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INHIBITORY EFFECT OF INORGANIC PHOSPHATE ON THE AXONEMAL ATPase OF CILIA FROM *TETRAHYMENA PYRIFORMIS*

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SUMMARY

An inhibitory effect of inorganic phosphate on the axonemal ATPase of cilia from *Tetrahymena pyriformis* was shown. P_i inhibited the terminal phosphate liberation from [γ-^{32}P]ATP by 30-S dynein and inhibited the conversion of [8-^{14}C]ATP to ADP and AMP by digitonin-extracted cilia.

Enzymic properties of axonemal ATPase of cilia and flagella have been reported in several respects[1–6]. However, an effect of inorganic phosphate (P_i), an end product of the reaction, on the ATPase has not been examined adequately.

In this paper, an inhibitory effect of P_i on the axonemal ATPase of cilia from *Tetrahymena pyriformis* is described. The axonemal ATPase was used in the form of either 30-S dynein or digitonin-extracted cilia prepared as previously described[7]. Effect of P_i on the axonemal ATPase was examined at first by an effect of P_i on the liberation of $^{32}P_i$ from [γ-^{32}P]ATP by 30-S dynein. The amount of liberated $^{32}P_i$ was measured by the method of Shnebli and Abrams[8]. As shown in Fig. 1, the amount

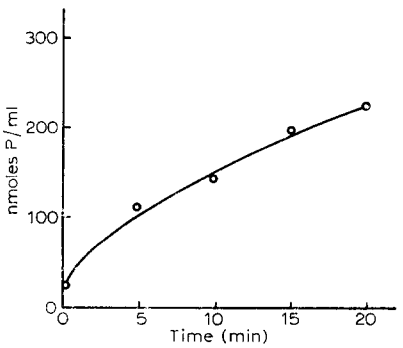

Fig. 1. Liberation of $^{32}P_i$ from [γ-^{32}P]ATP by 30-S dynein is shown as a function of time. Reaction was performed at 30 °C in a reaction mixture containing: 30 mM Tris–HCl (pH 7.5), 1 mM [γ-^{32}P]ATP ($1.3 \cdot 10^5$ cpm/ml), 2 mM $MgCl_2$, and 20 μg/ml. 30-S dynein. To measure liberated $^{32}P_i$, 1.0 ml of the reaction mixture was added with chilled 1 M HCl and with 200 mg of acid-washed Norit A in 1.0 ml of water. After 10 min, the suspension was centrifuged and the supernatant was filtered with Whatman glass fibre filter GF/C. The radioactivity of 1.0 ml of the filtrate was measured in a Beckman liquid scintillation counter Model 200B.

of liberated $^{32}P_i$ from [γ-^{32}P]ATP increases with the lapse of incubation time, although the rate of $^{32}P_i$ liberation decreases somewhat during this time. Such terminal phosphate liberation from [γ-^{32}P]ATP by 30-S dynein was evidently inhibited by P_i. As shown in Table I, the ATPase activity of 30-S dynein was inhibited in the presence of 5 and 10 mM P_i by 84.5 and 86.0%, respectively.

TABLE I

INHIBITION OF TERMINAL PHOSPHATE LIBERATION FROM [γ-^{32}P]ATP WITH 30-S DYNEIN BY INORGANIC PHOSPHATE

Reactions were performed in the same conditions as in Fig. 1, for 10 min, but in the presence of P_i.

P_i	*Liberation of terminal phosphate (nmoles [^{32}P]phosphate/min per ml)*
No addition	9.59
5	1.49
10	1.34

The inhibitory effect of P_i on the axonemal ATPase was also examined by the effect of P_i on the conversion of [8-^{14}C]ATP to ADP by using digitonin-extracted cilia as the axonemal ATPase. The ATPase activity was estimated from the amount of ADP converted from [8-^{14}C]ATP. Nucleotides in the reaction mixture were separated on a thin-layer polyethylenimine cellulose plate by chromatography. The amounts of the nucleotides, which were identified by correspondence with the marker nucleotides, were calculated from the radioactivity directly measured with an automatic gas-flow scanner (Aloka TLC-2B). A typical result of the separation and radioactivity scanning

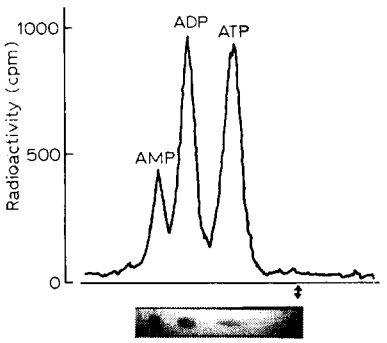

Fig. 2. Separation and quantitation of nucleotides in the reaction mixture after 10 min incubation with digitonin-extracted cilia. Reaction was performed at 30 °C in a 0.5-ml solution containing; 40 mM Tris-HCl (pH 7.5), 1.6 mM $MgCl_2$, 0.5 mM [8-^{14}C]ATP (1.0 μCi/ml), 0.5 mM P_i, and 0.4 mg/ml digitonin-extracted cilia. Reaction was terminated by charging 3 μl of the reaction mixture at the origin (arrow) on a polyethylenimine cellulose plate. Ascending separation was carried out by developing the plate with 0.2 M ammonium bicarbonate. A photograph of the plate under ultraviolet light at 253.6 nm is shown below the abscissa. On the graph scanned radioactivities are shown in correspondence with each spot shown in the photograph.

of the nucleotides is shown in Fig. 2. The radioactivity is observed exclusively in ATP, ADP, and AMP, and is not detected in any other region of the plate. This restricted nucleotide distribution was also confirmed from an observation of the plate under ultraviolet light at 253.6 nm. As shown in Fig. 3a, digitonin-extracted cilia converted [8-^{14}C]ATP to ADP and AMP. The production of AMP suggests the presence of adenylate kinase in the digitonin-extracted cilia. The conversion of ATP to ADP and AMP was not inhibited by 0.5 mM P_i (Fig. 3b). In case of 5 mM P_i, however, these conversions were strongly inhibited (Fig. 3c). During 10 min incubation, only 12.1% of ATP were converted to ADP, although more than 57% of ATP initially present were converted to other nucleotides in the absence of P_i. The production of AMP was inhibited almost completely by 5 mM P_i in the system. This complete inhibition of AMP production suggests an inhibitory effect of P_i on the adenylate kinase as observed in the ATPase activity. Another suggestion is also obtained that the adenylate kinase was inhibited by the higher concentration of ATP maintained as a result of the ATPase inhibition by P_i.

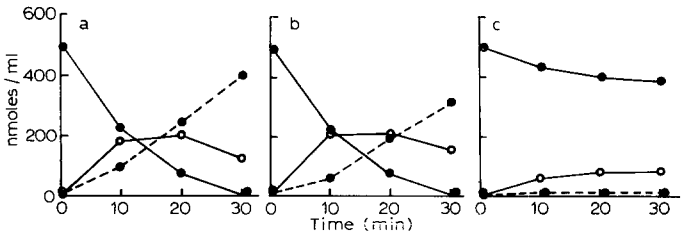

Fig. 3. Inhibitory effect of P_i on the conversion of [8-^{14}C]ATP to ADP and AMP by digitonin-extracted cilia. Conditions were the same as shown in Fig. 2, but initially contained P_i at (a) 0, (b) 0.5 mM, (c) 5 mM. ●—●, ATP; ○—○, ADP; ●---●, AMP.

A description about the effect of P_i on the axonemal ATPase of flagella of sea urchin sperm was presented by Gibbons and Fronk[6]. They observed no detectable effect of 1 mM P_i on the ATPase by their preliminary experiments. An inhibitory effect of P_i on their axonemal ATPase preparation may be observed at higher concentrations of P_i. A conclusion may be drawn at this stage that the axonemal ATPase of cilia is inhibited by P_i.

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