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Some Changes in the Properties of Dynein ATPase In Situ and After Extraction Following Heat Treatment of Cilia

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Glycerol-extracted cilia from Tetrahymena pyriformis were demembranated by treatment with Triton X-100 and then heated for up to 30 min at temperatures between 34–38°C. Heat treatment caused an uncoupling of the ATPase from motility as indicated by an increase in ATPase activity and a loss of pellet height response. After heat treatment, the ATPase activity of the dynein in situ differed from that in unheated cilia as shown by an increased sensitivity to a lower temperature of assay (0°C) and by a loss of the activation normally observed upon reaction with N-ethylmaleimide or p-phenylenedimaleimide. Upon extraction of the heat-treated cilia by Tris-EDTA, there was a large loss in ATPase activity so that the heat-treated cilia yielded a crude dynein fraction with a lower specific activity compared with that obtained from unheated controls. The difference was not due to a change in the amount of protein recovered or in the amount of ATPase activity which remained unextracted. Resolution of the crude dynein by sucrose density sedimentation indicated that activity was lost from both the 14S and 30S peaks but more so from the latter than from the former. Thus dynein in situ in cilia in which the ATPase has been uncoupled from motility by gentle heat treatment differs in several important respects from dynein inside unheated cilia.

Key words: cilia, dynein, N-ethylmaleimide, p-phenylenedimaleimide, uncoupling

INTRODUCTION

It is well established that bending waves in cilia and flagella are produced by a relative sliding of the outer tubules with respect to one another (1). Sliding is thought to result from a cyclic attachment and detachment of the dynein arms projecting from the A subfiber of one outer doublet onto the B subfiber of the adjacent doublet (2), so that the arms act in a manner superficially analogous to the cross bridges of striated muscle. Warner and Satir (3) suggested that there is also a cycle of detachment and reattachment of the radial spokes to projections from the central sheath which constitutes part of the mechanism for conversion of active interdoublet sliding into local bending. It is not yet certain whether an ATPase is localized at the region of the spoke head, although there are several indications in the literature that an ATPase other than 30S dynein may be present in cilia (4, 5).

Abbreviations: NEM, N-ethylmaleimide; PPDM, N,N′-p-phenylenedimaleimide; EGTA, [ethylenebis (oxyethylenenitrilo)]-tetraacetic acid

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Studies of mechanochemical coupling in flagella have shown that there are several states in which the axoneme may exist. Sperm tail axonemes that have been gently homogenized become nonmotile and dephosphorylate ATP at about one-fourth the rate of the motile axonemes (6, 7). The ATPase activity of cilia and of homogenized nonmotile axonemes can be increased by thiourea and by several extraction procedures, and this increased activity has generally been interpreted as an uncoupling of the dephosphorylation step(s) from its dependence on motility (8). Several studies have shown that the properties of dynein in situ in motile cilia differ in a number of ways from the properties of extracted dynein (4, 5, 8, 9). Similarly, the ATPase properties of extracted dynein differ in several ways from the properties of ATPase in situ in nonmotile coupled sperm tail axonemes (8, 9). In an earlier paper it was reported that heating glycerinated Tetrahymena cilia for 11 min at 40°C caused a complete loss of pellet height response but a slight enhancement of the ATPase activity, and this was interpreted as an uncoupling of the mechanochemical system (10). Since there have been no systematic studies on the properties of dynein extracted from cilia in which the ATPase had been uncoupled from motility prior to extraction, it seemed worthwhile to examine the effects of heating cilia at low temperatures on the relative amounts of 14S and 30S dynein which would be obtained on extraction. In this paper we report that extraction of dynein from such heat-treated cilia caused an appreciable loss of ATPase activity, the larger part of the loss occurring in the 30S dynein fraction. These results suggested that dynein in situ is held in a constrained configuration so that the damage caused by gentle heating was manifested as an increase in ATPase activity while the dynein remained in situ but as a loss of activity when the dynein was removed from its intra-axonemal environment. Evidence is also presented which shows that dynein in situ in heat-treated cilia differs from dynein in control cilia in its sensitivity to certain SH reagents and in its sensitivity to the temperature at which the ATPase activity is assayed, thus demonstrating that uncoupling by gentle heating causes a change in the properties of dynein in situ.

MATERIALS AND METHODS

Cilia were prepared from cultures of Tetrahymena pyriformis strain HSM as described earlier (4). The glycerinated cilia were demembranated by treatment with 0.1% Triton X-100 as described (11) in the morning of the day on which the heating experiment was to be conducted. After the demembranated cilia had been washed in IM 7.5 buffer (20 mM imidazole, 2.5 mM $MgSO_4$, pH 7.5), they were resuspended in slightly over 6 ml of buffer, warmed briefly to 25°, and then samples of 2.0 ml were pipetted into tubes containing 3.0 ml of IM 7.5 buffer at the desired temperature. After the desired time of heating, the tubes were placed in ice. (The control tubes contained 3.0 ml of ice-cold IM 7.5 buffer.) Samples were then taken from each tube for assay of ATPase activity, protein content, and pellet height response. Aliquots of 4.0 ml from each tube were also placed in dialysis sacking and dialyzed for about 22 hr at 4°C against a total of 2 liters of Tris-EDTA (1 mM Tris, 0.1 mM EDTA, pH 8.2). Following dialysis the contents of each dialysis sack were centrifuged at 0° for 30 min at 27,000 × g, and the pellet was resuspended in about 4 ml of Tris-EDTA. The supernatant (crude dynein) was recentrifuged for 15 min at 27,000 × g, and any pellet obtained was combined with the original pellet in Tris-EDTA and left at 0° for about 1.5 hr. The pellets were then centrifuged for 20 min at 27,000 × g and resuspended in 4.0 ml of IM 7.5. The supernatant of this step was saved and will be referred to as the wash. The resuspended pellet, the wash, and the original supernatant

containing the extracted dyneins were assayed for protein content and for ATPase activity. In some cases 3.0 ml of the crude dynein were layered onto 23 ml of a 5–30% (w/v) sucrose gradient prepared in IM 7.5 buffer as described elsewhere (12). After centrifugation at 23,500 rpm in an SW 25 rotor of a Spinco Model L centrifuge for 20 hr, fractions of 40 drops were collected and assayed for ATPase activity.

Cilia were also incubated with NEM or PPDM for various times prior to the addition of 0.2 ml of 5.0 mM ATP to the 0.8 ml volume of cilia plus NEM in IM 7.5 buffer. When specified, 0.088 mM EGTA was included in the reaction mixture. Both NEM and PPDM were used within 1 hr after they were dissolved. PPDM was prepared by dissolving it in warm acetone and then diluting that in IM 7.5 buffer.

ATPase activity was measured in IM 7.5 buffer essentially by the method of Taussky and Shorr (13), as described elsewhere (4). Duplicate determinations generally agreed to within 5%.

Protein was measured by the method of Lowry et al. (14), using bovine serum albumin as a standard.

The swelling response of the cilia was measured by adding ATP to a suitable aliquot of cilia and placing $\sim$ 1 ml in a Wintrobe hematocrit tube and centrifuging in a clinical centrifuge at room temperature for 3.5 min, as described in detail elsewhere (10). The pellet height ratio is defined as the height of the pellet in the tube containing ATP to that of the same amount of cilia centrifuged at the same time but without ATP.

ATP was purchased from Pabst, NEM and imidazole from Sigma, PPDM from Aldrich, EGTA from Eastman. All other chemicals were of the highest purity commercially available.

RESULTS

Table I shows the effects of heating demembranated cilia on the ATPase activity in situ and after extraction of the dyneins by Tris-EDTA. Four experiments are presented, covering a very narrow temperature range (34.2–38.4°), and we shall being by describing the features common to all of these experiments.

At each temperature in this range there was a considerable enhancement (up to $\sim$ 40%) of the axonemal ATPase activity, more than had previously been observed in cilia heated for 11 min at 40°C (10). The reason for this is that at any temperature there is first an increase in activity and then a gradual loss of activity from the peak activity with increasing time of heating. Correlated with the increase in ATPase activity was a loss of the pellet height response, as expected from our initial observation on the uncoupling of ATPase activity from the mechanochemical response. The loss of pellet height response was almost complete when the cilia were heated for 4 min at 38.4°C (expt. I, Table I) but was still incomplete after 18 min at 34.2°C (expt. IV, Table I). When control (unheated) cilia were extracted with Tris-EDTA, about 90% of the protein originally put into dialysis was recovered in the pellet, supernatant I, and wash, and about 80–90% of the original ATPase activity was accounted for. Clearly there was no appreciable loss of ATPase activity (over and above the slight loss of protein) as a result of the dialysis procedure. Since the ATPase activity of the heat-treated cilia had increased relative to the control values, one might have expected to obtain an increased ATPase activity in the crude dynein (supernatant I) fraction. Instead the specific activity of the ATPase recovered was less than that obtained from control cilia and, since the amount of protein recovered in each fraction was about the same for control and heated cilia, the total amount of ATPase activity re-

TABLE I. Distribution of Protein and of ATPase Activity in Extracts of Heat-Treated Cilia

Preparation	Temperature	Time of heating (min)	Pellet height ratio	ATPase activity in				% Initial ATPase activity recovered	Total Protein in			% Initial protein recovered
				Whole cilia	Supernatant I	Pellet	Wash		Supernatant I	Pellet	Wash	
				(μmoles P/min		mg protein)				(mg)		
		0	2.0	.171	.385	.0760	.155	84.1	1.40	4.14	0.28	93.4
I	38.4°	4	1.2	.226	.305	.0749	.131	55.4	1.46	3.97	0.29	91.8
		12	1.1	.254	.232	.0801	.101	42.7	1.43	4.04	0.21	91.2
		0		.164	.420	.0902	.0574	79.7	0.78	3.76	0.37	92.4
II	38.2°	20		.217	.302	.0784	.0351	46.6	0.74	3.87	0.23	90.9
		30		.220	.276	.0749	.0390	47.0	0.83	4.07	0.23	95.3
		0		.175	.362	.0930	.155	79.6	1.18	4.04	0.29	90.1
III	36.6°	6		.234	.358	.0937	.135	55.5	1.06	4.04	0.28	87.8
		14		.245	.270	.0930	.112	46.9	1.18	3.80	0.20	85.3
		0	2.3	.152	.485	.0855	.107	97.9	3.58	12.2	2.25	89.4
IV	34.2°	8	1.9	.183	.479	.0845	.111	79.1	3.39	12.8	2.02	90.2
		18	1.6	.202	.385	.0851	.136	66.0	3.39	14.0	1.30	92.8

Data are shown from four experiments in which cilia were heated at the indicated temperatures for the times shown. Since the temperature bath used in these experiments was held constant to within ± 0.1° only, the temperatures in experiments I and II do not differ significantly. After the heating, the tubes were chilled to 0° and samples taken for measurement of the pellet height ratio, ATPase activity, and protein content as described in Methods. Volumes of 4.0 ml were then dialyzed against Tris-EDTA and separated by centrifugation into 3 fractions designated Supernatant I, pellet, and wash, as described in Methods. The volume, ATPase activity, and protein content of each fraction was determined.

covered in supernatant I was less than that from control cilia. Since there were no appreciable changes in the specific activity or total amount of unextractable ATPase activity in the pellet fraction, and no reproducible changes in the small amount of ATPase activity in the wash fraction, it is apparent that heating the dynein in situ caused its ATPase activity to increase but that subsequent extraction of the heat-treated cilia caused a loss of the ATPase activity which not only nullified the increment in activity but reduced the activity to well below that of the control dynein.

These experiments suggested that heating demembranated cilia at low temperatures caused an appreciable change in some properties of the dynein in situ. It was possible, however, that the heat treatment caused the dynein to leak out of the cilia even during the relatively short times required for heating and for subsequent assay procedures (compared to overnight dialysis against Tris-EDTA). To check on this possibility, an experiment was performed in which duplicate aliquots were heated at 38.2° for 20 min and for 30 min. After heating, their average ATPase activities were, respectively, 182% and 190% higher than the average of the unheated controls. (This near doubling of the activity is the highest activation we have observed on heating.) Identical amounts of the heated and control cilia were then centrifuged at 12,000 × g for 10 min at 0° and the supernatants carefully decanted. The pellets were resuspended in 1.0 ml of IM 7.5 buffer, and both the supernatants and pellets were assayed for ATPase activity. The average percentages of the original activity recovered in the supernatants were 3%, 11%, and 10%, for the control, 20 min, and 30 min heated samples, respectively; 96%, 86%, and 87% of the activity present prior to centrifugation was recovered in the two fractions. An identical experiment on a different preparation of cilia yielded closely similar results: over 92% of the activity was recovered in each tube and 1.4%, 9.4%, and 10.9% of the initial activity centrifuged was recovered in the supernatant fractions of the control, 20 min, and 30 min heated samples, respectively. Thus, heating at 38.2° for up to 30 min does solubilize up to 11% of the ATPase activity of demembranated cilia. There are some indications of a loss of activity of the solubilized material, but whether because of the short time of handling after the heating or because of the presence of Mg^{++}, the amount of activity lost was much less than that observed after overnight dialysis against Tris-EDTA. Since about 90% of the ATPase activity remained with the pellet even after heating at 38.2° for 30 min (the maximum heat treatment used in any of the experiments reported in this paper), we shall, in what follows, refer to the changes in properties of the dyneins assayed in heat-treated cilia before extraction with Tris-EDTA as changes which occur in situ.

Effect of SH Reagents on the ATPase Activity of Heat-Treated Cilia

The large loss of ATPase activity which occurred upon extraction of dynein from heat-treated cilia suggested that some properties of the uncoupled dynein in situ might be different than those of control dynein in situ. Since it had been shown that there were at least two SH groups on 30S dynein which were involved in the ATPase activity and in the ability of the 30S dynein to rebind to extracted axonemes (11, 15), it seemed possible that a study of the effect of SH reagents on control and heat-treated cilia might reveal any changes in conformation that had occurred as a result of the heat treatment. Figure 1 shows that the ATPase activity of unheated cilia was slightly enhanced by brief exposure to 1.25 mM NEM, in agreement with earlier observations (16). With increasing time of exposure to the NEM in the absence of ATP, the enhancement of ATPase activity was lost and replaced by net inhibition. The ATPase activity of the cilia that had been heated for

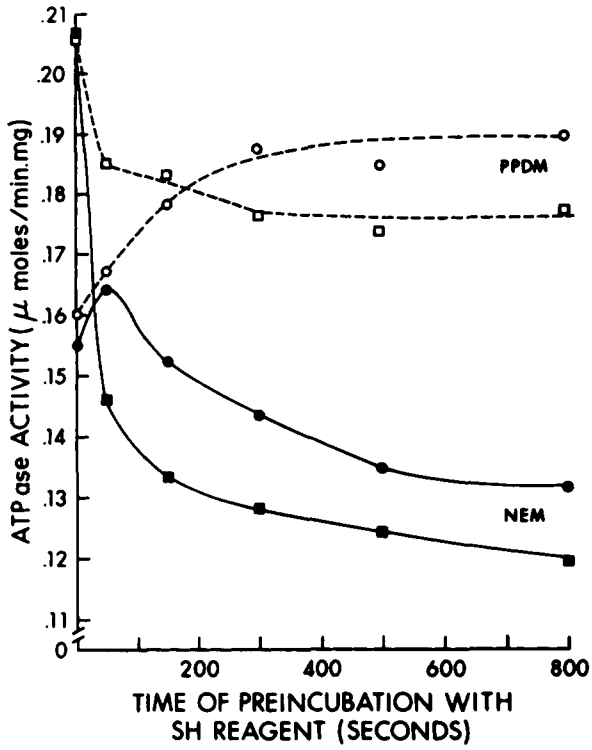

Fig. 1. Effect of NEM and of PPDM on the ATPase activity of heat-treated cilia. Demembranated glycerol-extracted cilia were heated for 20 min at 38.3°. Aliquots were then incubated at 25° in a total volume of 0.8 ml of IM 7.5 buffer containing 0.088 mM EGTA and either 1.25 mM NEM (solid symbols) or 6.25 μM PPDM (open symbols) for the times shown on the abscissa, except that the points shown at t = 0 contained neither NEM nor PPDM. The control tubes for the PPDM incubations did, however, contain 0.2 ml of a 1/20 dilution of acetone in IM 7.5 buffer, since that amount of acetone was present in the tubes containing PPDM. After preincubations with the SH reagent, 0.2 ml of 5 mM ATP was added, and the ATPase activity during a 15 min incubation was determined. Circles and squares show the results obtained with unheated and with heated cilia, respectively.

20 min at 38.3° was over 30% higher than that of the control cilia, as expected from the data shown in Table I, but when preincubated with NEM, only a very rapid decline of ATPase activity was observed. Thus the increased ATPase activity caused by gentle heating changes the reactivity of an SH group(s) of dynein in situ.

Very low concentrations of N,N′-p-phenylenedimaleimide, which has been shown by Reisler et al. (17, 18) to act as a bifunctional bridging reagent that can react with SH_1 and SH_2 of myosin, enhance the activity of 30S dynein but inhibit that of 14S dynein (Blum and Hayes, unpublished). It was therefore of interest to compare the effects of PPDM on the ATPase of control and of heat-treated cilia. Figure 1 shows that in the same preparation of cilia in which 1.25 mM NEM caused a slight enhancement of ATPase activity, 6.25 μM PPDM enhanced the activity about 20% over the course of an 800-sec preincubation. In the heat-treated cilia, however, only inhibition was observed, again indicating that the heating had caused a change in the properties of an SH group(s) on the dynein in situ.

TABLE II. ATPase Activity of Heat-treated and Control Cilia Assayed at 0° and at 25°

Preparation	% ATPase activity		Ratio of activity treated/control	
	25°	0°	25°	0°
A Control	100	14.0		
Heated	142	16.2	1.42	1.16
B Control	100	12.9		
Heated	124	13.4	1.24	1.04
C Control	100	12.4		
Heated	128	12.4	1.28	1.00
			1.31 ± 0.09	1.07 ± 0.08

Three preparations of Triton X-100 extracted cilia were heated at 38.2° for 20 min, and the ATPase activity was then assayed in duplicate (expt. A) or triplicate (expt. B and C) at 0° and at 25°. The mean values of the ATPase activity are shown as well as the mean ± S.D. of the ratio of the activities at 0° and at 25° of the heat-treated cilia to that of the control cilia. 100% ATPase activity corresponds to 0.155, 0.120, and 0.129 μmoles/min mg for preparations A, B, and C, respectively.

Effect of Assay Temperature on ATPase Activity of Heat-Treated Cilia

Table II shows the results of three experiments in which the ATPase activities of heat-treated cilia and of their unheated controls were assayed both at 25° (standard assay conditions) and at 0°. It can be seen that whereas at 25° there was an enhancement of about 1.3-fold, the enhancement measured at 0° averaged only 1.07-fold. Thus much more of an increment in ATPase activity is observed when the heat-treated cilia are assayed at 25° than when assayed at 0°.

Effect of Heating Cilia on the Amounts of 14S and 30S Dyneins Extracted by Tris-EDTA

An effort was made to determine whether heat treatment and the consequent uncoupling of ATPase activity from motility changed the relative amounts of 14S vs 30S dyneins extracted in comparison to unheated controls. For this reason, the cilia were heated for various times within the narrow temperature range reported on here, as well as at higher temperatures (data not shown). Figure 2 presents the ATPase activity profile in the gradients obtained when three of the preparations reported on in Table I were extracted by Tris-EDTA and the crude dynein resolved by sucrose gradient centrifugation.

When cilia preparation IV of Table I was heated for 8 min at 34.2°, or preparation III for 6 min at 36.6°, there was no change in the specific activity of the ATPase extracted, despite the increase in activity in the heated axonemes. In both of these cases, there was no change in the amount of 14S activity extracted from the heated cilia as compared to their controls (see Fig. 2). In one case (preparation III, heated 6 min at 36.6°) there was also no loss of 30S dynein, while in the other case (preparation IV, 8 min at 34.2°) there was a small loss of 30S dynein ATPase extracted. In both of these preparations when the cilia were heated for longer times and a clear decrease in the specific activity of the crude dynein occurred, there was an appreciable loss of activity in the 30S dynein peak but relatively small loss of the 14S dynein peak (Fig. 2). From those two experiments it appeared that the heat treatment caused preferential loss of the 30S dynein. The results obtained upon extraction of dynein from cilia heated at 38.4° show that after 4 min there was a roughly equal loss of

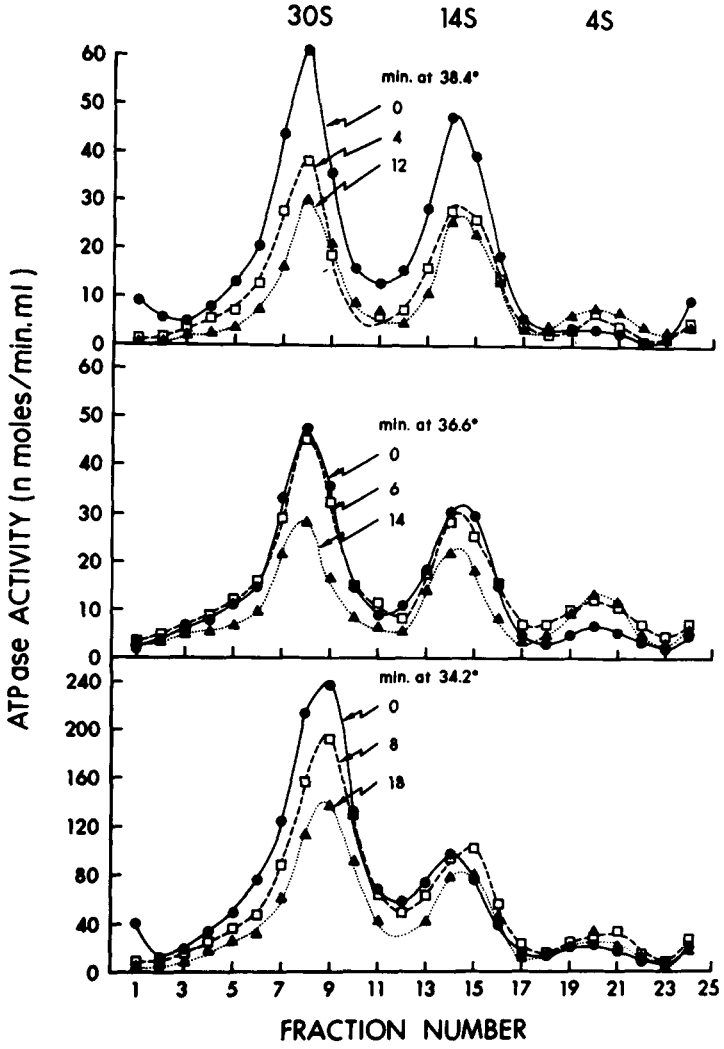

Fig. 2. Amount and distribution of ATPase activity in sucrose density gradients of the crude dynein extracted from heat-treated cilia. The crude dynein (supernatant I) from 3 of the preparations shown in Table I was resolved by sucrose density gradient sedimentation and the ATPase activity assayed as described in Methods. The peaks of ATPase activity are designated as 30S, 14S, and 4S for convenience; it is not implied that these are the exact sedimentation coefficients.

activity in each peak. After 12 min at 38.4°, however, it again appeared that there was a relatively larger loss of 30S dynein activity than of 14S. Thus in each of these preparations, as well as in a preparation heated for 4 min at 40.6° (data not shown), heating caused a preferential loss of activity under the 30S dynein peak as compared to the 14S peak, although the proportions lost are variable and depend on the time as well as the temperature.

It should also be noted that in each experiment there was an increase in the amount of ATPase activity recovered in the "4S" peak, as if heating had caused some 14S and/or 30S dynein to break into a smaller but still active fragment. Alternatively, it is possible that the heat treatment released some of the "residual" ATPase that ordinarily resists extraction, if one assumes that the residual ATPase is a low molecular weight enzyme.

DISCUSSION

The present results confirm and extend an earlier observation that brief heating of glycerinated Tetrahymena cilia enhances the ATPase activity and uncouples it from motility. The key observations in the present work are that in addition to the increase in ATPase activity, the dynein in the heat-treated cilia differs from dynein in unheated cilia in three important ways: (1) reaction of the dynein in situ with either the monofunctional sulfhydryl reagent NEM or the bifunctional reagent PPDM no longer causes an increase in ATPase activity, but only an inhibition; (2) the ratio of ATPase activity of heat-treated cilia to that of control cilia assayed at 25° is larger than the same ratio of activities assayed at 0°; (3) extraction of the dynein from the heated cilia causes a large loss of ATPase activity. We have shown earlier that when crude dynein binds onto EDTA-extracted axonemes, there is an enhancement of its ATPase activity (11). If the EDTA-extracted axonemes were heated at 42.2° for times up to 25 min, there was a rapid loss in the ability of the axonemes to cause enhancement of the dynein ATPase and a somewhat slower loss of the amount of dynein that could bind. Part of the uncoupling action of the heat treatment may therefore reside at a site or sites in the axoneme where the native dynein binds. This cannot be the entire effect of the heat treatment in the present study for two reasons. First, if the effect of heating the cilia were only on some structural proteins other than dynein, one would expect a decrease in ATPase activity, since, as just stated, heating EDTA-extracted axonemes caused a loss of their ability to enhance dynein ATPase. In fact, however, the heat treatment increased the ATPase activity of the cilia. Second, if the only effect of the heat treatment were on components other than dynein, one would expect that extraction of heated cilia by Tris-EDTA would yield the same amount of ATPase activity as extraction of unheated cilia. Instead, much less ATPase activity was obtained from the heated cilia, despite their increased ATPase activity relative to unheated controls. Since about the same amount of protein was extracted from the heat-treated cilia as from the control cilia, and there was no difference in the amount or specific activity of the residual ATPase remaining on the EDTA-extracted axonemes, it must be concluded that, in addition to any changes in properties of the nondynein proteins of the axonemes, the heat treatment caused changes in the dynein which, as stated above, include changes in the properties of some SH groups and in its sensitivity to the temperature of assay.

It has been shown that heating crude dynein at 41° causes an enhancement of its ATPase activity (11). It would seem reasonable, therefore, to ascribe the increase in ATPase activity observed on heating the dynein in situ to the same process, presumably a conformation change, that causes the increase in ATPase activity of dynein in solution. The effects of heating dynein in situ, however, differ from the effects of heating crude dynein in solution. Heating the solubilized dynein at 41° (or lower) causes an increase in ATPase activity. Heating the dynein in situ also causes an increase in ATPase activity, but subsequent extraction of the dynein from the heat-treated cilia causes a loss not only of the "extra" ATPase activity but of total activity, as compared to the amount extracted from unheated controls. Perhaps the simplest interpretation of this difference is that when dynein is heated in situ at these low temperatures it enters into a somewhat different state than when it is heated in solution. When heated in situ the uncoupled dynein is, apparently, maintained in a state which has high ATPase activity but which is unstable and reverts to a low activity state upon release from its in situ environment. It has been appreciated that native dynein in situ differed in several properties from dynein in solution (4, 5, 9). Brokaw and Benedict (7, 8) reported that when the ATPase of sperm tail flagella was uncoupled

from motility by gentle homogenization the ATPase differed in several properties from that of motile sperm tails. The uncoupling they studied, however, is probably quite different from that studied here. There is no evidence that the dynein in the small pieces of sperm tail was in any way altered by the gentle homogenization procedure. Rather, judging from the results of Lindemann and Rikmenspoel (20, 21) it would appear that bend initiation and coordinated propagation were prevented by the short length of the fragments, so that native dynein was being observed in situ under conditions where it could not interact effectively with microtubular protein to cause sliding. In the present study the dynein was both uncoupled and altered by the heat treatment.

When the crude dynein extracted from Tetrahymena cilia is subjected to sucrose gradient sedimentation, two forms are obtained, which are referred to as 30S dynein and 14S dynein (22); the latter appears to be a monomeric subunit of the former, but there are several indications in the literature that 14S dynein obtained by extraction may not be identical to that obtained by disaggregation of 30S dynein in vitro (23–25). Since heating cilia at low temperatures caused a loss in the ATPase activity subsequently extracted into the crude dynein fraction, it was of interest to establish whether the loss occurred primarily in the 30S or the 14S fraction. Our results do not permit an unequivocal answer to this question. Clearly, there was more activity lost from the 30S fraction than from the 14S fraction, but some loss also occurred in the 14S fraction. It is not surprising that the 14S lost some activity even at temperatures as low as 34.2°, since earlier studies had shown that 14S dynein was more thermolabile than 30S dynein, and 30% of its activity was lost within 5 min at 47° (4). Given that 14S dynein is somewhat more thermolabile in solution than is 30S dynein, one would not expect that much of the loss in activity after heating of whole cilia would occur in the subsequently extracted 30S dynein fraction. The present data therefore suggest that if the only form of dynein in situ is the 30S form, then heating at these low temperatures alters the 30S dynein in such a way that its ATPase activity increases while it remains subject to the constraints of its in situ environment, but both decays upon extraction and dissociates more readily into active 14S subunits than does 30S dynein extracted from unheated cilia. If the 14S dynein exists as such in situ (presumably in a location other than the arms) then it would appear that it is less thermolabile in situ than is 30S dynein even though it is more thermolabile than 30S dynein when in solution. The present data do not permit a choice to be made between these alternative interpretations. Further work would also be required to assess the significance of the increased amount of "4S" ATPase observed in gradients of material extracted from heat-treated cilia.

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