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INHIBITION OF AXONEME AND DYNEIN ATPase FROM SEA URCHIN SPERM BY FREE ATP

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Summary

Sea urchin sperm flagellar ATPase (EC 3.6.1.3) has magnesium-ATP as an effective substrate and is inhibited by free ATP. The inhibition is prevented by high concentration of KCl or NaCl. 0.4 M KCl extracts 48% of ATPase activity from axoneme. The 0.4 M KCl extract and 0.4 M KCl-treated axoneme are also inhibited by free ATP and this inhibition is reversed by KCl. Dynein purified twice by sucrose density gradient centrifugation is also inhibited by free ATP; this inhibition is also reversed by KCl.

Introduction

The ATPase dynein (EC 3.6.1.3), identified as "arm" in the 9 + 2 structure [1,2], can be obtained in two enzymatically active forms. Cilia from *Tetrahymena* yield both a 14 S form and a 30 S form, while sea urchin flagella yield only a 10—11 S form [3]. Dynein ATPase is known to be stimulated by Mg^{2+} or Ca^{2+} [4], and is influenced by binding of microtubules in its KCl dependency and pH dependency [5,6]. With a glycerine-model or Triton-model, the movement of flagella or cilia was found to couple with hydrolysis of ATP [7—9]. From the divalent cation requirement for the movement, Gibbons and Gibbons [9] and Douglas and Holwill [10] suggested that a magnesium-ATP complex was an effective substrate. Recently, our kinetic analysis of axoneme and dynein ATPase showed that the effective substrate of the ATPase was a magnesium-ATP complex and free ATP inhibited ATPase [11]. This report describes the reversal by KCl of the inhibition by free ATP.

Materials and Methods

Sperm were collected from sea urchin *Pseudocentrotus depressus*. Axoneme was prepared as previously reported [11]. Axoneme was treated with

0.4 M KCl, 20 mM Tris · HCl buffer (pH 8.0) and 0.1 mM dithiothreitol for 5 min at 0°C, and was then centrifuged at 8000 rev./min for 7 min with a Sorvall SS-34 rotor. The supernatant and precipitate were referred to as KCl-extract and KCl-treated axoneme, respectively. For the purification of dynein, Tris-EDTA extract [11] was fractionated by 5—20% (w/v) sucrose density gradient centrifugation with a SW 27 rotor at 74 900 × *g* for 43 h at 4°C. Fractions showing ATPase activity (fraction number 9—14 in Fig. 1, a) were collected and again centrifuged in the same sucrose density gradient with a SW 25.2 rotor at 75 500 × *g* for 35 h at 4°C. Fractions showing ATPase activity (fraction number 14—18 in Fig. 1, b) were collected and used as a purified dynein.

ATPase activity was measured at 30°C as previously reported [11]. Inorganic phosphate liberated was measured by the method of Murphy and Riley [12]. One unit of the enzyme activity was defined as the amount of enzyme which catalyzed the liberation of 1 μmol P_i per min. Protein concentration was determined according to the method of Lowry et al. [13].

Results

Our previous report shows that axonemal ATPase requires magnesium-ATP as an effective substrate and is inhibited by free ATP [11]. These effects were analyzed from the ATPase activities in 30 mM KCl and 20 mM Tris · HCl buffer (pH 8.0), when the magnesium-ATP concentration was fixed and free ATP concentration was varied. In the presence of 0.4 M KCl, however, axone-

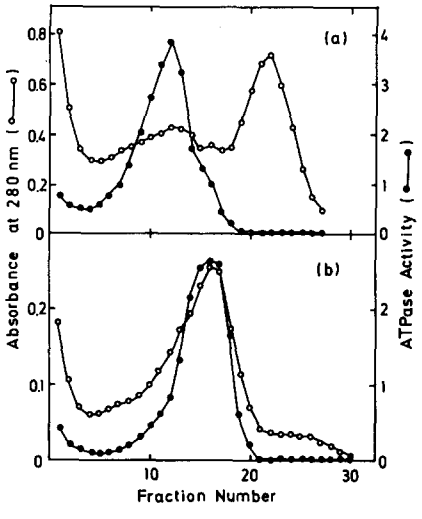

Fig. 1. (a) First sucrose density gradient centrifugation of Tris · EDTA extract. Two ml of Tris · EDTA extract of 6.24 mg/ml were applied on 5—20% sucrose containing 1 mM Tris · HCl buffer (pH 8.0), 0.1 mM EDTA and 0.1 mM dithiothreitol, and centrifuged at 74 900 × *g* for 43 h at 4°C. Each 34 drops were collected from the bottom of a tube. (b) Second sucrose density gradient centrifugation. A sample was collected, fractions showing ATPase activity in Fig. 1a (fraction number 9—14) and applied on 5—20% sucrose containing 1 mM Tris · HCl buffer (pH 8.0), 0.1 mM EDTA and 0.1 mM dithiothreitol. Centrifugation was performed at 75 500 × *g* for 35 h at 4°C. ○——○, absorbance at 280 nm; ●——●, ATPase activity (arbitrary unit).

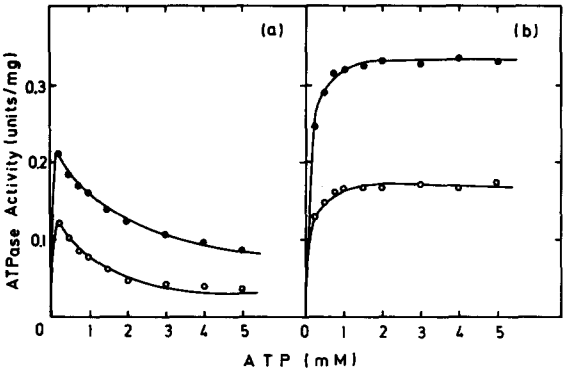

Fig. 2. (a) Axonemal ATPase inhibited by high concentrations of ATP. ATPase activity was measured in the presence of 20 mM Tris · HCl buffer (pH 8.0), ATP and 0.05 mM $MgSO_4$ (○) or 0.5 mM $MgSO_4$ (●) at 30°C. (b) Inhibition reversal of axonemal ATPase by KCl. ATPase activity was measured in the presence of 0.4 M KCl, 20 mM Tris · HCl buffer (pH 8.0), ATP and 0.05 mM $MgSO_4$ (○) or 0.5 mM $MgSO_4$ (●) at 30°C.

mal ATPase showed a typical Michaelis-Menten type relationship and was not inhibited by high concentrations of ATP (Fig. 2b) as compared with that in the absence of KCl (Fig. 2a). ATPase activity was always higher in the presence of 0.4 M KCl than in the absence of KCl at all range of ATP concentrations used. This suggests that 0.4 M KCl protected axonemal ATPase from free ATP.

Fig. 3 shows that axonemal ATPase was stimulated by KCl more strongly in the presence of 1.5 mM ATP and 0.05 mM $MgSO_4$ than that in the presence of 0.2 mM ATP and 0.05 mM $MgSO_4$. These two activity curves crossed at 0.2 M KCl. Axonemal ATPase started to decrease in the presence of KCl concentrations greater than 0.2 M. The difference between both activities changed gradu-

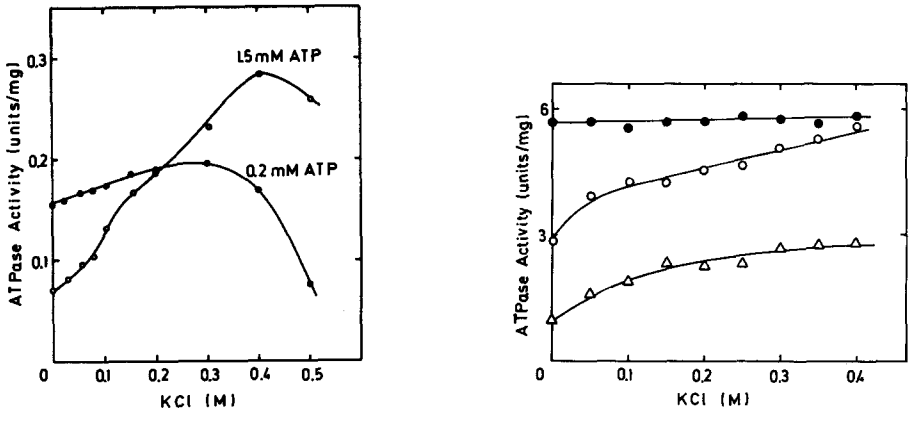

Fig. 3. Inhibition and reversal of inhibition of axonemal ATPase. ATPase activity with rising concentration of KCl was measured in the presence of 0.05 mM $MgSO_4$, 20 mM Tris · HCl buffer (pH 8.0) and 0.2 mM ATP (●) or 1.5 mM ATP (○) at 30°C.

Fig. 4. KCl stimulation of dynein ATPase. ATPase activity was measured in the presence of 20 mM Tris · HCl buffer (pH 8.0), 1 mM ATP, and 0.1 mM $MgSO_4$ (△), 1 mM $MgSO_4$ (○) or 20 mM $MgSO_4$ (●) with different concentrations of KCl at 30°C.

TABLE I

INHIBITION AND REVERSAL OF INHIBITION AT AXONEME, TRIS · EDTA EXTRACT AND PURIFIED DYNEIN

ATPase activity was measured in the presence of enzyme, ATP, KCl, 0.05 mM $MgSO_4$ and 20 mM Tris · HCl buffer (pH 8.0) at 30°C.

Conditions	ATPase activity (units/mg)		
	Axonême	Tris · EDTA extract	Dynein
0.2 mM ATP	0.125	0.248	0.620
1.5 mM ATP	0.0575	0.186	0.310
0.2 mM ATP and 0.4 m KCl	0.110	0.244	0.672
1.5 mM ATP and 0.4 KCl	0.150	0.398	1.21

ally with rising concentration of KCl. This suggests that inhibition by free ATP was strongest in the absence of KCl and was gradually reversed by KCl. The same profile was observed in the case of NaCl substituted for KCl.

From Fig. 3, a rough idea of inhibition and inhibition reversal of the ATPase could be deduced after comparison with four levels of activity. The results of the measurement of inhibition of axonemal ATPase in the presence of 1.5 mM and 0.2 mM ATP and the reversal of the inhibition produced by addition of 0.4 M KCl are shown in Table I.

Treatment with 0.4 M KCl at 0°C for 5 min extracted 48% of ATPase activity and 14% of protein from axoneme, and it was shown that both KCl extract and KCl-treated axoneme were inhibited by free ATP and that this inhibition was reversed by KCl, although the former ATPase activity was about 4 times higher than the latter's (Table II).

Usually, dynein was extracted from axoneme by dialyzing against 1 mM Tris · HCl buffer (pH 8.0) and 0.1 mM EDTA according to Gibbons [1,2]. 0.1 mM dithiothreitol was added in our system to prevent denaturation of dynein ATPase. Dithiothreitol did not influence the characteristics of inhibition or inhibition reversal of the ATPase. 0.4 M KCl could not extract more than 60% of the ATPase from sea urchin sperm axoneme, while dialysis against 1 mM Tris · HCl buffer (pH 8.0), 0.1 mM EDTA and 0.1 mM dithiothreitol extracted 90% or more ATPase. Tris · EDTA-extracted ATPase showed the same inhibition

TABLE II

INHIBITION AND REVERSAL OF INHIBITION OF KCl-EXTRACT ABD KCl-TREATED AXONEME

ATPase activity was measured in the presence of enzyme, ATP, KCl, 0.05 mM $MgSO_4$ and 20 mM Tris · HCl buffer (pH 8.0) at 30°C.

Conditions	ATPase activity (units/mg)	
	KCl extract	KCl-treated axoneme
0.2 mM ATP	0.281	0.0864
1.5 mM ATP	0.194	0.0381
0.2 mM ATP and 0.4 M KCl	0.393	0.0662
1.5 mM ATP and 0.4 M KCl	0.536	0.0876

and reversal of inhibition properties as axonemal ATPase (Table I). This suggests that the inhibition and inhibition reversal property of the enzyme was independent of microtubule binding.

Dynein was purified from Tris · EDTA extract by 2 sucrose density gradient centrifugations (Fig. 1). The ATPase fractions from the first sucrose density gradient centrifugation were pooled. ATPase activity of this sample showed 3.00 units/mg in the presence of 30 mM KCl, 2 mM $MgSO_4$, 1 mM ATP and 20 mM Tris · HCl buffer (pH 8.0) at 30°C. This sample was again centrifuged in a sucrose density gradient (Fig. 1b). From this figure double peaks of protein were observed; one near the bottom, the other in the middle. The protein concentration of the middle fractions was coincident with ATPase activity. Fractions 14—18 in Fig. 1b were used as purified dynein. ATPase activity of the purified dynein was 4.02 units/mg in the presence of 30 mM KCl, 2 mM $MgSO_4$, 1 mM ATP and 20 mM Tris-HCl buffer (pH 8.0) at 30°C. The purified dynein also exhibited inhibition by free ATP and inhibition reversal by KCl (Table I).

In sea urchin sperm flagella, KCl is known to stimulate axoneme and dynein ATPase [5]. Dynein ATPase at 0.1 mM and 1 mM $MgSO_4$ in the presence of 1 mM ATP increased with rising concentration of KCl, while the ATPase at 20 mM $MgSO_4$ in the presence of 1 mM ATP was independent of KCl concentration (Fig. 4). Free ATP concentration decreases with rising concentration of $MgSO_4$ and is negligible in the presence of 20 mM $MgSO_4$. This suggests that KCl stimulation of dynein ATPase can be interpreted as a result of a reversal of inhibition of the ATPase by KCl.

Discussion

Inhibition of flagellar ATPase by free ATP was reversed by KCl. With respect to the characteristics of the inhibition and inhibition reversal, there was no essential difference between axoneme and dynein.

In *Paramecium* demembraned by Triton X-100, calcium ions regulated the direction of ciliary beating at 10^{-6} M [14,15]. Brokaw et al. [16] recently reported that the mode of flagellar beats of Triton X-100 treated spermatozoa was also regulated by low concentration of calcium ions. However, calcium ions did not influence the inhibition and inhibition reversal of axonemal ATPase at low concentration.

Sea urchin sperm contained about 10 mM ATP [17]. But, there is no report about salt concentration and divalent cation concentration in sea urchin sperm flagella. Therefore, we cannot speculate on the biological function of the inhibition and inhibition reversal of dynein ATPase in vivo.

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