Volume 264, number 2, 179-182 FEBS 08424 May 1990

Microtubule translocation caused by three subspecies of inner-arm dynein from *Chlamydomonas* flagella

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Received 17 March 1990

To help understand the function of inner-arm dynein in flagellar motility, dynein samples from an outer arm-missing mutant of *Chlamydomonas* (*oda*1) were examined for the ability to translocate microtubules in vitro. High-salt extract of axonemes containing inner-arm dynein was separated by ion-exchange chromatography into 7 peak fractions with ATPase activities. Of these, three fractions containing different sets of dynein heavy chains translocated microtubules. The maximal velocities were all between 3 and 5 μm/s, which were comparable to the microtubule sliding rate in disintegrating *oda* axonemes.

Dynein; Microtubule; Motility; Translocation

1. INTRODUCTION

The inner and outer dynein arms in cilia and flagella are the key protein assemblies that cause sliding movements between outer-doublet microtubules [1]. Both kinds of arms have complex structures comprising as many as 10 protein subunits, including several high-molecular-weight peptides (HMWs) with enzymatic activities to hydrolyze ATP [2-4]. Moreover, a recent study indicates that three different species of inner arm exist, each containing two different HMWs [5]. Little is known, however, as to what functions those different HMWs carry out in the axoneme.

Functional assay of isolated dynein has recently been made possible by development of an in vitro system [6], in which microtubules are made to glide on a dynein-adsorbed glass surface. With this technique, microtubule translocation can be induced by outer-arm dynein from sea urchin sperm flagella [7] and *Tetrahymena* cilia [8], and by subsets of outer-arm HMWs [9,10]. In addition, *Tetrahymena* 14S dynein, a ciliary dynein whose localization is unknown, has been reported to cause both translocation and rotation of microtubules [8].

In this study, we investigated whether inner-arm dynein can translocate microtubules in vitro, using a *Chlamydomonas* mutant (*oda*1) that lack the outer-arm dynein [11]. We show here that three different inner-arm subspecies separated by ion exchange chromatography have such activities. This is the first report to demonstrate microtubule translocation caused by inner-arm dynein.

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2. MATERIALS AND METHODS

*Chlamydomonas reinhardtii oda*1 strain [11] was grown as described elsewhere [12]. Axonemes were prepared by the method of Witman [13]. Dynein was extracted on ice with 2 ml of HMDE solution (10 mM HEPES, 5 mM $MgSO_4$, 1 mM dithiothreitol and 0.5 mM EGTA, pH 7.4) containing 0.6 M KCl [14]. This extract was dialyzed against a solution containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 5 mM KCl and 0.1 mM PMSF (phenylmethylsulphonyl fluoride), and fractionated by fast protein liquid chromatography (FPLC) on a Mono Q column (Pharmacia). All the procedures for dynein extraction and fractionation were carried out at 4°C. ATPase activity in each fraction was measured by a Malachite green method [15]. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli [16], using a 3-5% acrylamide gradient and a 3-8 M urea gradient [17].

Tubulin was prepared from porcine brain and purified using phosphocellulose chromatography [18]. Microtubules were prepared by polymerizing porcine brain tubulin in the presence of 10 μM Taxol. For motility assay, a perfusion chamber was made from a glass slide and a cover slip, which are held apart with two cover slip strips. The chamber was perfused with a dynein preparation and then with HMDE solution containing the desired concentration of ATP and microtubules (about 20 μg/ml). The behavior of the microtubules was recorded with a dark-field microscope, equipped with a 100 W high-pressure mercury lamp, an SIT camera (Hamamatsu C2400-08), and a video recorder (Panasonic AG-3800). The velocity was measured on a computer monitor, on which the video image was superimposed. Usually 20 microtubules were analyzed to obtain the average velocity at each concentration of ATP.

3. RESULTS AND DISCUSSION

In a preliminary experiment, we examined whether a wild-type dynein preparation purified by sucrose density-gradient centrifugation [18] can translocate microtubules. We did find a microtubule-translocating activity in a fraction sedimenting at 12.5 S, which contained several inner-arm HMWs as major components (data not shown). Such a translocating activity was not

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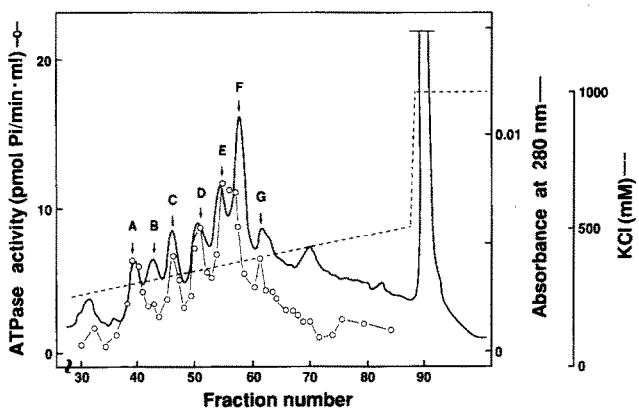

Fig. 1. Fractionation of high-salt extract from *oda* axonemes by fast-protein liquid chromatography on a Mono Q column. A–G, peak fractions assayed.

detected in 18S and 12S fractions containing outer-arm HMWs.

To see whether inner-arm dynein can actually cause microtubule translocation, we examined dynein samples obtained from *oda* mutant. High salt extract was subjected to a Mono Q column to separate inner-arm dynein into discrete subspecies [3]. Fig. 1 shows the elution profile and Fig. 2 the SDS-PAGE pattern to show MHWs contained in each peak fraction. The SDS-PAGE pattern of the *oda* axoneme had 6 bands (labeled 1–6) in the region where all the dynein HMWs would normally appear [2], whereas the pattern of its high-salt extract had a somewhat increased number of bands. The presence of extra bands in the extract is probably due to proteolytic degradation of some HMWs, although we took care to minimize proteolysis; such a degradation has been observed in studies on outer-arm dynein. This chromatography separated the presumptive inner-arm HMWs into several peak fractions (labeled A–G in Fig. 1). SDS-PAGE pattern (Fig. 2) demonstrates that each fraction has its own charac-

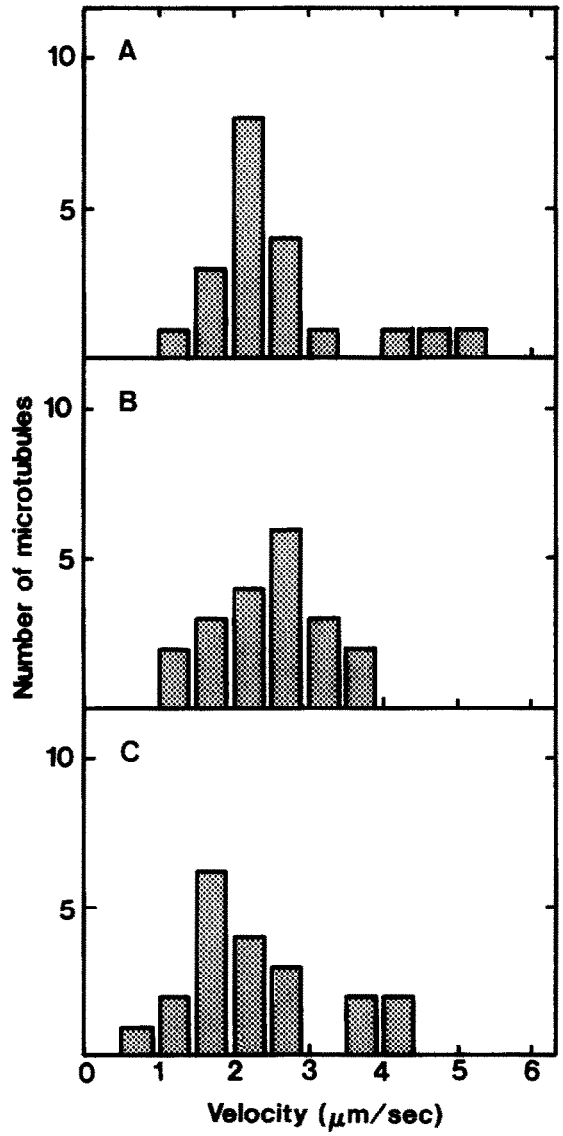

Fig. 3. Histograms of velocities at 0.1 mM ATP. A, fraction C; B, fraction D; C, fraction G. Temperature: 23°C.

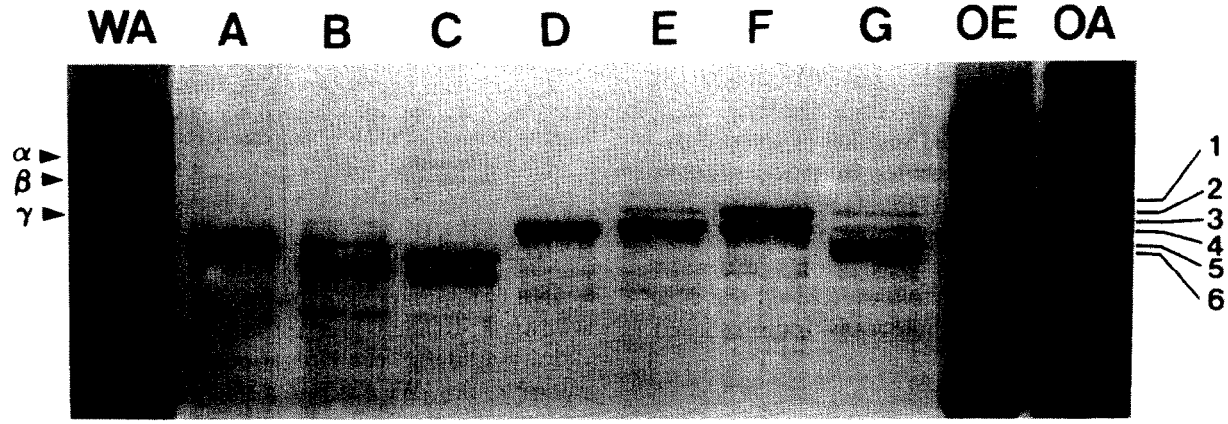

Fig. 2. SDS-PAGE pattern to show HMWs contained in each peak fraction. Silver-stained [20]. A–E, peak fractions as indicated in Fig. 1; WA, wild-type axoneme; OE, extract from *oda* axoneme; OA, *oda* axoneme. The bands labeled α, β, γ, and those labeled 1–6 are outer-arm and inner-arm heavy chains, respectively.

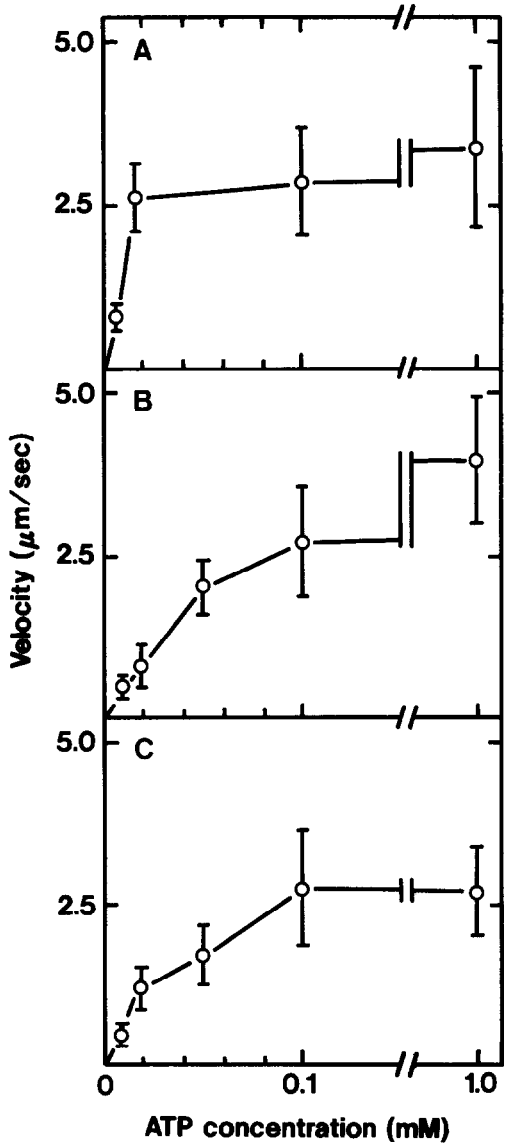

Fig. 4. ATP-dependence of the velocity. A, fraction C; B, fraction D, C, fraction G. Temperature: 23°C.

teristic HMW composition, although possibly some of them represent proteolysed HMWs.

We found that microtubule-translocating activities are present in fractions C, D and G. The velocity histograms at 0.1 mM ATP, and the ATP-dependence of the velocity for each fraction are shown in Figs 3 and 4. These data show that each species of dynein induces microtubule translocation with a maximal velocity of 3–5 μm, and an apparent Km for ATP of 10–50 μM. The maximal velocities are similar to the maximal sliding velocity of outer-doublet microtubules observed in disintegrating *oda* axonemes (5.2 μm/s), i.e. 1/8 to 1/5 of the maximal sliding velocity in wild-type axonemes (about 25 μm/s; Kurimoto and Kamiya, manuscript in preparation). The direction of the translocation with respect to the microtubule polarity has not been determined.

With fractions E and F, which contained no. 1 and no. 2 bands, microtubules stuck to the glass surface in the presence and absence of ATP, but no translocation was observed at 0.01–1 mM ATP. Microtubules never stuck to the glass unless the specimen chamber had been perfused with dynein. Such microtubule adhesion to the dynein HMW-adsorbed glass slide, with no translocation, has been observed with outer-arm HMWs [10]. The lack of translocation in these fractions may be due to the intrinsic nature of the HMWs contained, or to some technical problems in attaching HMWs to the glass surface in proper orientation.

Microtubules translocating in the presence of fraction G, which contained the band 3 as a major one, often appeared to be rotating. Rotation was particularly clear when the tubule had a kink or a small particle attached. The frequency was about 3 Hz at 1 mM ATP. Although the details of this movement and the exact location of the HMW contained in fraction G must be analyzed further, this observation raises the possibility that some of the inner-arm HMWs exert a force that has a rotatory component (see also [22]).

The presence of various inner-arm subspecies with different mechanochemical properties must be important for the flagellar beating mechanism. It will be of great interest to study how the in vitro properties of different dynein HMWs are correlated with the behavior of mutant axonemes lacking them [19].

Acknowledgements: We thank Professor Sho Asakura for critical reading of the manuscript, and Dr M. Suffness (National Cancer Institute, USA) for the gift of Taxol. This work has been supported by a grant-in-aid from the Ministry of Education, Science and Culture of Japan (01657001).

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