Nucleotide-metabolizing Enzymes in *Chlamydomonas* Flagella

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Nucleotides have at least two functions in eukaryotic cilia and flagella. ATP, originating in the cells, is utilized for motility by energy-transducing protein(s) called dynein, and the binding of guanine nucleotides to tubulin, and probably certain transformations of the bound nucleotides, are prerequisites for the assembly of microtubules. Besides dynein, which can be solubilized from *Chlamydomonas* flagella as a heterogeneous, Mg\(^{2+}\)- or Ca\(^{2+}\)-activated ATPase, we have purified and characterized five other flagellar enzymes involved in nucleotide transformations. A homogeneous, low molecular weight, Ca\(^{2+}\)-specific adenosine triphosphatase was isolated, which was inhibited by Mg\(^{2+}\) and was not specific for ATP. This enzyme was not formed by treating purified dynein with proteases. It was absent from extracts of *Tetrahymena* cilia. Its function might be an auxiliary energy transducer, or in steering or tactic responses. Two species of adenylate kinase were isolated, one of which was much elevated in regenerating flagella; the latter was also present in cell bodies. A large part of flagellar nucleoside diphosphokinase activity could not be solubilized. Two soluble enzyme species were identified, one of which was also present in cell bodies. Since these enzymes are of interest because they might function in microtubule assembly, we studied the extent to which brain nucleoside diphosphokinase co-polymerizes with tubulin purified by repeated cycles of polymerization. Arginine kinase was not detected in *Chlamydomonas* flagellar extracts.

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Nucleotides and nucleotide-metabolizing enzymes are important in at least two kinds of function in eukaryotic cilia and flagella. Flagellar microtubules are assembled from a 110,000 dalton dimeric protein subunit, composed of two similar monomers (α and β tubulin), which binds guanine nucleotides. The first study of intact outer doublets from sea urchin sperm reported the presence of about 1 GTP and 1 GDP per subunit (1). Recently cytoplasmic singlet microtubules polymerized in vitro from brain tubulin have been reported to contain either 1 GTP and 1 GDP (2) or 2 mol of GDP (3) per 110,000 g of protein.

ATP generated in the cell body diffuses into flagella and is utilized for motility by an energy-transducing enzyme(s) (dynein) localized in arms of the outer doublet microtubules (4). Dynein can be solubilized as a heterogeneous, high molecular weight ATPase by low ionic strength dialysis, a procedure we have used in all the work reported here, and which dissolves not only the arms but most other ultrastructural elements except the membrane, outer doublets, and portions of the radial spokes. Soluble dynein ATPase is quite specific for ATP and is activated by calcium nearly as well as by magnesium. Nothing is known about the structure of the native protein(s), nor is it certain that all of the dynein is confined to the outer doublet arms (5, 6).

We have previously reported (7) that *Chlamydomonas* flagella contain, in addition to dynein, a distinct low molecular weight, calcium-specific ATPase. No enzyme of this type has so far been described in other eukaryotic cilia or flagella, and we confirm here its absence from *Tetrahymena* cilia. ATPase assays of glycerinated *Euglena* flagella have been reported to show higher activity with calcium than with magnesium (8), but this could be due to a functionally altered dynein; indeed, we confirm here that *Chlamydomonas* dynein shows this behavior with certain batches of ATP. The 3s-CaATPase might be a second energy transducer, or have an unspecified membrane function or a role in steering or tactic responses. We report here some further properties of the enzyme and attempts to determine where it is located in the flagella.

Axonemal adenylate kinase activity, reported by several investigators since it was first described in *Tetrahymena* cilia (9), functions to potentiate the utilization of ATP for motility. We have previously reported (7) that *Chlamydomonas* flagella contain two species of adenylate kinase, and that the activity of one of these was very high in newly regenerating flagella and appeared to reside in a protein also having (10) nucleoside diphosphokinase activity (Equation 1), a circumstance which would be quite unprecedented.

\[
\text{ADP} + \text{GTP} \rightleftharpoons \text{ATP} + \text{GDP} \quad (1)
\]

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\[
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polymerize by accretion at the flagellar tips (19), and a special kind of nonciliary diphosphokinase reaction may be involved in this polymerization (4, 13). Tubulin dimer from brain binds guanine nucleotides at two sites, one exchangeable (E) and one not (N), and GDP bound to the N site can be transphosphorylated in a reaction analogous to Equation 1. It has not been established that this transphosphorylation

\[
\text{GDP-N-dimer-E-GTP} \rightarrow \text{GDP-N-dimer-E-GTP} + \text{ATP} \text{ (or GTP) } \rightarrow \text{GTP.}
\]

is a prerequisite for, or that both triphosphates are hydrolyzed during polymerization. It is also not known whether the unidentified enzyme catalyzing the transphosphorylation is tubulin itself, or whether it can alternatively use free GDP, as in Equation 1.

We have now found that the previously described flagellar tubulin diphosphokinase can, in fact, be separated from adenylate kinase, does not increase during flagellar regeneration, and is probably primarily a cell body enzyme. However, flagella do contain another species of nonciliary diphosphokinase not shared with the cell bodies, and therefore somewhat more likely to have a specific flagellar function such as the transphosphorylation shown in Equation 2.

**EXPERIMENTAL PROCEDURES**

**Methods**

Organisms—*Chlamydomonas reinhardtii* wild type +137c was obtained from Dr. R. P. Levine (Harvard University); +137c(2) was a wild type with the “brevi” mutation (20) but from a different source, Dr. N. Gillham (Duke University); -pf 18, a wild type with the “short flagellum” mutation (21) was obtained from Dr. W. Ebersold (University of California at Los Angeles). Plus and minus refer to mating type. *Tetrahymena pyriformis* strain W was obtained from American Type Culture Collection, No. 16542.

Culture Conditions and Isolation of Flagella or Cilia—Flagellated and ciliated cells were grown in synchronous culture as previously described (7, 14). Since during the course of harvesting the cells a DeLaval model 102 cream separator about one-half the flagella became detached (7), flagella were allowed to regenerate for 90 min by keeping a dense suspension of harvested cells, in 10 mM Tris-HCl, pH 7.9, at 100 rpm on a rotary shaker at 25°. This yielded 85 to 90% normal biflagellate cells. Both -pf 18 and +137c(2) failed to hatch synchronously, so that when harvested almost all the cells were in clumps of four to eight, unless otherwise specified. Cell pellets derived from 18 liters of culture medium were suspended in 100 ml of 5% sucrose in 10 mM Tris-HCl, pH 7.9, and the flagella were detached by the “pH shock” procedure of Witman et al. (16). Flagella and cell bodies were then separated by centrifugation; the flagella accumulate at and above the 25% sucrose (16). Flagella were then pelleted by high speed centrifugation (16), suspended in the Tris/sucrose buffer, and stored at 0° with an International rotor 269 for 5 min at 1,900 x g to remove residual cell bodies. The supernatant was centrifuged at 0° with a Sorvall SS-34 rotor for 20 min at 17,000 x g. The ciliary pellet, nearly free of cell bodies, bacteria, and other particles, was suspended in 5 ml of the above buffer and stored at 0°.

**Extraction of Enzymes**—Unless otherwise specified, flagella were extracted by the “low ionic strength dialysis” procedure (17) on the same day that they were isolated. Usually a flagellar suspension was dialyzed overnight at 4° against two or three changes of 200 volumes of 1 mM Tris-HCl, pH 8.3, containing 0.1 mM each of EDTA and diithiothreitol. Before use, the dialysis tubing was heated to the boiling point twice in 1 mM EDTA. The suspension, which was considerably less turbid after dialysis, was centrifuged at 0° in a 65 Ti rotor. Beckman L2-65B centrifuge, for 45 min at 105,000 x g. The precipitate was suspended in dialysis buffer and stored at 0° along with the supernatant. *Chlamydomonas* cell bodies were extracted by the same dialysis procedure. To extract *Tetrahymena* cilia the dialysis solution was supplemented with 5 mM KCl, and the centrifugation was for 30 min at 55,000 x g.

**Enzyme Assay**—All assays were done at 30°. A unit is defined as the amount of enzyme catalyzing the formation of 1 pmol of product in 1 min, and specific activity is units/mg of protein. Protein was determined by the Lowry method (18), or by absorbance at 280 nm for some column elutates. The fluorescamine method (19) was used for some very dilute solutions of purified protein in the absence of Tris buffer. Ultrafiltration was used to concentrate dilute protein using either Schleicher and Schuell No. 100 collodion bags with the vacuum from a Shimadzu ASW-65 pump, or Diaflo UM 20E filter discs (Amicon) with a pressure of 10 p.s.i. of nitrogen.

**ATPase**—This activity was assayed according to Gibbons (9) in 1-ml final volume containing enzyme extract and 27 mM Tris-HCl, pH 7.9, 1.2 mM MgCl₂ or CaCl₂, 1 mM ATP, and 0.15 mM EDTA. Reactions were started by adding ATP, and stopped after 20 min by adding 0.15 ml of 5 N H₂SO₄. Orthophosphate formation was determined by measuring the absorbance at 660 nm 15 min after adding 0.15 ml of 2.5% ammonium molybdate and 0.2 ml of freshly dissolved (1.9 mg/ml) reducing agent (20).

**Adenylate Kinase**—Adenylate kinase was routinely assayed by coupling the formation of ATP from ADP to TPN reduction in the presence of hexokinase and glucose-6-P dehydrogenase. The assay cuvettes contained, in 1-ml final volume, 55 mM of Tris-HCl, pH 7.9, 40 mM of glucose, 2 mM of MgCl₂, 1 mM of ADP, 0.18 mM of TPN, 1 unit of hexokinase, and 0.5 unit of glucose-6-P dehydrogenase. The mixtures were preincubated for 4 min to consume any ATP contaminating the ADP. When the absorbance at 340 nm became constant, the reaction was started by adding the enzyme fraction, and followed by recording the increase in absorbance with a Cary 16 spectrophotometer equipped with a thermostatted automatic sample changer and accessories for individual adjustment of scale expansion and zero suppression for each cuvette.

An alternative radioisotope assay was done with reaction mixtures containing, in 0.1-ml final volume, 10 mM of Tris-HCl, pH 7.9, 0.1 mM of MgCl₂, and 0.2 mM of [8-¹⁴C]ADP (0.03 μCi). The reaction was started by adding the enzyme fraction; after 30 min the tubes were placed in a boiling water bath, then cooled, and the samples were spotted on Whatman DE 81 paper together with 0.1 μl each of carrier ATP, ADP, and AMP. The nucleotides were separated by
Chlamydomonas Flagella

were from Calbiochem; pyruvate kinase was from Calbiochem and
mM EDTA. The column was eluted with a gradient generated from
potassium phosphate, pH 6.9, containing 2 mM dithiothreitol and 0.1
collected. The KC1 concentration in eluates was measured with a
collected. The KC1 concentration in eluates was measured with a
internal molecular weight standards. The gradients were centrifuged at
by adding 10 μl of 60% HClO4, followed by 10 μl of 8 N KOH, and after
be due to nucleoside triphosphatase activity and was subtracted from
for 4 min without either flagellar enzyme fraction or TDP, until GDP contaminating the GTP was consumed and there was no further decline in absorbance at 340 nm. Enzyme fraction was added next; any further decrease in absorbance was considered to be due to nucleoside triphosphatase activity and was subtracted from the final absorbance change. The nucleoside diphosphokinase reaction was then started by the final addition of TDP. It was also necessary to subtract the absorbance change in a companion cuvette to which TDP was added without flagellar enzyme, since some batches of TDP were contaminated with other nucleoside diphosphate(s) capable of being substrates for pyruvate kinase.

An alternative radioisotope assay was done with reaction mixtures containing, in 0.1 ml final volume: 5 μmol of Tris-HCl, pH 7.9, 0.5 μmol of MgSO4, 0.2 μmol of ATP, and 0.2 μmol of [U-14C]GDP (0.00 μCl). A companion reaction mixture without ATP was run as a blank for each assay. The reactions were started by adding enzyme, and the assay procedure was the same as for adenylate kinase except that the chromatographic carriers were GTP, GDP, and GMP, and the solvent was 200 mM ammonium formate + 5 mM EDTA, pH 3.1.

For nucleoside diphosphokinase assays of fractions from brain the radioisotope assay was modified as follows. The reactions were stopped by adding 10 μl of 60% HClO4 followed by 10 μl of 8 M KOH, and after the precipitate had settled, 10 μl of the supernatant were spotted on a polyethyleneimine cellulose thin layer plate (Polygram Cel 300 PEI, Brinkmann Instruments, Inc.). The plates were developed with 0.75 M potassium phosphate, pH 3.4 (21).

In all assays the reactions were shown to be proportional to reaction time and protein concentration.

**Enzyme Fractionation**

**Sucrose Density Gradient Centrifugation**—For analytical sucrose density centrifugation (22) 5 ml of a linear gradient from 5 to 20% sucrose was prepared in 5 mM Tris-HCl, pH 7.9, 5 mM KCl, 0.5 mM EDTA, and 0.1 mM dithiothreitol. Samples of 0.1 to 0.2 ml containing 200 to 400 μg of flagellar protein, were layered on top of the gradient together with catalase (11.2 s) and/or malic dehydrogenase (4.0 s) as internal molecular weight standards. The gradients were centrifuged at 180,000 × g for 5 hr in a Beckman SW 25.1 rotor at 25,000 rpm. Fractions of 11 drops were collected manually from a hole punched in the bottom of the tube.

For preparative purposes 30 ml of a similar gradient were prepared and overlayed with 1 ml of flagellar extract. Centrifugation was for 42 hours at 180,000 × g in a Beckman SW 26.1 rotor at 35,000 rpm. Fractions of 0.6 ml were collected with a Buccher Densi-Flow.

**DEAE-Sephadex Column Chromatography**—In a typical experiment a column (1 × 17 cm) of DEAE-Sephadex A-50 was equilibrated with 10 mM Tris-HCl, pH 7.8, containing 100 mM KCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol. Flagellar extracts or fractions were brought to the same buffer condition and applied to the column. The column was eluted with a concave KC1 gradient generated by a Technicon Autoprep, with three chambers filled with 70 ml of 0.1, 0.25, and 0.6 M KCl, respectively, all in the same buffer used to equilibrate the column. The flow rate was 10 ml/hour, and 2-ml fractions were collected. The KC1 concentration in eluates was measured with a Radiometer conductivity meter, type CDM 24.

**Hydroxyapatite Column Chromatography**—A column (1 × 7 cm) of hydroxyapatite (Bio-Gel HTP, Lot 7331) was equilibrated with 10 mM potassium phosphate, pH 6.9, containing 2 mM dithiothreitol and 0.1 mM EDTA. The column was eluted with a gradient generated from three chambers containing 40 ml of 0.91, 0.95, and 0.25 mM potassium phosphate, pH 6.9, respectively, containing EDTA and dithiothreitol as above. The flow rate was 15 ml/hour, and 2-ml fractions were collected.

**Materials**

Hexokinase, glucose-6-P dehydrogenase, and lactic dehydrogenase were from Calbiochem; pyruvate kinase was from Calbiochem and Sigma; trypsin and a-chymotrypsin were from Worthington; papain was from Mann. Radioactive nucleoside diphosphates were from Amersham/Seearle. Other nucleotides were from P-L Biochemicals; samples of ATP were obtained from Sigma and Schwarz/Mann, and of GTP from Calbiochem. Triton X-100 was from K and K or New England Nuclear; Sarkosyl was from K and K; Non Iden P-40 was from Shell Oil Co. Tris (base) and sucrose were Schwarz/Mann ultrapure.

**RESULTS**

**Enzyme Activities in Flagellar Extracts and Whole Flagella**—Table I summarizes the enzyme activities of whole flagella isolated by different methods from Chlamydomonas wild type +137c, and the activities in supernatant and particulate fractions after extraction by overnight dialysis. The results are expressed as total flagellar protein per 4 × 10^12 cells, and total specific activities of Mg^{2+}- and Ca^{2+}-activated ATPase, adenylate kinase, and nucleoside diphosphokinase. Except for adenylate kinase, the sum of activities in extract supernatant and pellet roughly equalled the activity in whole flagella, indicating that the enzymes were not cryptic or latent in the flagella. The apparent low activity of flagellar adenylate kinase was due to instability, and was not observed when the flagella were suspended in buffer containing dithiothreitol; Table I (Line 5) also shows that dynein ATPase activity in whole flagella was reduced in the presence of dithiothreitol. From the nature of the enzyme assays it is clear that during the procedure for isolating whole flagella the flagellar membrane must have been sufficiently damaged to become freely permeable to nucleotides.

**Dynemin ATPase** is characteristically activated slightly less well by calcium than by magnesium. The results with Tetrahymena (Table I) show this characteristic, and suggest that all the ATPase in the ciliary preparations could be attributed to dynemin. However, in all Chlamydomonas preparations the ATPase was activated at least twice as well by Ca^{2+} as by Mg^{2+}.

The extent to which flagellar enzymes were solubilized depended on the method by which they had been isolated. Extraction of pH shock flagella solubilized 90% of the ATPases and adenylate kinase, but only 80% and 60%, respectively, was solubilized from flagella isolated by the STEEP + Ca procedure (Table I). Much less nucleoside diphosphokinase was solubilized, especially in Tetrahymena cilia.

Since an arginine kinase had been described in Tetrahymena cilia (23), we also assayed for this enzyme in Chlamydomonas flagellar extracts. Addition of arginine to reaction mixtures did not yield any increase in phosphate liberated from ATP after heating for 1 min at 100° at pH 2.5 (conditions that would release orthophosphate from phosphoarginine). We did not repeat the experiment in Tetrahymena.

To determine the optimum conditions for solubilizing Chlamydomonas flagellar enzymes, we varied the dialysis time and pH and ionic strength of the extracting solution. Ca^{2+}-ATPase was solubilized more quickly than the other enzymes, and a 15-hour dialysis was required for maximal solubilization of all enzymes. When the pH of the dialysis buffer was varied from 6.4 to 9.1, the percentage of both ATPase activities solubilized increased progressively with increasing pH, but since adenylate kinase was inactivated above pH 8.6, we chose 8.3 for routine extraction. The percentage of all enzymes solubilized (nucleoside diphosphokinase was not tested) decreased progressively on addition of KCl above 4 mM; 50 mM KCl decreased the solubilization of protein and adenylate kinase by 40% and of both ATPases by 75%. EDTA and
Results are based on the amounts of flagella obtained from about $4 	imes 10^9$ cells of *Chlamydomonas* or of cilia from $2 	imes 10^9$ cells of *Tetrahymena*. Extracts were obtained by dialysis of flagella, suspended in the buffer indicated, against Buffer A (1 mM Tris HCl, pH 8.3, containing 0.1 mM each of EDTA and dithiothreitol) as described under the "Methods"; in the case of *Tetrahymena* cilia this was supplemented with 5 mM KCl. Extracts were divided into supernatant and pellet fractions by centrifuging for 45 min at $105,000 \times g$. Total enzyme units are expressed as micromoles of reaction product formed per minute, and specific activities as units/mg of protein.

TABLE I

<table>
<thead>
<tr>
<th>Organism</th>
<th>Buffer</th>
<th>Flagellar (or ciliary) fraction</th>
<th>ATPase</th>
<th>Adenylate kinase</th>
<th>Nucleoside diphosphokinase</th>
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<tr>
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<td>STEEP+Ca</td>
<td>Whole Flagella</td>
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<td>Specific Total activity units</td>
<td>Specific Total activity units</td>
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<td>Mg-activated</td>
<td>Ca++-activated</td>
<td>Mg-activated</td>
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<td>+2-activated</td>
<td>Specific Total activity units</td>
<td>Specific Total activity units</td>
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</table>

a Ten millimolar Tris HCl, pH 7.9, containing 5% sucrose.

b Same as a plus 0.1 mM dithiothreitol.

c Thirty millimolar Tris HCl, pH 8.0, 6.8% sucrose, 25 mM KCl, 2.5 mM MgSO4, and 1 mM β-mercaptoethanol.

dithiothreitol were included in the dialysis buffer although their omission had no immediate effect; however, adenylate kinase was unstable to storage if dithiothreitol was omitted.

Enzyme Fractionation—Sucrose density gradient centrifugation of the supernatant fraction of *Chlamydomonas* flagellar extracts showed the presence of at least six enzyme species (Fig. 1). The high level of Ca++-activated ATPase in the extract was clearly due to the presence of a low molecular weight enzyme quite distinct from dynein and completely inactive in the presence of Mg++. This 35-CaATPase (32,000 daltons) appeared monodisperse, in contrast to dynein which appeared as two components of 12s (260,000 daltons) and 18s (500,000 daltons), and adenylate kinase with a peak of activity at 4.3s (55,000 daltons) and a shoulder at about 2s (20,000 daltons). These values were calculated from the mobility of catalase (Fig. 1), and the molecular weights are very rough approximations assuming spherical molecules (22). In earlier experiments flagellar nucleoside diphosphokinase appeared to be a single 4.3s species (Fig. 1). The same pattern was observed after sucrose density centrifugation of a flagellar extract from another wild type, +137c(2) (Table I). The fact that flagellar nucleoside diphosphokinase was coincident with 4.3s adenylate kinase was intriguing because we had found the latter to be elevated during flagellar regeneration (7) and, as noted in the introduction, nucleoside diphosphokinase might have a role in microtubule assembly.

DEAE-Sephadex column chromatography of flagellar extract also failed to separate these two activities, which eluted

![Fig. 1. Distribution of enzymes in fractions obtained by sucrose density gradient centrifugation of a crude extract of *Chlamydomonas* flagella: Mg++-activated ATPase (1), Ca++-activated ATPase (2), adenylate kinase (3), nucleoside diphosphokinase (4). Enzyme activities are plotted as units per fraction that would have been observed if 1 mg of extract protein had been applied to the gradient. The arrow indicates the peak tube for catalase, added to the gradient together with the flagellar extract. DeoxyGDP was used as phosphoryl acceptor for nucleoside diphosphokinase in this experiment, which gives twice the activity observed with TDP. TDP was used in all other experiments.](http://www.jbc.org/)

![Fraction Number](http://www.jbc.org/)
together at a KCl concentration of 150 mM (Fig. 2). The adenylate kinase in these fractions was shown to be the 4.3s species by applying the concentrated pooled fractions to a sucrose gradient, and likewise the second peak of adenylate kinase eluted by 500 mM KCl (Fig. 2) was shown to be the 2s species. These species will be designated 2s(500)adenylate kinase and 4.3s(150)adenylate kinase, the numbers in parentheses being the mM KCl concentration at which they were eluted from DEAE-Sephadex.

DEAE-Sephadex did not completely separate the other enzymes from dynein, present at two very low peaks in Fig. 2 (dynein activity is unstable and recoveries were variable in purified fractions). All the other enzymes could be separated completely from dynein by taking the light fraction from a preparative sucrose gradient and applying it to DEAE-Sephadex. The same procedure for the heavy fraction yielded dynein, which eluted in two very distinct peaks with 190 and 240 mM KCl, respectively, free of the other enzymes. When these fractions were concentrated and reapplied to analytical sucrose gradients they both showed only 12s dynein, but only a trace of the activity of the 240 mM peak remained. Therefore, we could not correlate the two sucrose gradient components of dynein with the two from DEAE-Sephadex.

The 4.3s(150)adenylate kinase and 4.3s(150)nucleoside diphosphokinase were finally separated by applying the concentrated fractions from DEAE-Sephadex (Fig. 2) to a hydroxyapatite column and eluting with a gradient of potassium phosphate (Fig. 3). By these two steps, the nucleoside diphosphokinase species was recovered in a 35% yield with specific activity increased from 1.8 to 220 μmol/min/mg. The protein was too dilute to determine the specific activity of the adenylate kinase; the yield was 20%. Fig. 4 confirms the result reported previously (7) that 4.3s adenylate kinase is much elevated in newly regenerating (upper) as compared with normally generated (lower) flagella. Clearly the nucleoside diphosphokinase did not increase in parallel with adenylate kinase. Fig. 4 also shows a minor second 2s component of nucleoside diphosphokinase not seen in earlier experiments.

Properties of ATPases—The 3s-CaATPase was stable for months at 0° or -20°, but dynein was unstable to storage and was further inactivated by freezing.

The 3s-CaATPase was markedly dependent on the concentration ratio of Ca²⁺ to ATP; activity was optimal with Ca²⁺ concentrations 1 to 3 times that of ATP (Fig. 5). On both sides of this range, the activity dropped sharply and was zero with Ca²⁺ concentration one third of ATP. This ATPase was not activated by Mg²⁺ and was distinctly inhibited when Mg²⁺ was added in addition to Ca²⁺ (Fig. 6). Dynein ATPase, as

![Fig. 2. Separation of enzymes in Chlamydomonas flagellar extract by gradient elution from a DEAE-Sephadex column. Mg²⁺-activated ATPase (●), Ca²⁺-activated ATPase (▲), adenylate kinase (○), nucleoside diphosphokinase (▲).](#)

![Fig. 3. Separation of the 4.3s species of adenylate kinase and nucleoside diphosphokinase by gradient elution from a hydroxyapatite column, after DEAE-Sephadex chromatography. Adenylate kinase (○), nucleoside diphosphokinase (▲).](#)

![Fig. 4. Sucrose density gradient centrifugation of extracts of Chlamydomonas flagella which had newly regenerated for 90 min (upper) or had been formed normally in the cell cycle (lower). Mg²⁺-activated ATPase (●), Ca²⁺-activated ATPase (▲), adenylate kinase (○), nucleoside diphosphokinase (▲).](#)

![Fig. 5. Rate of ATP hydrolysis by 3s-CaATPase as a function of Ca²⁺ concentration.](#)
is the case with this enzyme from other sources (9), was activated by Ca\(^{2+}\) about 80% as well as by Mg\(^{2+}\), and the activity was much less sensitive to the ratio of either bivalent metal to ATP concentration (Fig. 7). Activity was maximal with a ratio of about 3:1; at high metal concentrations, activation by Ca\(^{2+}\) declined sharply and selectively (Fig. 7). Some time ago, Gibbons (9) reported that with certain batches of ATP a 30s preparation of dynein from *Tetrahymena* cilia was activated normally by Ca\(^{2+}\) but much less well by Mg\(^{2+}\). We have also noted very similar results with one commercial batch of ATP, Sigma highest purity (No. A3127) from equine muscle. *Chlamydomonas* dynein was activated normally by Ca\(^{2+}\) with this ATP, but activation by Mg\(^{2+}\) was only 15% of that by Ca\(^{2+}\). Since the "inhibitory" ATP had a low Ca\(^{2+}\) content, it seemed possible that traces of Ca\(^{2+}\) were required for the Mg\(^{2+}\) activation of dynein. Preliminary experiments indicate this is not the case.

The 3s-CaATPase showed a single broad pH optimum (Fig. 8), while dynein showed two pH optima at 6 and 9 (Fig. 8) as previously reported (9, 24). Increasing ionic strength progressively inhibited 3s-CaATPase, whereas KCl had complex effects on dynein ATPase, depending on whether it was activated by Mg\(^{2+}\) or Ca\(^{2+}\) (Fig. 9). \(K_m\) values for ATP were determined by varying ATP concentration in the presence of 1.2 mM Mg\(^{2+}\) or Ca\(^{2+}\); the values were 2 \times 10^{-4} \text{ M} for dynein, activated by either Mg\(^{2+}\) or Ca\(^{2+}\), and 4 \times 10^{-4} \text{ M} for 3s-CaATPase. The dynein \(K_m\) for ATP has been reported to vary greatly with pH (24).

The 3s-CaATPase was not specific for ATP, hydrolyzing other nucleoside triphosphates half as rapidly (Table II). Adenosine tetraphosphate was a poor substrate. Mg\(^{2+}\)-activated dynein was very specific for ATP (9), but Ca\(^{2+}\)-activated dynein was less so (Table II).

**Nature and Function of 3s-CaATPase**—The chloroplast coupling factor is a latent CaATPase. We have described several experiments indicating that the presence of 3s-CaATPase is not due to contamination of flagella with chloroplast fragments (7). The enzyme is not inhibited by oligomycin or ouabain (7), and does not resemble known calcium transport ATPases, which typically require a combination of Mg\(^{2+}\) plus trace amounts of Ca\(^{2+}\) (25, 26). Is it a proteolytic fragment of something else? Myosin has not been reported in flagella, and conditions which activate ATPase in muscle myosin fragments were ineffective with 3s-CaATPase (7). In preliminary experiments we have treated dynein, isolated from a Bio-Gel A-15m
Diphosphokinase-To study the kinetic properties of the three enzyme species which had so far been separated from each other and from ATPases as described above, radioisotope assays were used which measured the rate of formation of AMP and ATP from [\(^{32}\)P]ADP or of GTP from [\(^{32}\)P]GDP + ADP. All reactions showed the expected stoichiometry, and in comparing different enzyme preparations the rates were always proportional to those observed with the coupled enzyme assays. Neither species of adenylate kinase catalyzed any reaction with GDP. Nucleoside diphosphokinase was found able to utilize a wide range of nucleoside triphosphate substrates, like the enzyme from various animal tissues, but appeared relatively more specific for purine nucleotides, whereas UTP is often the best substrate for the animal enzymes (27, 28).

Kinetic constants were determined with the coupled enzyme assays. \(K_m\) values for ADP were 2.1 and 0.7 mM, respectively, for the 2s(500) and 4.3s(150) species of adenylate kinase. For the nucleoside diphosphokinase reaction, parallel families of lines were obtained when reciprocals of initial velocity were plotted against reciprocals of different concentrations of one substrate in the presence of several fixed concentrations of the other, indicating the formation of phosphoenzyme intermediate (29), as has been demonstrated for this enzyme from other sources (30). \(K_m\) values (0.17 and 0.39 mM, respectively, for GTP and TDP) were obtained by reploting the intercepts at the ordinates against reciprocals of fixed concentration of one or the other substrate.

**Localization of Flagellar Enzymes**—Dynein is present in the arms attached to the outer doublet microtubules, although there is still some uncertainty whether all of it is in this location (5, 9). An indirect approach to the function of the 3s-CaATPase is to determine where it is located.

One method of studying this is to see if the enzyme is absent or modified in mutants with specific structural derangements. We have examined one such mutant which is paralyzed and lacks the two central microtubules: -pf 18 was found to have no differences from wild type in the flagellar content of dynein, 3s CaATPase, and nucleoside diphosphokinase. The level of adenylate kinase was somewhat low in -pf 18 (Table I); sucrose gradient centrifugation of flagellar extracts showed this was due to diminution of the 4.3s species, which was expected since cells of this strain hatched only after harvesting and none of the flagella were newly regenerated (see under "Methods"). There are, however, at least three other genes, mutations in which also result in the "9 + 0" phenotype (31). In current experiments Dr. K. Summers has found that some characteristics of the 3s-CaATPase appear to be modified in flagella of +pf 15.

A second approach is to fractionate flagella by detergent treatment and see whether the enzymes are enriched in certain fractions. A difficulty has been that isolated flagella have been rather fragile or leaky in our experience. Flagella isolated by the STEEP + Ca procedure are less so, but even in this case brief washing solubilized some of each enzyme, and repeated washing continued to do so (Table III), indicating that this was not solely due to removal of contaminating soluble cell material. All the enzymes were largely solubilized when flagella isolated by pH shock were washed with Tris/sucrose; K. Summers and M. Flavin, unpublished results. Flagellar dynein was comparable to that of wild type, but in some extracts the levels of 3s-CaATPase, as well as both species of adenylate kinase, were reduced to only a few per cent of the wild type level. The absence of these enzymes might be due to increased flagellar fragility, and we cannot yet conclude that they are normally associated with central structures of the axoneme.

![Fig. 10. Sucrose density gradient centrifugation of an extract of Tetrahymena cilia. The catalase marker has moved a shorter distance into the gradient than in Fig. 1 because centrifugation was for 4 hours and the gradient was from 5 to 30% sucrose. Mg\(^{2+}\)-activated ATPase (O), Ca\(^{2+}\)-activated ATPase (D), adenylate kinase (O), nucleoside diphosphokinase (A).](http://www.jbc.org/)

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**Table II**

<table>
<thead>
<tr>
<th>Substrate*</th>
<th>Relative rate of inorganic phosphate formation</th>
<th>3s-CaATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg(^{2+})-activated dynein</td>
<td>Ca(^{2+})-activated dynein</td>
</tr>
<tr>
<td>ATP</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>ATPP</td>
<td>74</td>
<td>23</td>
</tr>
<tr>
<td>GTP</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>ITP</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>CTP</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>UTP</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

*No phosphate was liberated by either enzyme from: AMP, dAMP, GMP, IMP, CMP, or inorganic pyrophosphate.

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1. K. Summers and M. Flavin, unpublished results. Flagellar dynein was comparable to that of wild type, but in some extracts the levels of 3s-CaATPase, as well as both species of adenylate kinase, were reduced to only a few per cent of the wild type level. The absence of these enzymes might be due to increased flagellar fragility, and we cannot yet conclude that they are normally associated with central structures of the axoneme.
much of this leakiness could be eliminated by suitable modification of the washing buffer (Table III). Matrix protein would be expected to be easily lost from pH shock flagella (16), but Table II shows that dynein was solubilized as much as any other enzyme.

We attempted to determine the distribution of all of the enzyme studied, in matrix, membrane, and axoneme fractions, when the latter were isolated by the "STEEP minus Ca rinse" procedure, or by partial extractions with Triton X-100, Sarkosyl, or Non Idet P-40 (16). Unfortunately none of these procedures gave satisfactory results in our hands. The only conclusion that might be suggested by these experiments is that, since protein was solubilized relatively more than any of the enzymes, perhaps none of the latter is truly a matrix component.

Flagellar and Cellular Species of Adenylate Kinase and Nucleoside Diphosphokinase—Table IV shows the distribution of enzyme activities in flagella and cell body fractions prepared from the same total number of cells. Since we wanted to know whether any "flagellar" enzymes might have originated from contaminating cell bodies, we extracted the latter in the same way as flagella, although this would not otherwise have been the method of choice. Although both ATPase activities appeared to be present in cell bodies in total amounts comparable to those in flagella, the specific activities were extremely low, and we cannot actually be sure whether these activities are present in cell bodies at all. The supernatant from which flagella were originally pelleted was also assayed, after it had been concentrated; it contained only traces of ATPase (Table IV). The ATPases were clearly of flagellar origin.

This was not as clear for adenylate kinase and nucleoside diphosphokinase, since the amounts in cell bodies were 5 to 50 times greater than in flagella (Table IV). The activities in the flagellar supernatant were also high, especially of nucleoside diphosphokinase, suggesting that there might have been sufficient cell lysis during flagellar isolation to cause significant contamination of the latter with cell body enzymes. It was now necessary to determine whether the enzyme species we had so

<table>
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<th>Table III</th>
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<tbody>
<tr>
<td><strong>Fragility of isolated flagella</strong></td>
</tr>
<tr>
<td>Flagella freshly isolated by the STEEP + Ca or pH shock procedures were suspended in the indicated buffers and either kept 10 min at 0° (washing), or agitated further by three or four strokes at 0° with a loose fitting type A Dounce homogenizer (Kontes Glass Co.). The suspensions were then centrifuged for 20 min at 30,000 × g, and the supernatants were assayed.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method of flagellar isolation</th>
<th>Buffer used to suspend flagella</th>
<th>Supernatant from protein</th>
<th>ATPase Mg**-activated Ca**-activated</th>
<th>Adenylate kinase</th>
<th>Nucleoside diphosphokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEEP + Ca</td>
<td>A* First wash</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second wash</td>
<td>10</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>STEEP + Ca</td>
<td>A Dounce</td>
<td>15</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>pH shock</td>
<td>A First wash</td>
<td>24</td>
<td>50</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>A Dounce</td>
<td>32</td>
<td>71</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>pH shock</td>
<td>B* First wash</td>
<td>18</td>
<td>11</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>pH shock</td>
<td>B Dounce</td>
<td>15</td>
<td>5</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

* Ten millimolar Tris-HCl, pH 7.9, containing 5% sucrose and 0.1 mM dithiothreitol.

** Ten millimolar Tris-HCl, pH 7.9 containing 5 mM MgSO_4, 1 mM dithiothreitol, and 0.5 mM EDTA.

<table>
<thead>
<tr>
<th>Table IV</th>
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</thead>
<tbody>
<tr>
<td><strong>Enzyme activities in Chlamydomonas flagella and cell bodies</strong></td>
</tr>
<tr>
<td>Cell fractions were obtained from 4 × 10^4 cells (18 liters of culture medium). Deflagellation was by the STEEP + Ca procedure. Flagella were isolated as usual from a sucrose step gradient, and after centrifuging for 20 min at 31,000 × g, both pelleted flagella and supernatant solution were assayed. Cell bodies pelleting through 25% sucrose were extracted by the same low ionic strength dialysis procedure usually used to extract flagella.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chlamydomonas fraction</th>
<th>Total Protein</th>
<th>ATPase Mg**-activated</th>
<th>Ca**-activated</th>
<th>Adenylate kinase</th>
<th>Nucleoside diphosphokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mg</strong></td>
<td>Specific activity</td>
<td>Total units</td>
<td>Specific activity</td>
<td>Total units</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Flagella</td>
<td>Whole flagella</td>
<td>15</td>
<td>0.34</td>
<td>4.9</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Supernatant from pelleting flagella</td>
<td>7.5</td>
<td>0.06</td>
<td>0.43</td>
<td>0.08</td>
</tr>
<tr>
<td>Cell bodies</td>
<td>Extract supernatant</td>
<td>460</td>
<td>0.004</td>
<td>2.0</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Extract pellet</td>
<td>910</td>
<td>0.011</td>
<td>9.8</td>
<td>0.005</td>
</tr>
</tbody>
</table>
far studied were uniquely flagellar or were also in the cell bodies, by comparing extracts of the latter with those of "well washed" flagella.

Fig. 11 shows the sucrose density centrifugation pattern of an extract of flagella which had been isolated by pH shock and then washed by gently resuspending with a Dounce homogenizer, as described in Table III, i.e. flagella from which a considerable part of the original adenylate kinase and nucleoside diphosphokinase activities had been washed away (Line 4, Table III). Comparison with unwashed flagella (Fig. 4) shows that nucleoside diphosphokinase was mostly 2s, with a smaller component of the 4.3s species. DEAE-Sephadex chromatography (Fig. 12) showed three nucleoside diphosphokinase components: the 4.3s(150)nucleoside diphosphokinase seen in unwashed flagella (Fig. 2), another eluting at 330 mM KCl, and a small shoulder at 190 mM. The latter two were presumably not detected in unwashed flagella because the 4.3s(150)nucleoside diphosphokinase activity was much higher, and assays were not made sensitive enough to detect minor species. The component eluted at 380 mM KCl was shown to correspond to the 2s species, by applying the concentrated fractions to a sucrose gradient.

Similar extracts of cell bodies, from which flagella had been removed by the STEEP + Ca method, contained only 4.3s nucleoside diphosphokinase, which was eluted in two distinct peaks, at 150 mM and 190 mM KCl, from DEAE-Sephadex. At this point one might tentatively conclude that 2s(330)nucleoside diphosphokinase is uniquely flagellar, 4.3s(150)nucleoside diphosphokinase is present in large amounts in both cell bodies and flagella, and 4.3s(190)nucleoside diphosphokinase is more conspicuous in cell bodies (it might be in a cell particle not well removed from flagella by washing).

The results with adenylate kinase are unclear because the cell body enzyme (Table IV) was mostly inactivated by either sucrose gradient or DEAE-Sephadex fractionation. Cell bodies had mostly 2s adenylate kinase, but it appeared to elute with 280 mM KCl, in contrast to the flagellar 2s(500)adenylate kinase; a trace of the flagellar 4.3s(150)adenylate kinase was also present. Washed flagella had 4.3s(150)adenylate kinase and 2s(500)adenylate kinase (Fig. 11) like unwashed (Fig. 1), but in DEAE-Sephadex fractions very small amounts eluted with 500 mM KCl (Fig. 12 compared to Fig. 2). In summary, 2s(500)adenylate kinase has been seen only in flagella. 2s(280)adenylate kinase only in cells, and 4.3s(150)adenylate kinase is prominent in flagella and possibly present in cells.

Clearly more work is needed to determine how many flagellar adenylate kinase and nucleoside diphosphokinase species there are, and which ones are shared with the cell body or are unique to flagella. There may be more species than we described, and could be fewer if some are aggregates. The nonextractable moieties, especially of nucleoside diphosphokinase, may or may not be distinct species.

Copolymerization of Brain Nucleoside Diphosphokinase with Tubulin 4—As noted in the introduction, transphosphorylation of a tubulin-bound GDP, by ATP (Step 2, Equation 2), may be a prerequisite for polymerization. This reaction may be catalyzed by tubulin itself or by another protein; in the latter case the protein must copolymerize when tubulin is purified by cycles of assembly and disassembly. We do not know whether the enzyme catalyzing this reaction can utilize free GDP (Equation 1). We purified bovine brain tubulin by the glycerol assembly procedure (35), and assayed fractions for the ability to catalyze Reaction 1. The tubulin was purified by two cycles of assembly with an intervening "wash" step: pelleted microtubules from the first assembly were stored overnight at −20°C, the cold-disassembled tubulin was rewarmed without a cold spin, and the reassembled microtubules were used for the second cycle.

The nucleoside diphosphokinase specific activity of brain extract was 0.029 (3% of that of Chlamydomonas cells or flagella). This activity was extensively separated from tubulin (30) at each step of the purification: specific activity 0.011 in the first pellet of microtubules, 0.0044 in washed microtubules, 0.0029 in the disassembled tubulin, and 0.0014 in the second pellet of microtubules. Thus there was no evidence for a residual moity that polymerized together with tubulin. However, the residual specific activity, 1.4 nmol/min/mg, actually

![Fig. 11. Sucrose density gradient centrifugation of an extract of Chlamydomonas flagella, obtained after the flagella had first been washed by agitation with a Dounce homogenizer as shown in Table III. Enzyme activities are plotted as units per fraction that would have been observed if flagellar extract from 4 x 10^10 cells had been applied to the gradient. Mg^2+-activated ATPase (O), Ca^2+-activated ATPase (C), adenylate kinase (O), nucleoside diphosphokinase (A), malic dehydrogenase added as a reference marker (■).](http://www.jbc.org/content/330/1/190/F1.large.jpg)
specific for ATP. It was not formed after treating purified

The substrate specificity was compared for nucleoside
diphosphokinase of crude extract with that of the second
assembled microtubules, the latter showed a slightly greater
preference for purine nucleoside triphosphates.

**DISCUSSION**

Nucleotides have at least two functions in eukaryotic cilia
and flagella. Free ATP formed in the cells diffuses into the
flagella where it is utilized for motility by the action of dynein,
an energy-transducing protein or proteins in the arms attached
to the outer doublet microtubules. In addition, guanine nucleo-
tides are present, noncovalently bound at two sites on mi-
crotubule proteins. only one of which can exchange with free
nucleotide. There is some evidence, but no proof, that both
sites must be occupied by GTP in the microtubule subunit
before polymerization can occur; at the nonexchangable site
this is brought about by a transphosphorylation of GDP at the
expense of free ATP or GTP. The question of whether both
triphosphates are then hydrolyzed to GDP during polymeriza-
tion has not been settled (1-3). In any case, all components
needed for polymerization must be present in regenerating
flagella, since the microtubules grow by accretion at the tips
(12).

We have studied four enzymes involved in nucleotide trans-
formations in *Chlamydomonas flagella* and have purified, and
separated from each other and from dynein (which is solubili-
ized as a heterogeneous ATPase), a low molecular weight
Ca2+-specific ATPase, and two species each of soluble adenyl-
ate kinase and nucleoside diphosphokinase. Preliminary at-
tems have been made to determine where each of the
enzymes is localized in the flagella, by examining mutants with
structural defects or by detergent fractionation.

The low molecular weight Ca2+-specific ATPase, or 3s-
CaATPase, was homogeneous, inhibited by Mg2+, and not
specific for ATP. It was not formed after treating purified
dynein with several proteases. No enzyme of this kind had
previously been reported in flagella or cilia, and we found no
trace of it in *Tetrahymena* cilia. This species difference will be
more informative when we have surveyed other ciliates and
mono- and biflagellates. What is the function of this enzyme?
It might be a second energy transducer. During flagellar
beading there probably must be translocation of spoke heads
along the central sheath (as well as of arms along adjacent
doublets), and there is some cytological evidence for an
ATPase in the spoke heads (4). Such an ATPase should not be
Ca2+-activated in vivo, and the 3s-CaATPase would presum-
ably reflect some modification of the enzyme activity resulting
from extraction. These considerations would apply to
*Tetrahymena* also, but there are several reasons why such
spoke head energy transducer might not be extractable in the
same form in different species (4). Others have observed that
"dynein" is less extractable from cilia than from flagella, and
we have some evidence of this also (Table I).

Second, the enzyme might have an unspecified membrane
function. This is suggested only by the fact that the known
structural differences between *Tetrahymena* cilia and
*Chlamydomonas* flagella are in the membrane, the latter
having mastigonemes and gamete agglutinins (37). Third, the
enzyme might function in steering or tactic responses. Calcium
has been implicated in chemo- or phototactic responses in
many organisms (4), including *Chlamydomonas* (38). Involve-
ment of a Ca2+-activated ATPase might be suggested by the
dual requirement for Ca2+ + ATP for ciliary arrest and reversal
in *Paramecium* (39), although it has not been shown whether
the ATP is consumed under these circumstances. It would be
informative to determine whether the ATP could be replaced
by a nonhydrolyzable analogue. The species difference could be
explained in this case if the steering apparatus were confined
to the base of the cilium within the cell body in *Tetrahymena*, but
were located distally in the flagellum in *Chlamydomonas*.

The characterization of adenylate kinase and nucleoside
diphosphokinase enzymes in *Chlamydomonas* flagella was
hampered by the fact that cell bodies also contain very large
amounts of these enzymes, and further work will be needed to
be certain which enzyme species are uniquely present in
flagella, and which are shared with cell bodies or present in
flagellar preparations only due to contamination with cellular
material. The number of protein bands which have been
detected by sodium dodecyl sulfate gel electrophoresis of whole
flagella (16) suggests that cytoplasmic proteins cannot indis-
criminately penetrate the flagellar matrix, but no cytological
evidence of a barrier has been reported in *Chlamydomonas*.

One species of adenylate kinase, 2s(500)adenylate kinase,
was observed only in flagella, and a second, 4.3s(150)adenylate
kinase, seemed more conspicuous in flagella. The latter en-
zyme activity was markedly elevated in newly regenerating
flagella. We do not know why this should be so, since the only
function attributed to flagellar adenylate kinase is to potentiate the complete utilization of ATP for motility. It is possible that this species leaks out of flagella easily and that newly regenerated flagella are less fragile. It is interesting that some of the observed adenylate kinase species appear to be much larger protein molecules than is the case for this enzyme from other sources (40). After sucrose gradient centrifugation of Tetrahymena ciliary extract, adenylate kinase activity appeared in three peaks corresponding to \( s \) values of 11, 15, and 20 (Fig. 10).

A large proportion of flagellar nucleoside diphosphokinase activity was not solubilized under any conditions. One species of soluble nucleoside diphosphokinase, \( 2s(330) \) nucleoside diphosphokinase, appeared to be present only in flagella, and another, \( 4.3s(150) \) nucleoside diphosphokinase, showed high activity in both flagella and cell bodies. GTP is required for, and probably hydrolyzed during, microtubule assembly, and it seems likely that it needs to be replenished even in non-regenerating flagella, if there is some turnover of microtubule proteins (41). It GTP is not adequately replenished by diffusion from cell bodies, flagellar nucleoside diphosphokinase could function to reconstitute it from ATP and free GDP (Equation 1). Alternatively, a flagellar nucleoside diphosphokinase might function to transphosphorylate tubulin-bound GDP directly, as discussed in the introduction (Equation 2). We have not tested the second possibility directly, but we have investigated whether any enzyme catalyzing Reaction 1 copolymerizes when cytoplasmic microtubules are repeatedly polymerized in vitro. Ability to catalyze Reaction 1 was very extensively lost after repeated cycles of polymerization, but the trace of activity remaining was still comparable to the maximum observed rate of transphosphorylation of tubulin-bound GDP.

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T Watanabe and M Flavin


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