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STIMULATION OF ATPase ACTIVITY OF 30-S DYNEIN WITH MICROTUBULAR PROTEIN

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SUMMARY

Microtubule protein and 30-S dynein (an axonemal ATPase protein) were prepared from cilia of *Tetrahymena pyriformis* and their mutual interaction was investigated. Microtubular protein stimulated the ATPase activity of 30-S dynein. These observations give some support to models involving the interaction between tubules and dynein arms in ciliary movement.

Concerning the mechanism of ciliary and flagellar motility, observations suggesting an active mechanochemical role of dynein arms in ATP-induced sliding between tubules were recently presented[1]. Dynein arms and tubules have already been solubilized as 30-S dynein (an axonemal ATPase protein)[2] and tubulin (microtubular protein)[3], respectively. In this paper, observations indicating an interaction between 30-S dynein and microtubular protein are reported in preparations from a ciliated protozoan *Tetrahymena pyriformis*.

Isolation of cilia and purification of 30-S dynein and microtubular protein were performed fundamentally according to procedures previously reported[2-5]. Cilia were isolated by treating the cells with a solution containing 30 mM $CaCl_2$, 12 % ethanol, 1 mM 2-mercaptoethanol and 10 mM Tris–HCl buffer (pH 7.0). The isolated cilia were then treated with 0.5 % digitonin solution containing 2 mM $MgCl_2$, 30 mM KCl, 1 mM 2-mercaptoethanol and 10 mM Tris–HCl buffer (pH 8.2) for 2 h and dialyzed against 30 mM KCl containing 2 mM $MgCl_2$ and 10 mM Tris–HCl buffer (pH 7.5) for 18 h to remove ciliary membranes and matrix protein, respectively. After centrifuging the suspension, most of the 30-S dynein and a part of the microtubular proteins were extracted from the pellet by dialysis against 0.1 mM EDTA containing 1 mM 2-mercaptoethanol and 1 mM Tris–HCl buffer (pH 8.2) for 4 h and were purified by ultracentrifugation through a sucrose-density gradient. Their sedimentation properties and ATPase activities are shown in Fig. 1. Fractions indicated in Fig. 1. were used as 30-S dynein and microtubular protein after removal of sucrose.

In this paper, the interaction between 30-S dynein and microtubular protein was investigated through the effects of microtubular protein on the ATPase activity of 30-S dynein. As shown in Fig. 2, the ATPase activity of 30-S dynein was enhanced with an increase in concentration of microtubular protein despite the fact that microtubular protein itself showed no ATPase activity. Fig. 3 shows that the ATPase activity of the dynein increases linearly in proportion to dynein concentration. Such

a linear increase in activity corresponding to the amount of enzyme suggests that the activity is not so unstable as to become inactive in assay enzyme concentrations. Fig. 3 also shows that in the presence of microtubular protein the ATPase activity

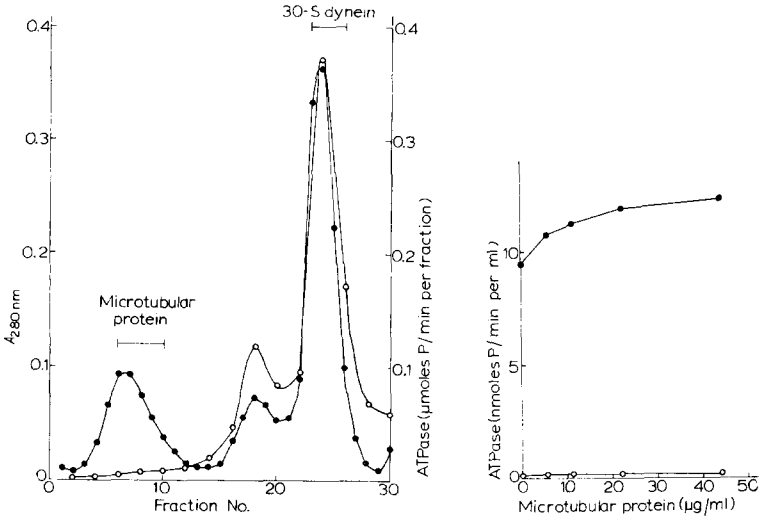

Fig. 1. Purification of microtubular protein and 30-S dynein by sucrose gradient centrifugation. 2.0 ml of 0.1 mM EDTA extract were layered onto a 7.5–30% linear sucrose gradient in 0.1 mM EDTA, 1 mM Tris–HCl buffer (pH 8.2). Gradients were spun at 24500 rev./min for 36 h on a SW 25.1 rotor in a Beckman ultracentrifuge L_2-65B at 4 °C. Each fraction in 1.0 ml was collected from the top of the tubes. Aliquots of each fraction were analyzed for protein concentration (●) and ATPase (○). ATPase assay was performed by incubating 2.0 ml of reaction medium containing 60 μmoles Tris–HCl buffer (pH 7.5), 4 μmoles $MgCl_2$, 1 μmole ATP and 0.1 ml of each fraction for 10 min at 30 °C. The reaction was stopped with 0.4 ml chilled 60% $HClO_4$. Liberated phosphate was determined by the method of Allen[6]. Fractions illustrated with bars were pooled as microtubular protein and 30-S dynein.

Fig. 2. Effects of microtubular proteins on the ATPase activity of 30-S dynein. Reaction media contained various amounts of microtubular protein with (●) or without (○) 10.6 μg 30-S dynein. Other conditions for ATPase assay were the same as in Fig. 1. Protein contents were determined by the method of Lowry *et al.*[7].

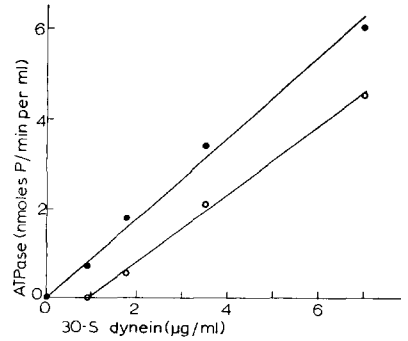

Fig. 3. Effects of 30-S dynein concentration with (●) or without (○) 47.3 μg/ml microtubular protein. Other conditions were the same as in Figs 1 and 2.

of the dynein was enhanced significantly and that a large amount of microtubular protein is more effective to activate the ATPase.

TABLE I

SPECIFICITY OF MICROTUBULAR PROTEIN FOR ACTIVATION OF 30-S DYNEIN ATPASE

Conditions for ATPase assay were the same as in Figs 1 and 2.

30-S dynein (5.3 μg/ml)	*Microtubular protein (33 μg/ml)*	*Bovine serum albumin (30 μg/ml)*	*ATPase (μmoles/min per ml)*
+	−	−	7
+	+	−	10.8
+	−	+	6.5
−	+	−	0
−	−	+	0

The specificity of microtubular protein for the activation is examined with bovine serum albumin. As shown in Table I, microtubular protein is specific for the ATPase activation, for the comparable amount of bovine serum albumin failed to stimulate the ATPase. The amount, however, of microtubular protein used seems too large to be considered as a physiological phenomenon. To understand the use of such a large amount of microtubular protein it may be helpful to note that microtubular protein is a mixture of A- and B-tubulin and other minor components, and that both A- and B-tubulins contain bound guanine nucleotides such as GTP or GDP[3]. Which component in the microtubular protein is effective for activation of ATPase activity of 30-S dynein is a very significant but still unsolved problem.

Any investigation on interaction between dyneins and microtubules will assist in the elucidation of the mechanochemical role of the proteins in ciliary movement.

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