Two-headed dynein arm

YOKO YANO* and TAIKO MIKI-NOUMURA**

Department of Biology, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo 112, Japan

ABSTRACT

The outer dynein arms in 21S dynein fraction from sea urchin sperm axonemes and those in NaCl-extracted 50,000 g supernatant fraction from *Tetrahymena* ciliary axonemes were examined by electron microscopy applying a negative staining technique. The extracted outer dynein arm was not seen as a linear polymer of several subunits, but rather had a heart-like or Y-shaped structure, with dimensions of approximately 20×25 nm. The distal region of the dynein arm was split with a shallow cleft into two heads, each being a globular subunit of about 10 nm in diameter.

KEY WORDS dynein / sea urchin sperm / *Tetrahymena* cilia

Gibbons and Rowe (4) first used the term 'dynein' to describe an axonemal ATPase from *Tetrahymena* cilia which forms the structures called 'arms' on the outer doublet microtubules. The 30S fraction of ciliary dynein consisted of rod-like particles of 70-90 Å in diameter, which were considered as linear polymers of globular 14S subunits, by shadow-casting electron microscopy with platinum. Although many works have been reported on the enzymatic characteristics of dynein ATPase since then, the morphological nature of the dynein arms has not been well established. According to negative contrast electron microscopy by Mohri *et al.* (8), the globular units of 100 Å in diameter were observed evenly in the 11S dynein fraction of sea urchin sperm flagella, and there were no figures of the linear polymer of dynein. The globular units were reported to resemble the arms attaching to the A-tubule of outer doublet microtubules in their shape and size. On the other hand, Warner *et al.* (11) observed that the ciliary dynein fraction contained a homogeneous array of 93 Å particles and that the individual arms seemed to comprise three or four 93 Å subunits, suggesting that each arm is a multisubunit polymer.

In a previous paper (12) we reported that the NaCl-extracted supernatant fraction from sperm flagellar axonemes recombined again with arm-depleted axonemes, which restored their function of extruding the doublet microtubules in the presence of ATP. In the present experiment, we have attempted to observe a whole individual arm of sea urchin sperm flagella and *Tetrahymena* cilia in more detail by negative contrast electron microscopy. The outer dynein arm was not seen as a linear polymer, but had a heart-like structure, the distal region of which was divided with a shallow cleft into two heads.

Sperm flagella of the sea urchin, *Pseudocentrotus depressus* were demembranated with a Triton X-100 solution (0.25% Triton X-100, 0.15 M KCl, 2 mM MgSO₄, 2 mM CaCl₂, 0.5 mM EDTA, 1 mM dithiothreitol, 10 mM Tris-HCl buffer, pH 8.3). The demembranated axonemes were then suspended in a 0.5 M NaCl solution (0.5 M NaCl, 4 mM MgSO₄, 1 mM CaCl₂, 1 mM dithiothreitol, and 10 mM Tris-HCl buffer, pH 8.0) for 10 min at 0°C, to remove the outer dynein arms from the axonemes. The suspension was centrifuged at 50,000 g for 30 min. The 50,000 g supernatant was further centrifuged by sucrose density-gradient centrifugation at 60,000 g for 24 hr at 4°C. The collected 21S dynein fraction from the flagellar axonemes was used as the sample for further observation. The

*Present address: Department of Biology, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

**To whom correspondence should be addressed

Fig. 1

composition of the 21S fraction was surveyed by SDS-urea polyacrylamide gel electrophoresis. The electrophoretic profile of the 21S fraction is shown in Fig. 1.

Electron micrographs were taken with a JEM 100CX at 80 kV and a Hitachi HS-9 at 75 kV at a magnification of 50,000. The magnification was calibrated by using the 24 nm arm period (2).

A drop of the 21S dynein fraction from the flagellar axonemes was applied to a carbon-coated collodion grid or a microgrid and stained with 1% uranyl acetate of pH 3.8 or 0.5% uranyl oxalate of pH 6.0. Negative contrast electron microscopy revealed that the larger particles of around 20 nm in diameter and the smaller ones of around 10 nm were distributed on the grid.

Fig. 1 The electrophoretic patterns of flagellar axonemes (50 μg) (a) and 21S dynein fraction (20 μg) (b) from sea urchin sperm. Electrophoresis was carried out according to the method of Laemmli (6). The separation gel had two linear gradients of a 4–15% acrylamide and a 1–8M urea.

Fig. 2 21S dynein fraction of sea urchin sperm axonemes, fixed with 1% glutaraldehyde and stained with 1% uranyl acetate. ×200,000

Fig. 2

When the 21S fraction was previously fixed with 1% glutaraldehyde, a relatively homogeneous population of larger particles was observed as shown in Fig. 2. The larger particles might separate into the smaller particles during specimen preparation. Comparing the size of the larger particle with that of the outer arm recombined with the outer doublet microtubules (see Fig. 7), we regarded the larger particles in the 21S fraction as a whole outer arm.

As can be seen from Fig. 3, the outer arm had a heart-like or Y-shaped structure with dimensions of approximately 20×25 nm. The distal region of the outer arm was constricted into two parts by a shallow cleft, that is, two heads, while the

Fig. 3 Outer dynein arms in 21S dynein fraction of sea urchin sperm axonemes, stained with 1% uranyl acetate (a), fixed with 1% glutaraldehyde and stained with 1% uranyl acetate (b), fixed with 1% glutaraldehyde and stained with 0.5% uranyl oxalate (c), and stained with 1% uranyl acetate on the microgrid (d). $\times 200,000$

Fig. 4 Outer dynein arms in 21S dynein fraction from sea urchin sperm axonemes, mixed with 1% uranyl acetate and 20 μ g bacitracin. $\times 200,000$

Fig. 5 Outer dynein arms in NaCl-extracted 50,000 g supernatant fraction from *Tetrahymena* ciliary axonemes, stained with 1% uranyl acetate. The photograph at the right is a superimposed image of the three photographs at the left. $\times 400,000$

proximal region was slender and appeared to attach to the A-tubule of the outer doublet microtubules (see, Fig. 7). Sometimes a pear-like shape with dimensions of 12×25 nm, having no cleft, was observed in the 21S dynein fraction. The heart-like structure may give the pear-like image from the lateral view.

To obtain images of higher resolution, a negative staining procedure using bacitracin (antibiotic polypeptide), which requires no special preparation of film-coated grid, was examined according to the method of Malech and Albert (7). Fifty μ g/ml of the 21S fraction in 1% uranyl acetate and 20 μ g/ml bacitracin were applied to a clean, naked 400 mesh copper grid. Electron microscopy revealed that the outer dynein arms on the grid had also the heart-like structure (Fig. 4).

Ciliary dynein arms were extracted with 0.5 M NaCl solution from the axonemes of *Tetrahymena pyriformis* strain W. After centrifugation at 50,000 g for 30 min, the supernatant fraction was examined by negative contrast electron microscopy, which revealed a heart-like structure similar to that of the flagellar outer arms. Three photographs of individual dynein arms arranged in the same direction were superimposed one over other. The method reinforced the image of the heart-like structure with a shallow cleft, as shown in Fig. 5.

Next, the structure of the outer dynein arms bound to the outer doublet microtubules was observed. For this purpose, the doublet microtubules extruded from ciliary axonemes by ATP and the arm-recombined doublet microtubules of flagellar axonemes were prepared.

The suspension of demembrated axonemes of *Tetrahymena* cilia in the medium (25 mM KCl, 2.5 mM $MgSO_4$, 0.2 M sucrose, 1 mM dithiothreitol, and 30 mM Tris-HCl buffer, pH 8.3) was reactivated with 0.1 mM ATP. The extruded doublet microtubules after active sliding movement by ATP were placed on a microgrid and stained with 1% uranyl acetate. Fig. 6a shows the dynein arms bound to the A-tubule of the extruded doublets. Fig. 6b is an image of two-step translation of Fig. 6a, which was moved by one arm period. While the distal region of the outer arm seems to be round, having a cleft (arrows in Fig. 6b), the basal region bound to the A-tubule was slender.

Both outer and inner arms are arranged so closely with each other in two rows on the doublet microtubule that the shape of the individual outer dynein arm *in situ* was difficult to observe exactly. Therefore, we tried to observe the

Fig. 6 A partially disintegrated ciliary axoneme from *Tetrahymena* after active sliding movement, stained with 1% uranyl acetate (a). Image of photographic translation with two steps of Fig. 6a (b). Arrows indicate the heart-like structures with a shallow cleft. $\times 200,000$

doublet microtubules recombined with the outer dynein arm fraction. The demembranated flagellar axonemes were dialysed against a solution (1 mM EDTA, 1 mM dithiothreitol, and 10 mM Tris-HCl buffer, pH 8.0) for 18 hr at 4°C, to remove both types of arms, radial spokes and nexin links. After the dialysis, the doublet microtubules were incubated with the NaCl-extracted 50,000 *g* supernatant fraction in a protein ratio of 5:1 (doublet microtubules: supernatant) in a 0.15 M NaCl solution containing 4 mM $MgSO_4$, 1 mM $CaCl_2$, 1 mM

dithiothreitol and 10 mM Tris-HCl buffer, pH 8.0. The doublet microtubules recovered partially their outer arms in groups here and there as shown in Fig. 7. Whereas the basal region appeared to be slender, attaching to the A-tubule, the distal region was also divided into two heads (arrows in Fig. 7), each of which appeared to be in a parallel array to the long axis of the doublet microtubules.

Fig. 8 shows a transverse image of a flagellar axoneme of sea urchin sperm. The axonemes were fixed with 2.5% glutaraldehyde in 0.1 M

Fig. 7 Arm-recombined doublet microtubule of sea urchin sperm axoneme, stained with 1% uranyl acetate. Arrows indicate the heart-like structures with a shallow cleft. $\times 200,000$

Fig. 8 Transverse sectional image of sea urchin sperm axoneme (a). Typical forms of outer dynein arms with two heads. Doublet microtubules are arrayed in the same direction as the outer arm at the left (b). $\times 200,000$

phosphate buffer (pH 7.5) and postfixed with 1% OsO_4 . Thin-sectioned specimens were double stained with uranyl acetate and lead citrate. Applying the Markham rotation technique to the cross-sectioned ciliary axonemes, Allen (1) reported that the outer dynein arm at its distal end appeared to hook sharply toward the center of the axoneme. However, as shown in Fig. 8, many images of the outer arms in cross-sectioned flagellar axonemes were seen as a heart-like or Y-shaped structure. The proximal region of the outer arm was bound to the A-tubule and the distal region was divided into two parts, each projecting toward the adjacent B-tubule.

On the basis of analysis by SDS-gel electrophoresis and negative contrast electron microscopy, Warner *et al.* (11) presented evidence that individual outer arms of ciliary axonemes comprise three or four $93 \text{ \AA}$ subunits, and suggested that each arm was a polymer of dynein molecules. These findings are in general agreement with the early description by Gibbons and Rowe (4), who reported that 30S dynein of *Tetrahymena* cilia consisted of rod-like particles of various lengths and of $70\text{--}90 \text{ \AA}$ in diameter.

In the present study, negative contrast electron microscopy revealed that the outer dynein arm in

the 21S fraction from flagellar axonemes and in the NaCl-extracted $50,000 g$ supernatant from ciliary axonemes, had the heart-like or Y-shaped structure, which was quite different from the linear polymer form of three spherical particles suggested by Warner and Mitchell (10). When the two-headed arm bound to the doublet microtubules is viewed from the oblique side, the two headed arm may give an image similar to the 'three morphological subunits' as described by them. However, nothing definite can be said now, for the direction and the angle of the outer arm to the doublet microtubules have not yet been determined: for example, while the outer arms in cross-sectioned axonemes had the clear image of the two heads perpendicular to the long axis of the doublet microtubules (Fig. 8), each head in the recombined doublet microtubules appeared to be arranged parallel to the axis (Fig. 7). In a study on the structure of flagellar microtubules, Grimstone and Klug (5) observed many rods in the background of the micrographs of outer doublet microtubules. They reported that the rods were identified as arms, which had a repeating globular structure of $70 \text{ \AA}$ in diameter and often had V-shaped form. The heart-like or Y-shaped structure presented here might be consistent with the V-shaped form, although the dimensions found in the present work are larger than those reported by them.

The head region of the myosin molecule of skeletal muscle consisted of two globular subunits (9). A possibility was suggested recently that the two heads of myosin may interact in a co-operative fashion (3). Muscle myosin and the dynein arm have a similar function: transforming the chemical energy of ATP to mechanical work in cellular movement, forming cross-bridges with the actin filament or with the doublet microtubule. From this point of view, the structural conformation of the two-headed dynein arm is an interesting problem to be elucidated in more detail.

The authors wish to thank Dr H. Mohri of University of Tokyo for his encouragement and for reading through the manuscript. Thanks are also due to the Director and Staff of Tateyama and Sugashima Marine Biological Laboratory for their kind help during the course of this work. This work was supported in part by grants-in-aids from the Ministry of Education, Science and Culture, Japan and from the Naito Foundation.

Received for publication 29 November 1980

REFERENCES

1. ALLEN R. D. (1968) A reinvestigation of cross-sections of cilia. *J. Cell Biol.* **37**, 825-831
2. AMOS L. A., LINCK R. W. and KLUG A. (1976) Molecular structure of flagellar microtubules. In *Cell Motility* (Book C) (ed. GOLDMAN R., POLLARD T. and ROSENBAUM J.) Cold Spring Harbor Laboratory, pp. 847-867
3. CRAIG R., SZENT-GYÖRGY A. G., BEESE L., FLICKER P., VIBERT P. and COHEN C. (1980) Electron microscopy of thin filaments decorated with a Ca^{2+} -regulated myosin. *J. Mol. Biol.* **140**, 35-55
4. GIBBONS I. R. and ROWE A. J. (1965) Dynein: A protein with adenosine triphosphatase activity from cilia. *Science* **149**, 424-426
5. GRIMSTONE A. V. and KLUG A. (1966) Observation on the substructure of flagellar fibers. *J. Cell Sci.* **1**, 351-362
6. LAEMMLI U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature* **227**, 680-685
7. MALECH H. L. and ALBERT J. P. (1979) Negative staining of protein macromolecules: A simple rapid method. *J. Ultrastruct. Res.* **69**, 191-195
8. MOHRI H., HASEGAWA S., YAMAMOTO M. and MURAKAMI S. (1969) Flagellar adenosine-triphosphatase (dynein) from sea-urchin spermatozoa. *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo* **19**, 195-217
9. SLAYTER H. S. and LOWEY S. (1967) Substructure of the myosin molecule as visualized by electron microscopy. *Proc. Natl. Acad. Sci. USA* **58**, 1611-1618
10. WARNER F. D. and MITCHELL D. R. (1978) Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* **76**, 261-277
11. WARNER F. D., MITCHELL D. R. and PERKINS C. R. (1977) Structural conformation of the ciliary ATPase dynein. *J. Mol. Biol.* **114**, 367-384
12. YANO Y. and MIKI-NOUMURA T. (1981) Recovery of sliding ability in the arm-depleted flagellar axonemes after recombination with extracted dynein 1. *J. Cell Sci.* (in press)