PROPERTIES OF AN ANTISERUM AGAINST
NATIVE DYNEIN 1 FROM SEA URCHIN SPERM FLAGELLA

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ABSTRACT
Effects of an antiserum against native dynein 1 from sperm flagella of the sea urchin *Strongylocentrotus purpuratus* were compared with effects of an antiserum previously obtained against an ATPase-active tryptic fragment (fragment 1A) of dynein 1 from sperm flagella of the sea urchin, *Anthocidaris crassispina*. Both antisera precipitate dynein 1 and do not precipitate dynein 2. Only the fragment 1A antiserum precipitates fragment 1A and produces a measurable inhibition of dynein 1 ATPase activity. Both antisera inhibit the movement and the movement-coupled ATP dephosphorylation of reactivated spermatozoa. The inhibition of movement by the antiserum against dynein 1 is much less than by the antiserum against fragment 1A, suggesting that a specific interference with the active ATPase site may be required for effective inhibition of movement. Both antisera reduce the bend angle as well as the beat frequency of reactivated *S. purpuratus* spermatozoa, suggesting that the bend angle may depend on the activity of the dynein arms which generate active sliding.

Study of dynein, the ATPase believed to be responsible for the generation of motility in flagella and cilia, has recently revealed the existence of two forms of dynein, called dynein 1 and dynein 2 (9, 11), which are immunologically distinct. Dynein 1 comprises the major portion of the flagellar dynein, and is found in the arms which generate movement by an active sliding process involving the formation of cross-bridges between flagellar tubules. The particular function of dynein 2 has not been identified. A tryptic fragment (fragment 1A) containing the ATPase activity of dynein 1 was prepared by Ogawa (10). An antiserum against fragment 1A was found to inhibit the movement of reactivated spermatozoa and the ATPase activity of fragment 1A and dynein 1, but not of dynein 2 (9, 11, 13, 14). As part of the continued study of the functions of flagellar dyneins, we have obtained an antiserum against native dynein 1. In the present paper, the properties of this antiserum are described and compared with those of the antiserum obtained previously against fragment 1A.

MATERIALS AND METHODS

**Material**
Concentrated spermatozoa were collected from the aboral surface of the sea urchin, *Strongylocentrotus purpuratus*, after injection of 0.6 M KCl to induce shedding. For experiments with reactivated spermatozoa, a stock sperm suspension was prepared by dilution with 1-2 vol of cold 0.5 M NaCl. The concentration of the sperm suspension was adjusted until a 10 μl portion, diluted with 5.0 ml of 0.5 M NaCl, produced an optical density reading of 0.24-0.26 at 550 nm (5). For preparations of axonemes and axonemal proteins, the spermatozoa were...
washed once with sea water, and then either used at once for preparation of axonemes, or stored with 50% glycerol at -15°C (12).

Preparation of Axonemes

Axonemes were isolated from freshly shed or glycerinated spermatozoa by procedures based on those used by Gibbons and Fronk (8), modified for treatment of 50-100 ml of packed spermatozoa. The spermatozoa were demembranated with 150 ml of a 1% solution of Triton X-100 in solution A: 0.1 M KCl, 5 mM MgSO4, 0.2 mM EDTA, and 1 mM dithiothreitol (DTT) buffered with 10 mM Tris and 10 mM NaH2PO4 at a pH of 7.8. All steps were carried out at 0-4°C. The suspension was homogenized gently with seven strokes of a Teflon homogenizer, and then centrifuged at 800 g for 5 min. The supernate (I) was saved and the pellet (I) was suspended in 150 ml of Triton X-100 in solution A. Supernate (I) was centrifuged at 13,000 g for 10 min, and the pellet (II) was saved. The suspension of pellet (I) was centrifuged at 800 g for 5 min, and the supernate (II) was combined with pellet (II), homogenized gently to disperse the pellet, and centrifuged at 13,000 g for 10 min. The resulting pellet consists of a pinkish axoneme layer on top of a white head layer. A spatula and pipette were used to remove only the axoneme layer, which was then suspended in 100 ml of solution A and centrifuged at 13,000 g for 10 min. This process was repeated two more times to yield a pellet of axonemes with negligible head contamination.

Preparation of Dyneins and Tryptic Fragments

Dynein 1 was obtained by low ionic strength extraction of axonemes from freshly shed spermatozoa, followed by purification by column chromatography on Sepharose 4B and hydroxyapatite, as described by Ogawa and Mohri (12). Dynein 2 was prepared by low ionic strength extraction of axonemes which had previously been extracted with 0.6 M KCl to remove dynein 1. The extract was purified by column chromatography on Sepharose 4B and hydroxyapatite, as described by Ogawa and Gibbons (11). SDS gel electrophoresis, shown in Fig. 1, verifies that these procedures, applied to axonemes from 

\textit{S. purpuratus}

spermatozoa, yield preparations of dyneins of high purity having the relative electrophoretic mobilities expected for dynein 1 and dynein 2.

Fragment A used in these experiments was prepared previously by trypsin-digestion of dynein from spermatozoa of the Japanese sea urchin, \textit{Anthocidaris crassispina} (13). Following identification of dyneins 1 and 2, this fragment is now referred to as fragment 1A (11). The result of SDS gel electrophoresis of fragment 1A is shown in Fig. 5 H.

A trypsic fragment of dynein 1 from \textit{S. purpuratus} spermatozoa was prepared by mixing 16 ml of a concen-
FIGURE 2 Immunodiffusion tests. AS is antiserum obtained from the rabbit immunized with dynein 1. AD-1 is the antiserum following absorption with impurity protein prepared by method II (before (NH₄)₂SO₄ fractionation). I is impurity protein prepared by method I (0.3 mg protein). II is impurity protein prepared by method II (0.39 mg protein). D₁ is purified dynein 1 (0.053 mg protein). DX is a low ionic strength extract of axonemes, used to prepare D₁ (0.17 mg protein). Antisera wells in this figure and in Fig. 3 received 25 μl antisera.

FIGURE 3 Immunodiffusion tests. AF-1A is the antifragment 1A serum. AD-1 is the antidynein 1 serum absorbed with impurity protein prepared by method II. F₁A is fragment 1A (0.008 mg protein). D₁ is purified dynein 1 from a low ionic strength extract of axonemes (0.053 mg protein). D₁' is purified dynein 1 from a 0.5 M KCl extract of axonemes (0.06 mg protein).

Antiserum against fragment 1A from Anthocidaris was that previously described by Ogawa and Mohri (13) and used for experiments with reactivated spermatozoa (7, 9, 14). Serum obtained from a nonimmunized rabbit from the same group as the rabbit immunized with dynein 1 was used for a control.

Before use in these experiments, a partially purified antibody preparation was obtained from each of these sera. 10 ml of serum was mixed with 10 ml of saturated (NH₄)₂SO₄ at 0°C. The precipitate was collected and dissolved in 8 ml of the buffer solution appropriate for a particular experiment, and dialyzed against 1 liter of the buffer solution, which was changed twice during a 16-h period of dialysis at 4°C. The dialyzed solution was then centrifuged at 13,000 g for 20 min and the supernate was used as the purified preparation. The preparation obtained from the nonimmune serum will be designated NI. The preparation obtained from the antifragment 1A serum will be designated AF-1A. The preparation ob-

FIGURE 4 Sepharose 4B column chromatography of dynein 1 and its tryptic fragment. Measurements of protein and ATPase activity of fractions obtained after applying a tryptic digest of dynein 1 to the column, as described in Materials and Methods, are shown by the circles. The open circles represent ATPase activity and the solid circles represent protein. The x's show results obtained from another experiment with the same column, in which 15 ml of a low ionic strength extract of axonemes, containing 13.2 mg/ml protein, was applied to the column. In this case, the ATPase peak represents intact dynein 1. Fraction 34 was the void volume, and the tubulin peak, located by SDS gel electrophoresis of the fractions, was at fraction 68.

Antisera was obtained from rabbit foot pads, femoral muscles, and the back of the neck. After 7 wk, a booster injection of 4.6 mg of dynein 1 without adjuvant was given intraperitoneally. 1 wk after this booster, a serum sample showed ability to precipitate dynein 1. Serum collected from this rabbit 4 wk after the booster injection was used in the experiments described in this paper. After addition of 1% NaN₃, the serum was stored at 4°C until use.

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Figure 5 SDS gel electrophoresis using 5% polyacrylamide gels. Gel A is the precipitate of dynein 1 and NI. Gel B is the precipitate of dynein 1 and AD-1. Gel C is the precipitate of dynein 1 and AF-1A. Gel D is a sample of the fragment 1-AS preparation (18 μg protein). Gel E is the precipitate of fragment 1-AS and NI. Gel F is the precipitate of fragment 1-AS and AD-1. Gel G is the precipitate of fragment 1-AS and AF-1A. Gel H is a sample of fragment 1A (1.6 μg protein). Gel I is a mixture containing both G and H. D₁ indicates a subunit polypeptide of dynein 1; F₂ and F₃ indicate the principal component polypeptide subunits of fragment 1A with molecular weights of 190,000 and 135,000, respectively (13). IgG indicates the immunoglobulin bands from serum, with molecular weights of approximately 60,000 and 20,000.

Antigen-antibody precipitates were obtained from the experiments described in Table I, and similar experiments with a fragment 1-AS preparation containing 1.8 mg ml⁻¹ protein. Each precipitate was washed with 3 ml of 0.85% NaCl buffered with 10 mM sodium phosphate at pH 7.0 and containing 0.1% sodium azide. The precipitate was then dissolved in 1.0 ml of SDS-buffer solution (13), and 50 μl was applied to the gel.

Ogawa, Asai, and Brokaw Antiserum against Dynein 1 185

Preparation of Impurity Antigen and Purification of Antidynein 1

A second precipitation band was regularly found when the antidynein 1 serum was tested by immunodiffusion against the low ionic strength extract used to obtain dynein 1, and was usually visible when the serum was tested against purified dynein 1. This impurity band was much more prominent when the antidynein 1 serum was tested against the axonemal extracts obtained in the course of the procedure that we used for purifying dynein 2. We found that the impurity antigens in these extracts could be readily separated from dyneins 1 and 2 by Sepharose 4B chromatography. Two methods were used. For method I, we used the 0.6 M KCl extract which was used to remove dynein 1 from axonemes before the extraction of dynein 2. The extract was dialyzed against Tris-EDTA solution and concentrated with an Amicon membrane filter (XM 100A; Amicon Corp., Scientific Sys. Div. Lexington, Mass.). 15 ml of this concentrate was applied to a 2.5 × 90 cm Sepharose 4B column equilibrated with Tris-EDTA solution, eluted with the same solution, and 3.75 ml fractions were collected. When the void volume was at fraction 34, dynein 1 ATPase activity was eluted at fractions 40–64, as in Fig. 4, and the impurity antigen detected by immunodiffusion was eluted at fractions 64–80. Fraction 74–80 were pooled, concentrated by collodion bag ultrafiltration, and stored in 50% glycerol at −15°C. Approximately 3 ml, containing 12 mg/ml protein, was obtained. For method II, a similar procedure was used, except that the starting material was the low ionic strength extract of...
dynein 2, and 0.1 M KCl was added to the Tris-EDTA solution. In this case, dynein 2 ATPase activity appeared in fractions 58-70 and the impurity antigen was found in fractions 78-88, which were pooled and concentrated. Approximately 3 ml, containing 16 mg/ml protein, was obtained. Fig. 1 C shows the results of SDS gel electrophoresis of a sample of impurity antigen prepared by method II. The major component of the impurity antigen preparation migrates in the position expected for tubulin, but we have not determined whether tubulin is responsible for the cross-reaction seen in the immunodiffusion tests. Since gel electrophoresis of the purified dynein 1 used as antigen (Fig. 1 A) does not reveal significant contamination by proteins similar to those seen by gel electrophoresis of the impurity antigen preparation (Fig. 1 C), one or more of the impurity proteins may be highly antigenic even in low concentration.

Impurity antibody was removed from the antidynein 1 serum by mixing 10 ml of the antisemur with 2.0 ml of the concentrated impurity in 50% glycerol prepared by method II. After 3 days at 4°C the mixture was centrifuged at 13,000 g for 20 min to remove the antigen-antibody precipitate and the supernate was purified by ammonium sulfate precipitation as described in the preceding section, to obtain the antibody preparation, AD-1, used for experiments.

Observation of Motility of Reactivated Spermatozoa

Demembranated spermatozoa of S. purpuratus were prepared and reactivated using extraction and reactivation solutions with the same compositions used for previous experiments with Lytechinus spermatozoa (1) with an ATP concentration of 0.2 mM in the reactivation solution. A 10-μl portion of a standardized stock sperm suspension was mixed into 1.0 ml of extraction solution. After 60 s a 10-μl portion of extracted spermatozoa was added to 1.0 ml of reaction solution containing either AD-1, AF-1A, or NI, and gently mixed. A drop of this mixture placed in a well slide, covered with a cover glass, and examined with dark-field illumination using a × 40 oil immersion objective. Stroboscopic illumination was used, with the flash frequency adjusted to exactly 4 × the beat frequency of the spermatozoon which was being observed. A spermatozoon beating with its head attached to the bottom surface of the well slide and with its beat plane parallel to the surface was selected as quickly as possible, and photographed at intervals until its movement stopped. The time and flash frequency corresponding to each photograph was recorded. All work was carried out in a room maintained at 16°C.

The photographs were printed to give a final magnification of × 2,000, and these prints were used for measurement of bend angles with a protractor. Since the bend angle may decrease near the distal end of the flagellum, especially in spermatozoa exposed to antisera, the bend angle measurement was routinely made as near as possible to halfway along the length of the flagellum.

**Measurements of ATP dephosphorylation**

Measurements of ATP dephosphorylation by suspensions of demembranated spermatozoa were made using standard pH-stat techniques for S. purpuratus spermatozoa (5), except for the addition of antibody preparations to the reactivation solution at the beginning of an experiment. An ATP concentration of 0.33 mM and a MgSO₄ concentration of 1.3 mM were used for these experiments.

Similar methods were used for measurements on axonemes, except that the axonemal samples were 10 μl aliquots containing 15 mg/ml axonemal protein.

Measurements of the effect of antisera on dynein ATPase activities were also carried out with the same pH-stat techniques, but in these experiments a simpler assay solution was used, which contained 0.05 M KCl, 1.3 mM MgSO₄, 0.33 mM ATP, 0.2 mM EGTA, and 1 mM thiglycollate. The measurements were made at pH 8.4 and 16°C. The reaction was started by the addition of a 0.4 ml sample of the enzyme pretreated with antibody preparations, as described in Table I.

**Other Procedures**

Methods used for double diffusion in agar (Ouchterlony's test), SDS polyacrylamide gel electrophoresis, determination of protein by the Lowry method, and assay of ATPase activity by measurement of inorganic phosphate were standard methods which have been referenced in previous work (13).

**RESULTS**

**Immunodiffusion**

Fig. 2 shows that the unpurified antidynein 1 serum (AS) forms two precipitation bands against purified dynein 1 (D1) and against the low ionic strength extract (DX) obtained in the first step of the dynein 1 preparation. The multiple reactivity of the antidynein 1 serum was also confirmed by

**Table I**

|                  | Mixture | Supernate |
|------------------|---------|-----------|
|                  | NI      | AD-1      | AF-1A      | NI      | AD-1      | AF-1A      |
| Dynein 1         | 30      | 31        | 24         | 31      | 9         | 9          |
| Dynein 2         | 13      | 13        | 12         | 13      | 13        | 12         |
| Fragment 1A      | 41      | 41        | 2          | 41      | 41        | 2          |

1.0 ml of purified preparations of antidynein 1 serum (AD-1), antifragment A serum (AF-1A), or nonimmune serum (NI) was mixed with 0.1 ml of dynein 1 (2.1 mg ml⁻¹), 0.2 ml of dynein 2 (1.5 mg/ml), or 0.1 ml of fragment 1A (0.3 mg ml⁻¹). Duplicate mixtures were prepared and incubated for 6 h at 4°C. One of the mixtures was then centrifuged at 1,300 g for 20 min, and the ATPase activity of 0.4 ml portions of the supernate was assayed. Similar ATPase measurements were performed on 0.4 ml portions of the uncentrifuged mixtures, after uniformly resuspending any antigen-antibody precipitate. The values represent the mean of two ATPase measurements, in units of μM/min.
immunoelectrophoretic analysis. Precipitation also occurred between the antidynein 1 serum and the highly concentrated impurity protein preparations obtained by methods I and II. Under these conditions, this precipitate formed two closely spaced bands, as in Fig. 2. After absorption of the antidynein 1 serum with the impurity protein preparation obtained by method II, there is no precipitation with either impurity preparation, confirming the equivalency of the preparations obtained by these two methods. The absorption-purified antidynein 1 serum, which will now be referred to as AD-1, shows a single precipitation band against both the purified dynein 1 and the low ionic strength dynein 1 extract.

The reactivities of AD-1 and AF-1A, the antibody preparation obtained from the antifragment 1A serum, are compared in Fig. 3. As previously reported, AF-1A precipitates with dynein 1 and with fragment 1A, but not with dynein 2. AD-1 is also specific for dynein 1, showing no precipitation with dynein 2, but, in contrast to AF-1A, it fails to precipitate with the fragment 1A preparation from *Anthocidaris* spermatozoa.

#### Effects on ATPase Activities of Purified Dynesins

The ability of AD-1 and AF-1A to inhibit and precipitate the ATPase activity of dynein 1, dynein 2, and fragment 1A was compared by the experiments described in Table I. Under the conditions of these experiments, there was no detectable inhibition of ATPase activity of any of the enzymes by AD-1. The ATPase activity measurements indicate that AD-1 precipitated about 70% of the dynein 1 and none of the dynein 2 or fragment 1A, in agreement with the results in Fig. 3. In contrast, AF-1A, which also precipitates about 70% of the dynein 1, inhibits about 30% of the dynein 1 ATPase activity and almost all of the fragment 1A ATPase activity. A stronger inhibition of dynein 1 ATPase activity by AF-1A was obtained in previous measurements of the effect of AF-1A on dynein 1 from spermatozoa of *Anthocidaris* and *Tripneustes* (11, 13).

#### A Tryptic Fragment of Dynein 1 from *S. purpuratus* Spermatozoa

A single peak of ATPase activity was found by Sepharose 4B chromatography of dynein 1 from *S. purpuratus* spermatozoa after digestion with trypsin (Fig. 4). The preparation obtained by pooling fractions 55–66 from the Sepharose 4B column is assumed to contain a single ATPase-active species, which will be referred to as fragment 1AS. Other tryptic digestion products without ATPase activity may also be present in this fragment 1AS preparation. As seen by comparing gels D and H in Fig. 5, SDS gel electrophoresis of the fragment 1AS preparation produces a band pattern significantly different from that obtained with the original fragment 1A from *Anthocidaris* sperm dynein, which was obtained after digestion of the dynein with trypsin obtained from Worthington Biochemical Corp. (Freehold, N. J.). Further work will be required to determine whether the difference between fragment 1AS and fragment 1A is the result of a species difference or differences in the conditions for trypsin-digestion.

With a procedure similar to that used for the experiments in Table I, neither AD-1 nor AF-1A inhibited or precipitated the ATPase activity of the fragment 1AS preparation, although, under the same conditions, AF-1A almost completely inhibited the ATPase activity of the original fragment 1A. However, even though the ATPase activity was not precipitated, a visible precipitate was formed when the fragment 1AS preparations were mixed with AD-1 or AF-1A. These precipitates were collected by centrifugation, redissolved, and analyzed by SDS gel electrophoresis. The results are shown in Fig. 5. Comparison of gels D and G in Fig. 5 shows that at least one of the bands obtained with the fragment 1AS preparation, corresponding to the largest peptide chain, is not precipitated by AF-1A, and may therefore be derived from the fragment 1AS retaining ATPase activity, since the ATPase activity is not precipitated by AF-1A. This band is not found in the SDS gel of fragment 1A (Fig. 5 H). These results suggest tentatively that fragment 1AS is a smaller tryptic fragment than the original fragment 1A, which lacks the principal antigenic determinants of fragment 1A but retains the active ATPase site.

#### Effects on ATP Dephosphorylation by Demembranated Sperm Suspensions

After addition of demembranated spermatozoa to reactivation solution containing 2 or 5 µl/ml of AD-1 or AF-1A, a gradually decreasing rate of ATP dephosphorylation was observed. When the reactivation solution contained 10, 20, or 30 µl/ml of AD-1 or AF-1A, the rate of ATP dephosphorylation decreased during the first 5 min, and then leveled off to give a reasonably constant rate.
which was only slightly lower at the higher antiserum concentrations. Microscope observations on samples taken from these sperm suspensions during the period of steady dephosphorylation rate showed that with 20 µl of AD-1, most of the spermatozoa showed erratic, low amplitude beating. Only a few showed motility that was regular enough to measure a beat frequency; in those cases the frequency was in the neighborhood of 10 Hz, about half the frequency obtained in control experiments. With 20 µl AF-1A, the amount of motility appeared less, and was usually limited to low-frequency (≤1 Hz) bending near the basal end of the flagellum.

Table II compares the effects of the antibody preparations on motile demembranated spermatozoa, nonmotile broken spermatozoa, and axonemes. Both AD-1 and AF-1A inhibit the movement-coupled ATP dephosphorylation, if this is defined as the difference between the rates of ATP dephosphorylation by unbroken and broken sperm preparations (2). In addition, AF-1A, but not AD-1, appears to inhibit a significant portion of the ATP dephosphorylation by broken spermatozoa and by flagellar axonemes.

**Effects on the Motility of Reactivated Spermatozoa**

Figs. 6 and 7 show the results of measurements of beat frequency and bend angle of spermatozoa exposed to AD-1, AF-1A, or NI. Examples of the photographs used to obtain these measurements are shown in Fig. 8. Both antibody preparations inhibit both the frequency and amplitude of flagellar beating, in contrast to earlier observations of the effect of AF-1A on reactivated spermatozoa from another sea urchin, *Colobrocentrotus atratus*, where inhibition of beat frequency with little change in amplitude was obtained (7). During the first 2 min of exposure to 20 µl/ml of AD-1, both the beat frequency and bend angle decrease by about 10%. After this initial decrease, there is

| NI  | AD-1   | AF-1A  |
|-----|--------|--------|
| Unbroken spermatozoa | 54 ± 2.2 | 23 ± 4 | 14 ± 4.9 |
| Broken spermatozoa  | 20 ± 2.2 | 18 ± 1.6 | 13.5 ± 3.6 |
| Axonemes            | 23 ± 0.5 | 23 ± 1.4 | 15 ± 0.2 |

Each value represents the mean of 4-6 pH-stat measurements, and the standard deviation is indicated. For axonemes and for unbroken spermatozoa, the reactivation solution contained 20 µl/ml of antibody preparation. For the broken spermatozoa, 10 µl/ml was used. Units are μM/min.

![Figure 6](image-url)  
**Figure 6** Effects of antibody preparations on the beat frequency of individual reactivated spermatozoa. The curves labeled AD-1 were obtained with reactivation solution containing 20 µl/ml of the purified antidynein serum, AD-1. The curves labeled AF-1A were obtained with reactivation containing 5 µl/ml of the purified anti-fragment 1A serum, AF-1A. The demembranated spermatozoa were added to the reactivation solution at time 0. Each set of points connected by a line represents measurements on one spermatozoon. Two spermatozoa were measured in each antibody preparation; they are distinguished by dashed and solid lines which identify the same spermatozoon in Figs. 6 and 7.

![Figure 7](image-url)  
**Figure 7** Effects of antibody preparations on the bend angles of the same reactivated spermatozoa studied in Fig. 6. The curves are labeled as in Fig. 6, and dashed and solid lines are used as in Fig. 6 to identify the two spermatozoa studied with each antibody preparation.
FIGURE 8 Samples of the photographs used to obtain the bend angle measurements shown in Fig. 7. NI identifies the spermatozoon exposed to 20 μg/ml of the nonimmune serum preparation, NI. AD-1 identifies the spermatozoon exposed to AD-1 and identified by the open circles with solid lines in Figures 6 & 7. AF-1A identifies the spermatozoon exposed to AF-1A and identified by the x’s with dashed lines in Figures 6 & 7.

only a very slow decrease in beat frequency, while the bend angle continues to decrease until it is about 30–40% less than the original value. The movements then become very erratic and the flagellum soon sticks to the surface of the glass slide, terminating the experiment. With 5 μl/ml of AF-1A, the beat frequency continued to decrease and reached a value about 40% less than the original frequency by the time the movements became so erratic that the flagellum stuck to the surface. In terms of movement-inhibitory activity for a given concentration, AD-1 appears to be almost an order of magnitude weaker than AF-1A, except for the initial decrease in beat frequency, where the effects of AD-1 are relatively greater and appear to correspond to about one-half the activity of AF-1A.

DISCUSSION

Antigenic Determinants

AD-1 and AF-1A have approximately equal abilities to precipitate dynein 1, but AD-1 has much less effect on motility, and its ability to precipitate fragment 1A and to inhibit the ATPase activity of dynein 1 or fragment 1A was too low for us to detect. Presumably most of the antigenic determinants for AD-1 on the dynein 1 molecule are too far from the active ATPase site to induce the formation of antibody molecules which modify the function of the active ATPase site. With the smaller tryptic fragment of dynein 1, fragment 1A, a larger fraction of the antigenic sites must lie sufficiently close to the ATPase site so that they induce antibodies which modify the function of the ATPase site. However, the ability of dynein 1 to give rise to another tryptic fragment (fragment 1AS), which retains ATPase activity, but which was not precipitated or inhibited by AF-1A, indicates that the major antigenic sites on fragment 1A do not include the active ATPase site itself, which may be a highly conserved and poorly antigenic region of the dynein 1 molecule.

The much lower inhibition of movement which was obtained with AD-1, compared to AF-1A, indicates that antibody binding by dynein 1 is not, by itself, sufficient to give a strong inhibition of movement. Inhibition of movement may require a more specific interference with functionally active sites on the dynein molecule. However, this conclusion must be accepted only tentatively, as we have not excluded the alternative explanation that
the principal antigenic sites on the isolated dynein molecule are not exposed when dynein is in situ in the axoneme.

Some species specificity has previously been found for the effect of AF-1A on the movement of reactivated spermatozoa (14). Complete inhibition of the movement of reactivated spermatozoa by AF-1A occurred only in the same species used to obtain the antigen for preparation of AF-1A. In other species, including S. purpuratus, low frequency (<1 Hz) bending near the basal end of the flagellum continues even after extended exposure to high concentrations of AF-1A. This species specificity may be related to the differing capabilities of AF-1A to inhibit the ATPase activity of dynein 1 from various species, although little difference was found between the three Japanese species studied by Ogawa and Mohri (13). Such a correlation would strengthen the conclusion that inhibition of movement requires interference with the ATPase active sites on the dynein 1 molecule.

If allowance for the effects of species specificity is made, the relative effectiveness of an antifragment 1A preparation and an antidynein 1 preparation for inhibition of movement would show an even greater difference than is indicated by our results (Figs. 6 and 7) comparing the effects of AD-1 on the homologous species and AF-1A on a heterologous species.

**Inhibition of Movement-Coupled ATP Dephosphorylation**

AD-1 can be added to the list of agents which inhibit the movement-coupled ATP-dephosphorylation of suspensions of reactivated spermatozoa but do not directly inhibit dynein ATPase activity. If movement-coupled ATP-dephosphorylation is considered to be measured by the difference between the rates of ATP dephosphorylation by motile and broken sperm preparations (2), both AD-1 and AF-1A appear to inhibit the movement-coupled ATP dephosphorylation. The additional inhibition of the ATP dephosphorylation of broken spermatozoa by AF-1A but not by AD-1 may then be associated with the ability of AF-1A to directly inhibit some part of the ATPase activity of dynein 1.

The movement-coupled ATP dephosphorylation represents an activation of the ATPase activity of flagellar dynein by tubule interactions which occur during movement. When the motility of a reactivated sperm suspension is eliminated by 0.1 M NaHCO₃ (4) or by trypsin-disintegration (5), a larger fraction (77% or 82%) of the ATP-dephosphorylation is inhibited, compared to the 67% inhibition obtained by sperm breakage. Since these agents, like sperm breakage, do not directly inhibit dynein ATPase activity, these results indicate that some part of the ATP-dephosphorylation measured with broken spermatozoa represents activation of dynein ATPase by interaction with flagellar tubules, and should perhaps be included in the movement-coupled ATP dephosphorylation (4, 5). In this case, our results (Table II) with the antibody preparations indicate that AF-1A inhibits a larger fraction of the tubule-activated or movement-coupled ATP dephosphorylation than does AD-1, which is consistent with our observations on the inhibition of motility by these preparations.

**Comparison with Colobocentrotus**

Our observation that AD-1 and AF-1A inhibit the amplitude of flagellar bending as well as, and sometimes more strongly than, the beat frequency, contrasts with observations on Colobocentrotus spermatozoa, where AF-1A could reduce the beat frequency by more than 90% with little change in waveform (7). We have made some preliminary observations on reactivated spermatozoa from another sea urchin, Lytechinus pictus, which also indicate a strong inhibition of bend amplitude by AF-1A. The response of Colobocentrotus spermatozoa may be a relatively unique feature of this species.

Colobocentrotus spermatozoa have previously been found to be unique in their insensitivity to calcium ion concentrations (3). Their response to KCl extraction of the outer dynein arms by a reduction in beat frequency with no change in waveform (6), which is similar to their responses to antifragment A serum, may also be unique, as attempts to obtain this result with spermatozoa from several other sea urchin species have been unsuccessful. The mechanisms which regulate bend angle and symmetry appear to be less easily disrupted in Colobocentrotus spermatozoa than in other species.

**Inhibition of Bend Angle by Antidynein**

The inhibition of bend angle by AD-1 and AF-1A is a significant new result. Previously, the bend...
angle, but not the beat frequency, of reactivated spermatozoa was found to be inhibited by CO₂ (4). Flagellar bending was completely inhibited in solutions containing 0.1 M NaHCO₃, while the active sliding of tubules from trypsinized axonemes and the ATPase activity of dynein 1 were not inhibited. It was concluded that CO₂ acted on some part of the axoneme which is involved in converting active sliding into flagellar bending. This conclusion agreed well with the observations on Colobocentrotus spermatozoa where the frequency, but not the amplitude, of beating was reduced by antifragment 1A and by extraction of dynein 1. It supports a model in which the dynein arms produce active sliding, and other structures, such as the radial spokes and their connections to the central pair of tubules which have been described in detail by Warner and Satir (15), convert active sliding into oscillatory bending and regulate the amplitude of bending. This conclusion and model must now be reexamined, since we have found that bend angle can be inhibited by AD-1 and AF-1A, which are expected to act only on dynein 1 of the flagella. Figs. 6 and 7 show, in fact, that a large decrease in bend angle can be caused by AD-1 with little change in beat frequency, and the results are not very different from similar measurements on spermatozoa exposed to NaHCO₃.

The possibility that the amplitude of flagellar bending may be determined in part by the activity of the dynein arms is strengthened by the observation that AF-1A, which inhibits ATPase activity of dynein, is more effective than AD-1 at inhibiting the bend angle. However, further supporting evidence is required before this conclusion can be fully accepted. The exact mechanism of the inhibitory effects of AF-1A and AD-1 is not known, nor is it known with certainty that the dynein arms which generate active sliding are the only locus of proteins which bind these antibodies. Additional information might be gained by preparing antibodies to a smaller fragment of the dynein molecule containing the active ATPase site, such as fragment 1AS, and by electron microscope examination of axonemes which have been incubated with ferritin-labeled antibody.

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OGAWA, ASAI, AND BROKAW Antiserum against Dynein 1 191

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