antiserum against C heavy chain and that against A heavy chain revealed that the 210 kDa or 190 kDa fragment is derived from C or A heavy chain, respectively. The weight ratio of 235 kDa:210 kDa:190 kDa fragments was 1:0.48:0.63. On the other hand, 41% or 61% of C or A heavy chain, respectively, was cleaved. These results suggest that the 235 kDa fragment was produced from both C and A heavy chains and has no epitope recognized by each antiserum. Therefore, C or A heavy chain was cleaved into 235 kDa and 210 kDa or 235 kDa and 190 kDa fragments, respectively. Assuming that there is only one cleavage site in each heavy chain like the outer arm dynein (Lee-Eiford et al., 1986; Gibbons and Gibbons, 1987b; King and Witman, 1987) or cytoplasmic dynein (Paschal et al., 1987; Porter et al., 1988), the molecular mass of C or A heavy chain would be 445 kDa or 425 kDa, respectively. This value of 425 kDa of A heavy chain is close to those of A heavy chains of 21 S dynein, which were estimated in a similar manner (Lee-Eiford et al., 1986).

The mobility of A heavy chain of C/A dynein on SDS-gel electrophoresis was similar to A, especially Aα heavy chain of 21 S dynein. However, UV-cleavage patterns and antigenicity of A heavy chain of C/A dynein were different from those of A heavy chains of 21 S dynein. C/A dynein was extracted from salt-extracted axonemes depleted of outer arms. Concomitantly, about 22% of inner arms disappeared from the outer doublet microtubules. Furthermore, the C/A dynein molecule appears as a bouquet-like structure that is characteristic of outer and inner arms in axoneme (Goodenough and Heuser, 1985, 1989). Sale et al. (1989) also suggested that I heavy chain (C heavy chain in this study) may consist inner arms in flagellar axonemes of sea urchin sperm. From these results and reports, we consider that C/A dynein is one of the components of the inner arms.

We detected seven heavy chains, namely C, Aα, Aβ, D (doublet) and B (doublet), in sperm axonemes of the sea urchin *Anthocidaris crassispina* as also were found in the axonemes of *Tripneustes gratilla* (Gibbons et al., 1976; Gibbons and Gibbons, 1987a). Parts of Aβ and two D heavy chains and all B heavy chains, respectively, remained in the NaCl-ATP-extracted axonemes lacking both outer arm 21 S dynein and C/A dynein, although a small amount of D heavy chains was detected in the NaCl-ATP extract. Ogawa and Gibbons (1976) purified D band dynein (dynein 2) and showed that it has an ATPase activity. B heavy chains were purified by Inaba et al. (1988), but this heavy chain fraction had no detectable ATPase activity. On the other hand, Gibbons and Gibbons (1987a) and Sale et al. (1989) revealed that B heavy chains were cleaved by UV irradiation in an ATP- and vanadate-dependent manner as well as D heavy chains. Furthermore, Pratt (1986) showed that the B heavy chain has an ability to bind ATP by using a photoaffinity analog of ATP (8-N_3-[α-^{32}P]ATP). It could be that this protein loses its ATPase activity during extraction and purification. Concerning Aβ heavy chain that remained in the axonemes from which both 21 S dynein and C/A dynein had been extracted, no information has been available about its structure and function. It is possible that the remaining Aβ and D heavy chains and B heavy chains consist of the inner arm fraction that remains after extraction of C/A dynein.

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