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Immunological detection of actin in the 14S ciliary dynein of *Tetrahymena*

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Abstract

The association of actin with *Tetrahymena* ciliary dyneins was examined using a polyclonal antibody against *Tetrahymena* actin. Western blotting shows that actin is present in the 14S dynein fraction, but not in the 22S dynein fraction, which comprises the outer arm. By anion-exchange chromatography, 14S dynein can be further separated into three major fractions that contain four distinct heavy chains in total. When each fraction was tested by anti-actin immunoblotting, all three fractions contained actin in nearly stoichiometric amounts with the heavy chain. Since *Tetrahymena* actin differs significantly from actins of other species, the association with inner-arm dynein may be a conserved property of actin.

Key words: Cilia; Dynein; Actin; *Tetrahymena*

1. Introduction

The actin-based and the microtubule-based systems are usually considered to be independent of each other, although there are a few cases of motility systems in which both cytoskeletal proteins appear to participate. For example, Piperno et al. have shown that actin is associated with the inner dynein arms in *Chlamydomonas* flagella [1–4], and recent studies have suggested that actin and an actin-related protein are contained in a molecular complex (dynactin) that activates the function of cytoplasmic dynein [5–7]. However, it remains to be established whether or not association of actin with dynein is a general phenomenon.

In this study, we examined whether actin is present in *Tetrahymena* ciliary dyneins using polyclonal antibodies specific for *Tetrahymena* actin. *Tetrahymena* was chosen because it is a popular organism in the study of axonemal dynein, and because its actin has been shown to differ significantly in properties from actins from other species so far studied [8–10]. We found that multiple kinds of dyneins sedimenting at 14 S (14S dynein) contain actin, whereas the dynein sedimenting at 22 S (22S dynein) does not. The finding that even such a divergent actin is associated with 14S dynein suggests that the dynein association may be one of the most conserved properties of actin and that this association might be inner-arm dynein specific.

2. Materials and methods

2.1. Preparation of 14S dynein

Dynein was prepared from *Tetrahymena thermophila* (strain B) according to [11] with minor modifications. Ciliary axonemes were obtained from a 7-liter culture of cells, collected by centrifugation, and extracted by suspending the pellet in 3 ml of 0.6 M NaCl made in HMDE solution (10 mM HEPES, 4 mM $MgSO_4$, 1 mM dithiothreitol, 1 mM EGTA and 0.5 mM PMSF, pH 7.4). The extracted axonemes were sedimented by centrifugation at 40,000 × *g* for 30 min. The supernatant containing dynein was diluted with TMDE (10 mM Tris-HCl, 4 mM $MgSO_4$, 1 mM dithiothreitol, 0.1 mM EGTA and 0.2 mM PMSF, pH 8.0) to bring the final NaCl concentration to 80 mM, centrifuged at 40,000 × *g* for 30 min to remove small precipitates, and applied to a DEAE-Sephacel column (bed volume: 1 ml). Dynein was eluted with a TMDE solution containing 0.2 M NaCl. The column-purified dynein was further purified by sucrose density gradient centrifugation; the dynein sample was loaded onto an 11-ml 5–20% linear gradient of sucrose in HMDE plus 0.1 M NaCl and centrifuged at 250,000 × *g* for 14 h (4°C) in a Hitachi RPS-40T rotor.

2.2. Subfractionation of 14S dynein by ion-exchange chromatography

The 14S dynein fraction was subfractionated using a high-pressure liquid chromatography (HPLC) system (FPLC system, Pharmacia LKB, Uppsala) on a MonoQ HR5/5 analytical anion-exchange column, essentially according to the method described in [12]. The 14S dynein fraction was diluted with HMDE solution to bring the final NaCl concentration to 0.1 M and loaded on the column at a flow rate of 0.5 ml/min. The protein was then eluted with a linear 100 to 450 mM KCl gradient. Fractions of 0.5 ml were collected.

2.3. Polyclonal antibody against Tetrahymena actin

Tetrahymena actin prepared as described previously [8] was subjected to SDS-PAGE and cut out from the gel. After dialysis against phosphate-buffered saline, the gel was homogenized with complete Freund's

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adjuvant and subsequently injected into a rabbit at weekly intervals. Antiserum was obtained after the antigen had been injected five times. Immunoblot analyses of the whole cell extract with this antiserum showed that it reacted only with a single band of 43.5 kDa, which corresponded to the band of *Tetrahymena* actin. In some experiments, a polyclonal antibody raised against the N-terminal portion of actin [8] was used.

2.4. Gel electrophoresis and immunoblotting

For detection of the band for actin, a Laemmli SDS-PAGE system was used [14]. When dynein heavy chains were analyzed, gels with a 3–5% polyacrylamide gradient and a 3–8 M urea gradient were used [12]. Gels were stained either with Coomassie brilliant blue or with silver [15]. To determine the molar ratio of actin to a dynein heavy chain, Coomassie-stained gel was scanned with a LKB 2202 ULTROSCAN laser densitometer. Immunoblotting was performed according to [16] with slight modifications.

2.5. Photocleavage of heavy chains

UV-induced photocleavage of dynein heavy chains was performed essentially after [17]. Portions of MonoQ fractions containing dynein heavy chains were diluted with HMDE solution containing ATP and vanadate, to bring the final concentration of KCl to 120 mM, ATP to 100 μM, and Na_3VO_4 to 2 μM. Samples were irradiated for 1 h by an EN-280L UV lamp (Spectronics, New York) placed 3 cm above them, while being kept at 15°C and vigorously stirred.

3. Results

Sucrose density gradient centrifugation of the crude dynein extract from *Tetrahymena* cilia resolved three peaks as reported in previous studies [18]: a 22S peak containing the so-called 22S dynein, a peak containing 14S dynein and a fraction mainly composed of tubulin (Fig. 1A,B). Anti-actin immunoblotting detected, a band for actin in the 14S dynein fraction. In contrast, the 22S dynein, which comprises the outer-arm dynein [19], did not contain actin.

We found that the 14S dynein fraction contained at least four high-molecular-weight polypeptides when examined on 3–5% polyacrylamide gels (Fig. 1C). Because these multiple polypeptides may be due to the presence of multiple species of dyneins in this fraction, we next subjected the 14S fraction to ion-exchange chromatography on a MonoQ column. As shown in Fig. 2A, MonoQ chromatography separated the 14S dyneins into several peaks. SDS-PAGE analysis indicates that the five fractions contained distinct heavy chains, although we cannot rule out the possibility that some of them are proteolysis products (Fig. 2B). No other heavy chain bands were found in all fractions 1 to 70. Two heavy chains in peak c were separated from each other at slightly different ionic strength (data not shown). Hence they may not constitute a dimer. Of the five fractions, our study was focused on the three major fractions a, c, and d, because fractions b and e were minor and variable from preparation to preparation.

Irradiation of the three fractions with UV light in the presence of MgATP and vanadate cleaved all the heavy chains, showing that these heavy chains actually are dynein (Fig. 3). One of the heavy chains in fraction c apparently has a molecular weight similar to that of a heavy chain in fraction d, but UV–vanadate produced different sets of photocleavage products. This indicates that these heavy chains differ. Hence, it is likely that the

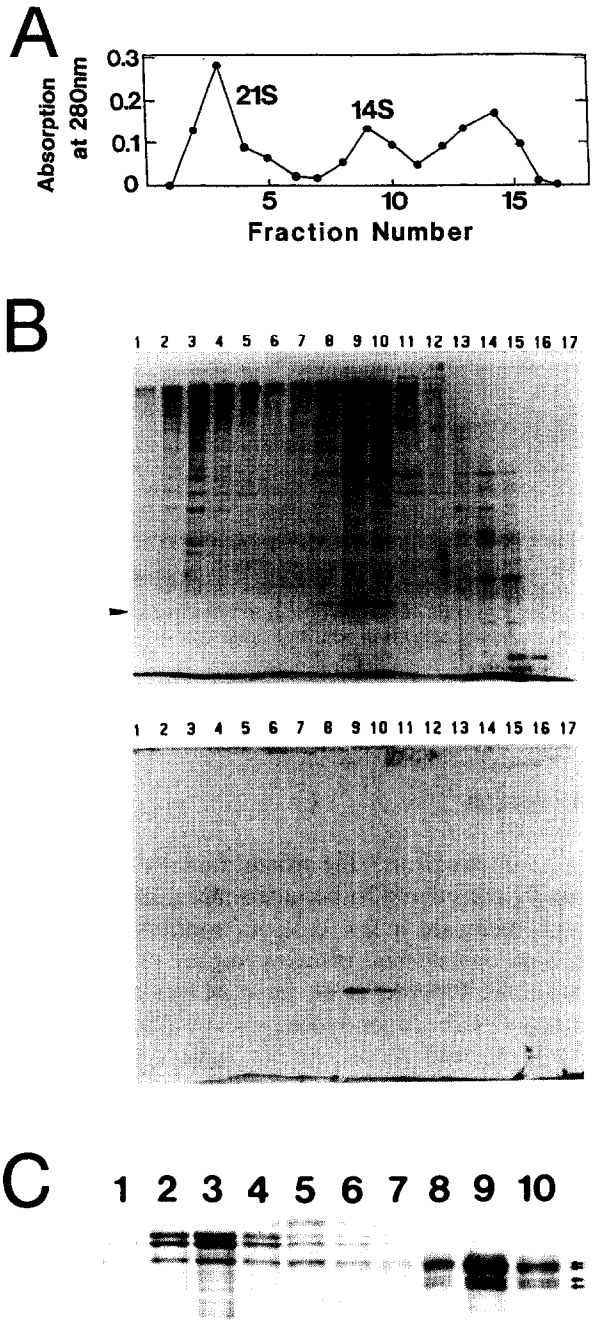

Fig. 1. Sucrose density gradient centrifugation of the high-salt extract from *Tetrahymena* axonemes. (A) Absorbance at 280 nm. The direction of sedimentation was from right to left. (B) Top, silver-stained patterns of SDS-PAGE in a 7.5% gel. Bottom, distribution of actin detected by anti-actin immunoblot. Arrowheads indicate the position of actin from rabbit skeletal muscle run under the same conditions. (C) Heavy chain composition of the sucrose gradient fractions analyzed by 3–5% SDS/urea-PAGE. Only the high molecular weight region (about 400 kDa) is shown. The 22S peak (fractions 2–4) shows three bands and the 14S peak (fractions 8–10) shows four bands as indicated by arrows.

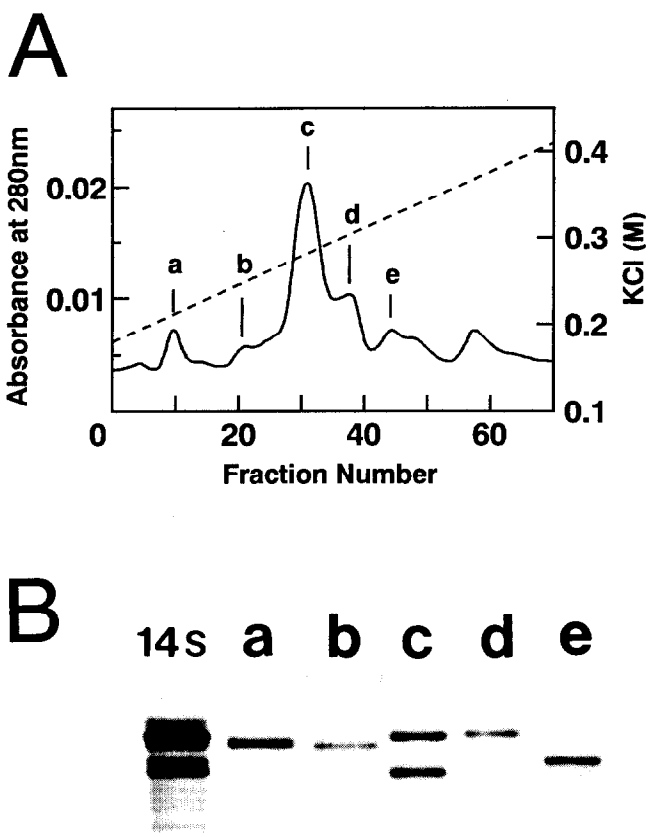

Fig. 2. Fractionation of the 14S dynein by HPLC on a monoQ column. (A) Absorbance at 280 nm. Dashed line, KCl concentration. a–e, peak fractions that contained high-molecular-weight polypeptides. (B) SDS/urea-PAGE patterns of the peak fractions in a 3–5% gradient gel. Each fraction contains one or two distinct heavy chains.

fractions a, c, and d contain four different heavy chains in total.

Actin was detected in all three fractions by anti-actin immunoblot (Fig. 4B). In addition to actin, each fraction commonly contained two polypeptides of about 30 kDa and 35 kDa (Fig. 4A). The apparent molar ratio of actin to each heavy chain obtained by the densitometry of a Coomassie-stained gel was 0.7, 0.7, 0.8 for fractions a, c, and d, respectively. These values are similar to those obtained for *Chlamydomonas* inner-arm dyneins (our unpublished data).

4. Discussion

We have shown that the 14S dynein of *Tetrahymena* contains several different species of dyneins, of which at least three contain a 43.5 kDa polypeptide that reacts with anti-actin polyclonal antibody. Identical results were obtained with a polyclonal antibody raised against the N-terminal portion that is unique to *Tetrahymena* actin (Muto, E. and Hirono, M., unpublished result). Hence it is most likely that the 14S dynein of this organism contains true actin, although we cannot rule out the possibility that it contains some actin-related proteins in addition to actin. Together with *Chlamydomonas* [1] and sea urchin sperm [20], *Tetrahymena* provides the third example in which an association of actin with axonemal dyneins has been demonstrated.

Tetrahymena has a single species of actin gene and its predicted amino acid sequence has appeared to be most divergent among the actins so far studied: its homology to skeletal muscle actin is about 75%, which is much lower than that of *Dictyostelium* (91%) or *Saccharomyces* (87%) (see [8]). Furthermore, although *Tetrahymena* actin has the basic properties of actin to undergo reversible polymerization and to activate myosin ATPase, it does not bind to some proteins and reagents that bind to typical actin: for example, α-actinin, DNaseI, tropomyosin and phalloidin [9,10]. Its molecular weight and isoelectric point also differ significantly from those of other actins [8]. The finding that such a divergent actin is associated with 14S dynein suggests that dynein binding is one of the most conserved properties of actin.

Actin is associated only with the 14S dynein, and not with the 22S dynein, which comprises the outer-arm dynein. Although the location of the 14S dynein within the axoneme has not been established, this association may be analogous to the association of actin with the inner-arm dyneins of *Chlamydomonas* [1] and sea urchin [20]. *Tetrahymena* 14S dynein can rotate microtubules in vitro [21], similar to *Chlamydomonas* inner-arm dynein [12], suggesting that some or all of the dynein species in the 14S may comprise the inner-arm dynein. Therfore, it seems likely that actin association is an inner-arm specific phenomenon.

What is the role of actin in the inner-arm function? Unfortunately, we have yet to obtain data relevant to this question. In *Chlamydomonas*, Piperno et al. [4] have indirectly shown that six axonemal proteins including

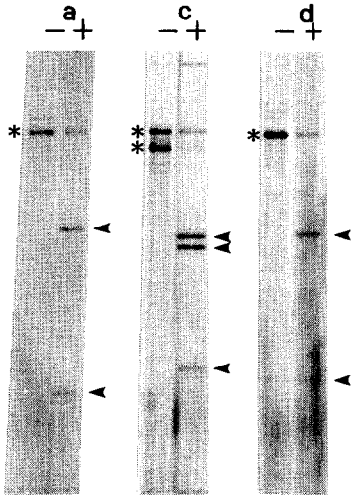

Fig. 3. Vanadate/UV-induced photocleavage of heavy chains a, c, and d. SDS/urea-PAGE patterns before (−) and after (+) photocleavage. Samples subjected to photocleavage are marked with asterisks and the cleaved products with arrowheads. In fraction c, one of the photocleavage products was unstable and very faint in the gel.

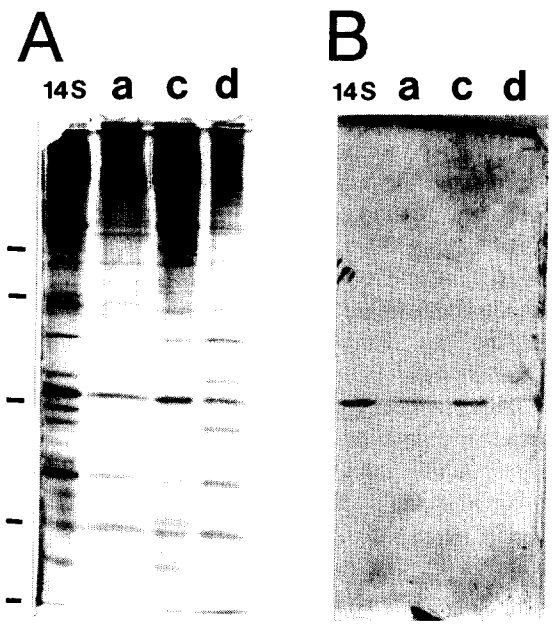

Fig. 4. Detection of actin by SDS-PAGE in a 10% gel. (A) Polypeptide composition of 14S dynein and MonoQ fractions revealed by silver staining. Bars on the left side indicate the position of the molecular weight markers with the size of 97.4, 66, 45, 31, and 21.5 kDa, respectively. To clearly resolve the low-molecular-weight polypeptides, the sample was overloaded for dynein heavy chains. (B) Distribution of actin detected by anti-actin immunoblot.

actin are located in the close proximity to inner dynein arms in situ and suggest that they may regulate the function of inner-arm dynein, possibly through interaction with the central pair/radial spoke system. In cytoplasmic dynein, a multiprotein complex, named dynactin, containing actin and an actin-like protein was found to enhance the dynein-based organelle transport in vitro [5–7]. Although the mechanism by which dynactin activates dynein-driven motility is unknown, the actin in the inner-arm dynein may well operate as an activator of motility.

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