INHIBITION OF MOVEMENT AND ATP-ASE ACTIVITY OF DEMEMBRANATED SEA URCHIN SPERMATOZOA BY ANTI-DYNEIN ANTISERUM

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SUMMARY: Anti-dynein antiserum was prepared in rabbits against a tryptic fragment (Fragment A) of dynein ATPase. The antiserum inhibited the ATP-induced movement of demembranated sea urchin spermatozoa. The complete inhibition of progressive movement occurred at relatively low concentrations of the antiserum, which affected the ATPase activity to a rather small extent. The anti-dynein antiserum seems to inhibit not only ATPase activity but also bending-wave formation, in some way.

Dynein ATPase, which corresponds to the arms projecting from the A-tubules of outer doublet microtubules in flagella and cilia, is considered to play an important role in their movement (1).

We have prepared an anti-dynein antiserum in rabbits using the purified tryptic fragment (Fragment A) of dynein ATPase from sperm flagella of the sea urchin, Anthocidaris crassispina (2, 3). The antiserum inhibits the ATPase activity of dynein and Fragment A, and forms a single precipitin line between the antigens from the same and other sea urchins, Pseudocentrotus depressus and Hemicentrotus pulcherrimus (3). Then, a question arises as to whether the antiserum inhibits not only the ATPase activity but also flagellar movement, because dynein is considered to generate active bending force by its ATPase activity. In this paper, we report the effects of the antiserum on glycerinated and Tritonated sea urchin spermatozoa.

MATERIALS AND METHODS

Anti-dynein antiserum was prepared in rabbits using a tryptic fragment (Fragment A) of sea urchin (A. crassispina) dynein ATPase, as described previously (3). The antiserum and non-immune serum were dialysed against a

medium consisting of 0.15 M KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 10 mM Tris-HCl buffer, pH 8.3 and 1 mM dithiothreitol (DTT) before use.

For observation of motility, spermatozoa of the sea urchins, H. pulcherrimus and A. crassispina, were used. Preparation of sperm models was made by demembranating the sperm with either Triton X-100 or glycerol. The Tritonated sperm suspension was prepared according to the method of Gibbons and Gibbons (4) with a slight modification: a small drop of semen was mixed with 2 ml of an extraction medium containing 0.15 M KCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 0.5 mM EDTA, 2 mM Tris-HCl buffer, pH 8.3, 0.5 mM ATP and 0.04 % (v/v) Triton X-100 at room temperature for 40 seconds, and stored at 0° C until use. The glycerinated sperm suspension was prepared as follows; a small drop of semen was suspended in a medium containing 0.3 M KCl, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM Tris-HCl buffer pH 8.3, 1 mM β-mercaptoethanol and 0.1 mM ATP. The suspension was then mixed with an equal volume of glycerol at 0° C for 1 hour and stored at -20° C.

The sperm models were reactivated as follows: 50 µl of the stored sperm suspension was added to 0.9 ml of a medium containing 0.15 M KCl, 2 mM $MgCl_2$, 0.5 mM EDTA, 1 mM EGTA, 5 mM DTT and 20 mM Tris-HCl buffer pH 8.3 and either anti-dynein antiserum or non-immune serum. After 2 hours' preincubation at 0° C, 0.1 ml of 10 mM ATP was added. The medium containing ATP is referred to hereafter as the "reactivation medium". The suspension was then poured into a trough for observation. Motility of the sperm models was examined by phase contrast microscopy, and flagellar beat frequency was counted by a stroboscopic method.

The ATPase activity was assayed with glycerinated spermatozoa of A. crassispina prepared as described previously (5). The spermatozoa were washed with and suspended in filtered sea water. The assay medium contained 0.3 ml of 0.5 M Tris-HCl buffer, pH 8.3, 0.1 ml of 0.3 M $MgCl_2$, 0.3 ml of filtered sea water, 0.1 ml of 0.15 M NaCl plus serum and 0.2 ml of 12.5 mM ATP. The reaction was started by the addition of ATP and terminated by the

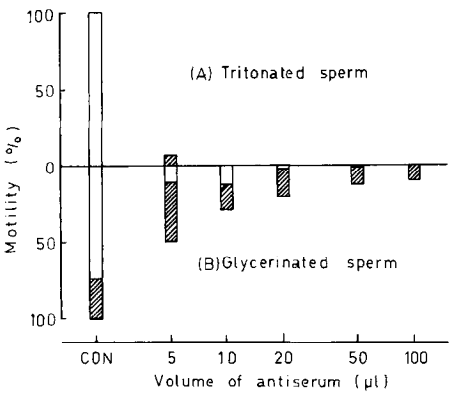

Fig. 1

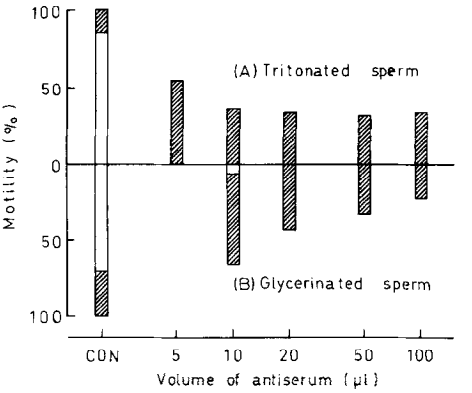

Fig. 2

Fig. 1. Effects of anti-dynein antiserum on the motility of Tritonated (A) and glycerinated (B) spermatozoa of A. crassispina. The motility is defined as Ne/Nc x 100 (Ne and Nc represent the percentages of motile sperm in the experimental and the control, respectively). In the control, 200 µl of non-immune serum is added. More than 400 spermatozoa from 3 individuals were counted in each sample. Open portion represents progressive swimming and hatched portion head oscillating.

Fig. 2. Effects of anti-dynein antiserum on the motility of Tritonated (A) and glycerinated (B) sperm of H. pulcherrimus.

addition of 0.5 ml of trichloroacetic acid. The liberated inorganic phosphate was determined by the method of Lohmann and Jendrássisk (6).

RESULTS AND DISCUSSION

The majority of the spermatozoa extracted with Triton X-100 and reactivated in the reactivation medium without serum progressed through the medium along a straight path, with a beat frequency of about 30 Hz. The addition of 200 µl of non-immune serum had no effect on their movement, but it was markedly inhibited in the presence of the anti-dynein antiserum, as summarized in Figs. 1 and 2.

The effects of the antiserum on the two species of sea urchin were very different. In A. crassispina (Fig. 1, A), the sperm models were very sensitive to the antiserum. The addition of 5 µl of antiserum completely inhibited their progressive movement; only a small number of sperm models oscil-

lated their heads. In the case of head-oscillating sperm models, only the proximal part of the flagellum exhibited bending, the distal three-quarters remaining straight. As the amount of antiserum increased, the number of motile spermatozoa decreased, and aggregation was observed when more than 100 µl of antiserum was added.

If the preincubation was omitted or antiserum was added to reactivated sperm models, they swam progressively for a short time just after the addition of ATP or antiserum. The motility then gradually diminished. Within several minutes, most of sperm models stopped swimming and either became immotile or showed head oscillation if the medium contained a sufficient amount of the antiserum (more than 10 µl of antiserum/ml of the reactivation medium).

On the other hand, in H. pulcherrimus (Fig. 2, A), head oscillation of 55 % of the Tritonated sperm persisted with 5 µl of antiserum and the value was still 35 % with 100 µl, although the progressive motion was completely inhibited. As the volume of added antiserum increased, the oscillation became irregular and its frequency decreased (less than 5 Hz with 50 µl of antiserum). More than 100 µl of antiserum caused the aggregation of sperm models as in A. crassispina. The difference in the effect of the antiserum is considered to be due to antigenic species specificity.

The glycerinated spermatozoa appeared to incur some damage during the extraction process. Even in the control, only a few sperm models swam in a straight path and most of the motile models followed a circular course. Swimming inhibition by the antiserum, however, occurred in the same manner as that observed in the Tritonated sperm (Fig. 1, B and Fig. 2, B), although more antiserum was needed to cause a degree of inhibition similar to that in the Tritonated sperm models.

The effects of the antiserum on ATPase activity are given in Fig. 3. When the glycerinated sperm of A. crassispina were assayed for ATPase activity after a 24 hr preincubation with the antiserum, it was found to be in-

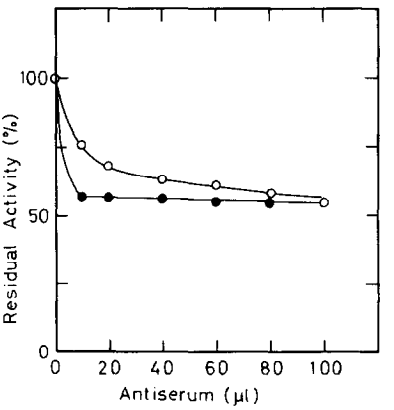

Fig. 3. Effects of anti-dynein antiserum on the ATPase activity of the glycerinated spermatozoa of A. crassispina. The percentage of residual activity is defined as (ATPase activity in the presence of antiserum/ ATPase activity in the presence of the same amount of non-immune serum) x 100. The specific activity of the glycerinated spermatozoa was 0.15 μmole Pi/min/mg protein.
—o— : After 10 min preincubation with the antiserum.
—●— : After 24 hr preincubation.

hibited by 45 %. With a short preincubation, a relatively large amount of antiserum was necessary to produce the same inhibition. The degree of inhibition remained at 45 % even after preincubation for one week.

We have recently demonstrated that an excess of anti-dynein antiserum inhibits the ATPase activity of purified Fragment A and dynein by 95 - 98 % within 10 min at 0° C (3). In the present experiment, however, excess antiserum inhibited only 45 % of the ATPase activity of demembranated spermatozoa. This suggests that the antiserum can attack only half of the total dynein ATPase when the dynein molecules are bound to the outer doublets, implying that some of the dynein, for example, that of the outer arms, is accessible to the antiserum and the rest inaccessible. Alternatively, there may be non-dynein ATPase in the axonemes which is not affected by the anti-dynein antiserum (see 7).

Gibbons and Gibbons (1) demonstrated that demembranated sea urchin spermatozoa further extracted by 0.5 M KCl exhibited swimming activity, but the beat frequency and ATPase activity decreased to less than half of the pre-

extraction values. In the present experiment, on the other hand, a relatively small amount of the antiserum, which attacked less than 50 % of the ATPase, inhibited the swimming of demembranated spermatozoa. It appears, therefore, that the atniserum inhibits not only the ATPase activity of dynein but also the bending wave formation in some manner, for instance, by inhibiting the conformational change of the dynein molecules which would be necessary for carrying out the sliding of adjacent microtubules.

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