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Effect of Extraction Time on Ability of Calmodulin to Activate 30S and 14S Dynein ATPases

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Cilia from the protozoan Tetrahymena pyriformis were demembranated and then extracted for 5 min with a buffer containing 0.5 M NaCl. The briefly extracted axonemal pellet was then reextracted for about 20 hr. The soluble material obtained from each extraction was resolved into 14S and 30S dynein ATPases by sedimentation on sucrose density gradients and tested for sensitivity to added calmodulin. The 14S dynein obtained by a 5-min extraction was generally insensitive to added calmodulin, whereas that obtained by 20-hr extraction of the 5-min extracted axonemes was activated by calmodulin, the activation being much larger in the "light" 14S fractions than in the "heavy" fractions. The 30S dynein ATPase obtained by a 5-min extraction was generally activated over 1.6-fold by added calmodulin, whereas that obtained by the subsequent long extraction was usually activated only 1.3-fold. After further purification of the 5-min extracted 30S dynein and of the 5-min to 20-hr-extracted 14S dynein on DEAE-Sephacel, these dyneins retained much of their calmodulin activatability. The ATPase activity of both 14S and 30S dyneins was inhibited more strongly by erythro-9-[3-(2-hydroxynonyl)] adenine and by vanadate in the presence of added calmodulin than in its absence. These data suggest that the only ATPase activity present in the fractions studied is that of the dyneins and demonstrate that both the 14S and 30S dynein ATPases may be obtained in forms that are activated by added calmodulin as well as in forms that are insensitive to added calmodulin.

Key words: dyneins, calmodulin, cilia

In a preceding paper we have shown that when demembranated cilia of Tetrahymena are extracted with high concentrations of KCl for times ranging from 2 hr to about 20 hr, the 14S* dynein ATPase activity is increased markedly by physiologically low concentrations of CA^{++} in the presence of added calmodulin [1]. The ATPase activity of the 30S dynein obtained by these extractions was generally increased about 1.3-fold by addition of calmodulin. Such an activation could, of course, have resulted

*In this paper we use the designations "14S dynein" and "30S dynein" merely to refer to location in a sucrose density gradient.

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from a nonspecific interaction of the calmodulin with the 30S dynein. The fact that both 14S and 30S dyneins would bind to calmodulin-Sepharose 4B columns in the presence of Ca^{++} and were eluted in the presence of EGTA, however, suggested that both dyneins had specific binding sites for calmodulin. More recently it has been shown by photoaffinity labeling studies using modified calmodulins that some heavy chain components of both the 14S and 30S dynein ATPases interact with calmodulin [2]. The finding that the heavy chains of the 14S dyneins were much more labeled than those of the 30S dyneins was in accord with the substantially greater activation by calmodulin of the 14S dynein ATPase activity than of the 30S dynein. One hypothesis consistent with these observations was that some factor(s) normally present in the 30S dynein complex and required for the activation of ATPase activity by calmodulin was lost during the extraction. If so, extracting the axonemes for much shorter times might lead to the isolation of 30S dynein ATPase somewhat more sensitive to added calmodulin. This line of reasoning was reinforced by private communications from several colleagues who were unable to find an activation of 14S dynein ATPase by calmodulin. Among other differences that might account for this discrepancy was the fact that they extracted the cilia for only 5 min rather than for many hours. The failure of such brief extractions to yield a 14S dynein with a calmodulin-activatable ATPase also raised the possibility that even though both the 14S and 30S dyneins had specific binding sites for calmodulin [1,2], some other (calmodulin activated) ATPase (perhaps a different kind of dynein) might have been extracted from the axonemes during the long extractions employed by us. We therefore decided to extract axonemes for 5 min and then to reextract the pellet for about 20 hr and to compare the calmodulin activatability of the 30S and 14S dyneins obtained from the two extractions. To test for the possibility that some ATPase other than dynein was being extracted, we employed the ATPase inhibitors erythro-9-[3-(2-hydroxynonyl)] adenine (EHNA) and vanadate. EHNA is a selective inhibitor of dynein ATPase [3]; of approximately one dozen ATP-metabolizing enzymes tested, only dynein ATPase was inhibited. Furthermore, kinetic analysis indicated a linear mixed-type of inhibition, suggesting that the binding site for EHNA was separate from the ATPase site. Vanadate, on the other hand, is a very potent inhibitor of dynein ATPase that acts at the active site [4,5] and so should be a useful complement to EHNA in studies designed to test whether an ATPase activity is due to dynein.

In this paper we report that brief (5 min) extraction of demembranated axonemes yields a 14S dynein ATPase that is either insensitive to added calmodulin or is only activated to a small extent, while the 30S dynein ATPase so obtained is often activated over 1.6-fold and, occasionally, manyfold. The ATPase activity of the 30S dynein obtained by a 20-hr extraction of the 5-min extracted axonemal pellet is, however, generally insensitive to added calmodulin, while the 14S dynein so obtained is activated as previously reported.

MATERIALS AND METHODS

The preparation of cilia from cultures of Tetrahymena pyriformis (strain HSM) and their demembranation by treatment with 0.5% (v/v) Triton X-100 was performed as described [6]. Crude dynein was prepared by extracting the axonemes for 5 min at 0°C in 10 ml of extraction solution (0.5 M NaCl/0.5 mM dithiothreitol/0.25 μg/ml of α_2-macroglobulin in IMT/6 buffer). The suspension was then centrifuged for 10

min at 11,000g at 0°C, and the pellet was reextracted with 10 ml of extraction solution for about 20 hr. Samples of 3 ml of the supernatant from the 5-min extraction and, a day later, from the 5-min to 20-hr extraction were placed on sucrose density gradients and centrifuged for ≈ 18 hr at 0°C at 23,500 rpm (about 55,000g average) in a Spinco Model L ultracentrifuge. The sucrose (5% to 30%, w/v) was dissolved in IMT/6 buffer (8.33 mM Tris/8.33 mM imidazole/1.25 mM $MgCl_2$/0.067 mM EGTA, pH 7.5) containing 0.5 mM dithiothreitol (DTT), 0.2 mM $CaCl_2$, and 5 μg/ml of leupeptin. Fractions of about 1 ml were then collected and stored at 4°C for use.

DEAE-Sephacel Chromatography

Pooled fractions from the desired regions of the sucrose gradients were loaded onto a DEAE-Sephacel column (1.5 × 8 cm) that had been equilibrated with a buffer consisting of 0.05 M NaCl, 4 mM $MgCl_2$, 0.5 mM DTT, 2.5 μg/ml α_2-macroglobulin, and 10 mM trishydroxymethylaminomethane, pH 7.5. Elution was carried out with a linear gradient of NaCl (0.05 M to 0.6 M) in this buffer. About 21 fractions of 2.4 ml each were collected.

ATPase Activity

ATPase activity was measured as previously described [7] as was protein concentration [8]. All tubes were preincubated for 4 min at 25°C before the reaction was started by addition of ATP (final concentration 1 mM unless otherwise specified).

Polyacrylamide Gel Electrophoresis

Samples were treated with sodium dodecyl sulfate and mercaptoethanol and electrophoresed on 7.5% polyacrylamide gels and stained with silver nitrate as previously described [7].

Materials

Bovine brain calmodulin was prepared as described [6]. α_2- Macroglobulin was purchased from Boehringer-Mannheim, DEAE-Sephacel from Pharmacia, dithiothreitol from Sigma, ATP from P and L, and leupeptin from the Protein Research Foundation. EHNA was the generous gift of Dr Gertrude Elion of the Burroughs-Wellcome Co, Research Triangle Park, NC. All other reagents were of the highest purity commercially available.

RESULTS

Figure 1 shows the results obtained from an experiment in which demembranated axonemes were extracted for 5 min with an extraction solution containing 0.5 M NaCl (see Methods) and the axonemal pellet remaining after the brief extraction then extracted for about 20 hr. The material obtained from the first (5 min) and second (20 hr) extractions was then resolved on sucrose density gradients as described in Methods. Except for a very small amount of ATPase activity in fractions 19 and 20 which is completely insensitive to calmodulin (not shown in Fig. 1; see [7]), all the "dynein" ATPase activities are distributed in fractions 1–18. The ratio of the sum of the protein concentrations in fractions 1–18 of the material obtained in the 5-min to 20-hr extraction to that obtained in the 0–5-min extraction was 3.2 ± 0.2 (SEM). Thus if

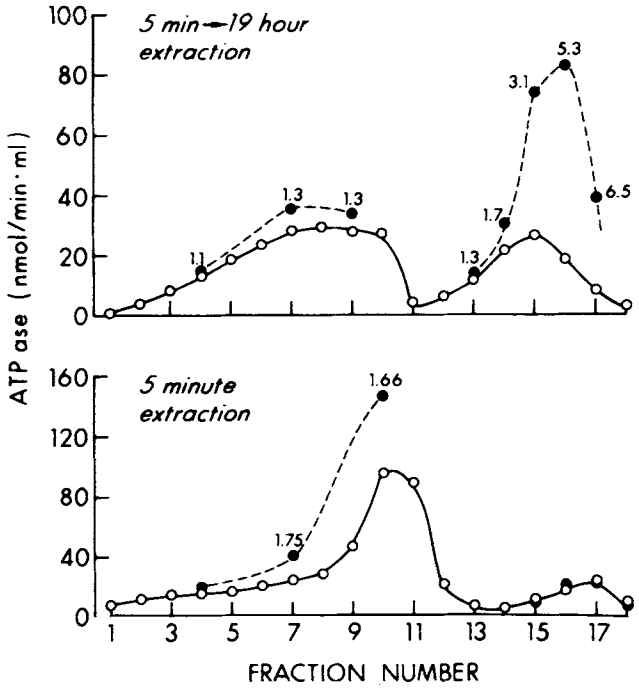

Fig. 1. Activation by calmodulin of ATPase activities of 14S and 30S dyneins extracted sequentially from ciliary axonemes. Demembranated cilia were extracted for 5 min in an 0.5 M NaCl buffer and the briefly extracted pellet was then reextracted for 19 hr as described in Methods. The supernatants obtained from each extraction were resolved on sucrose density gradients and assayed for ATPase activity in the absence of added calmodulin (○———○) and in the presence of 20 μg calmodulin/ml (●----●). The numbers next to several of the points are the values of the calmodulin activation ration (CAR), defined [7] as the ratio of ATPase activity with 20 μg/ml added calmodulin to that without. Although the effect of calmodulin on the ATPase activity of fractions 10–12 of the 5-min–19-hr extraction was not assayed in this experiment, the CAR for these fractions in other experiments was always less than 1.4. Fraction 1 is the bottom of the gradient, ie, highest sucrose density. Peak 30S dynein activity is in fractions 7–10 in the 5-min–19-hr-extracted material and in fractions 9–11 in the 5-min-extracted material. This difference in location is because the gradient with the 5-min-extracted material was centrifuged for 17 hr instead of the usual 20 hr.

in a typical experiment the 5-min extraction yielded 1.5 mg of protein sedimenting in the region of interest in the sucrose gradient, the subsequent 20-hr extraction yielded about 4.8 mg. As shown in Figure 1, sucrose density gradient sedimentation of the 5-min extract yielded a 30S dynein which was activated 1.7-fold by calmodulin, and a 14S dynein which was completely insensitive to added calmodulin. Eight axonemal preparations were treated by this sequential extraction procedure. In four out of these eight, the 5-min 14S ATPase was not activated by calmodulin (the calmodulin activation ratio [CAR] was less than 1.2). The other four had CAR values of 1.4, 1.9, 2.0, and 3, but, in contradistinction to the uniformly increasing CAR values of the 20-hr extracted 14S dynein (Fig. 1) [7,9], there was no systematic increase in CAR value as one progressed from the heaviest to the lightest fractions.

In six out of eight extractions the 30S dynein ATPase obtained by the 5-min extraction was activated about 1.6–1.8-fold. In one preparation it was 1.3-fold, and

in one preparation it was 6-fold. In the latter case, the CAR value dropped to 1.7 within 24 hr of storage at 4°C.

The response of the 5-min to 20-hr extracted 14S and 30S dynein ATPase activities to added calmodulin (Fig. 1) was quite similar to that reported earlier for dyneins obtained by extracting axonemes for times ranging from 2 to 20 hr. The 30S dynein ATPase was activated about 1.3-fold. In one preparation, however, a CAR value of 1.6 was observed, thus demonstrating that a 30S dynein ATPase that is activatable by calmodulin may be obtained from both long and short extractions. The response of the 14S dynein ATPase obtained by the 5-min to 20-hr extraction showed the same heterogeneity of CAR values as previously reported [7]; the CAR value increased steadily in going from fractions 13 to 17, ie, with decreasing sucrose density.

Retention of Calmodulin Sensitivity of 14S and 30S Dynein ATPases After Purification on DEAE-Sephacel

We have shown elsewhere that 14S dynein obtained from a single 20-hr extraction of axonemes can, in part, be retained on a calmodulin-Sepharose 4B affinity column [9] (Fig. 1). The ATPase activity that did not bind to the column in the presence of Ca^{++} was only slightly activated by addition of calmodulin. The ATPase activity that was bound to the column in the presence of Ca^{++} and was eluted by buffer containing EGTA retained its sensitivity to calmodulin. An alternative method of purification of the 14S and 30S dyneins is by chromatography on DEAE-Sephacel [10]. When 14S dynein obtained from a 20-hr extraction of axonemes that had already been extracted for 5 min was eluted from a DEAE-Sephacel column by a gradient of NaCl, a single peak of activity was obtained. The leading edge of this peak had a CAR value of 6 (Fig. 2, upper panel), which appeared to decline in subsequently eluted fractions. This decline, however, is in part artifactual, since increasing NaCl in the range from 0 to 0.2 M increasingly inhibits the activatability of 14S dynein by calmodulin with little effect on basal activity (unpublished data). During the assay of the fractions from the DEAE-Sephacel column, the NaCl concentration was diluted 5–10-fold, so that the inhibition of calmodulin activation by NaCl during the assay was small. Correction of the results for this inhibitory effect of NaCl would shift the peak of the ATPase activity curve measured in the presence of calmodulin slightly to the right so that it more closely matched the peak of the basal ATPase activity curve, but we have not done this in Figure 2 because of the uncertainty introduced by correcting for the effects of the NaCl.

The ability of 30S dynein ATPase obtained by a 5-min extraction of axonemes to be activated by calmodulin appears to be rapidly lost with time of storage (see above). Because some of this instability may be due to proteolytic activity despite the inclusion of protease inhibitors in the buffers used in the present study, it was of interest to ascertain whether the 5-min-extracted 30S dynein would retain sensitivity to calmodulin after further purification on DEAE-Sephacel. The elution pattern of a pooled sample of 30S dynein, with an average CAR value of 1.8, is shown in the lower panel of Figure 2. It can be seen that the 30S dynein eluted as a single peak and that the CAR value for the two fractions with highest activity was 1.4. This value, however, is also slightly low due to the effect of NaCl on 30S dynein activity. Unlike 14S dynein, where NaCl inhibits the activation by calmodulin without appreciably affecting basal ATPase activity, NaCl increases the basal ATPase activity of 30S dynein without much effect on the activity in the presence of calmodulin (unpublished

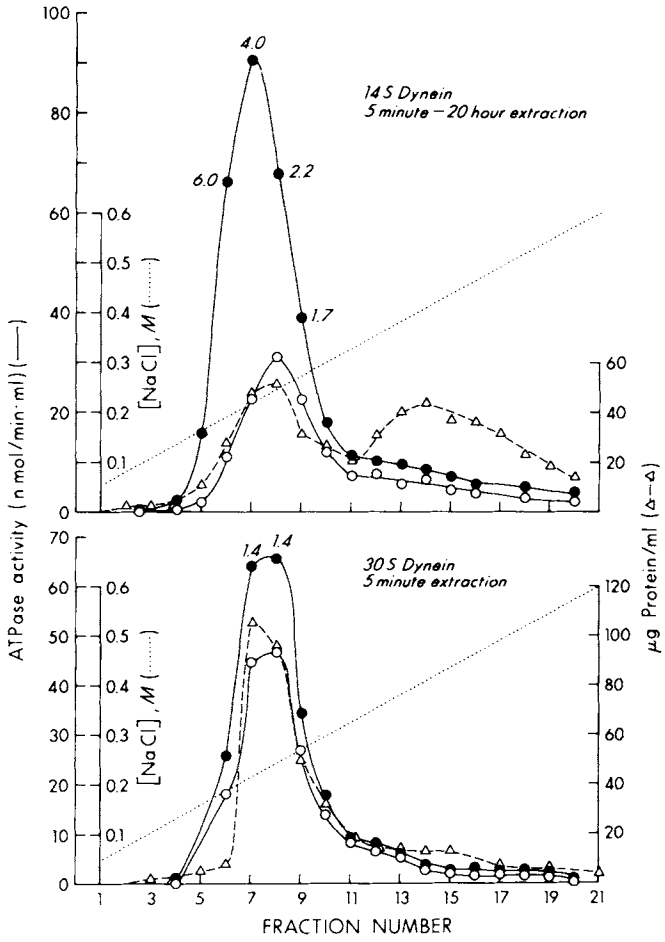

Fig. 2. Activation by calmodulin of 14S and 30S dynein ATPase activities after DEAE-Sephacel chromatography. Dyneins, prepared as described in Figure 1, and with properties closely similar to that shown in Figure 1, were further purified by chromatography on DEAE-Sephacel as described in Methods. ○——○, basal ATPase activity, ie, no added calmodulin; ●——●, with added calmodulin; △---△, protein concentration; . . . . . , nominal NaCl concentration.

data). Correction of the CAR values shown in the lower panel of Figure 2 would raise them to approximately the CAR value of the 30S dynein put onto the DEAE-Sephacel column. In any case, it is clear that both the 5-min-extracted 30S dynein and the 5-min–20-hr-extracted 14S dynein ATPases retain much of their sensitivity to activation by calmodulin after purification on DEAE-Sephacel.

The polypeptide composition of fractions near the peak of the ATPase activity eluted from the DEAE-Sephacel columns is in Figure 3. This preparation of 14S dynein is similar in composition but has somewhat fewer polypeptides than that obtained in an earlier study of a 20-hr extracted 14S dynein that had been resolved first on DEAE-Sephacel and then on a sucrose gradient (Fig. 3, lane C, of reference [9]). Fractions 6 and 7 are very similar in peptide composition except for the polypeptide of approximately 84 kilodaltons present in fraction 7 but absent in fraction

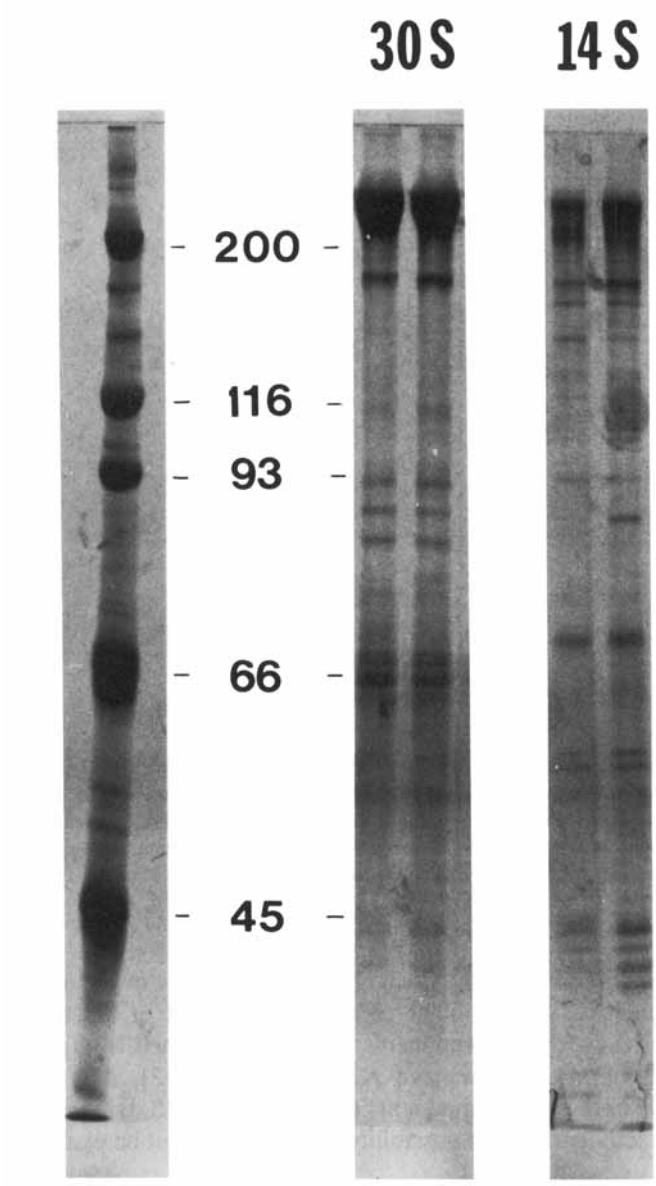

Fig. 3. Polyacrylamide gel electrophoresis of 30S and 14S dyneins after elution from DEAE-Sephacel columns. Polyacrylamide gels (7.5%) were silver stained as described in Methods. The left lane shows the molecular weight markers used. Light bands are trace contaminants. The dynein fractions shown are, from left to right, fractions 7 and 8 of the DEAE-Sephacel gradients of 30S dynein and fractions 6 and 7 of the 14S dynein of the experiment shown in Figure 2.

6. Since fraction 7 had a slightly higher ATPase and lower CAR than fraction 6 (see top panel of Fig. 2) this polypeptide is not likely to be a calmodulin sensitive ATPase.

Johnson and Wall [11] have shown that 30S dynein of Tetrahymena consists of three heavy chains of about 300–320 kilodaltons, one each at 86 and 73 kilodaltons, and about six light chains. The 30S dynein obtained from fractions 7 and 8 of the

TABLE I. Effect of EHNA on 14S and 30S Dynein ATPase Activities in the Presence and Absence of Added Calmodulin*

EHNA	Calmodulin	ATPase (nmol/min·mg)	Inhibition (%)	CAR
I. 5-min extraction				
A. 30S dynein				
−	−	354	—	
+	−	337	5	
−	+	1,334	—	3.8
+	+	1,033	23	3.1
B. 14S dynein				
−	−	141	—	
+	−	108	24	
−	+	481	—	3.4
+	+	268	54	2.4
II. 5-min–20-hr extraction				
A. 30S dynein				
−	−	161	—	
+	−	137	15	
−	+	226	—	1.4
+	+	159	30	1.1
B. 14S dynein				
−	−	100	—	
+	−	47	51	
−	+	445	—	4.4
+	+	189	58	4.0

*Two different preparations of demembranated axonemes were extracted for 5 min and then reextracted for about 20 hr as described in Methods. The crude extracts were resolved by sedimentation on sucrose density gradients and individual fractions from the 30S and 14S dynein regions were assayed in the presence or absence of 0.62 mM EHNA and 20 μg/ml calmodulin as indicated. For these experiments, the ATP concentration during the assay was 0.33 mM instead of 1.0 mM.

DEAE-Sephacel column contain, in addition, polypeptides of about 180, 90, and 60 kilodaltons as well as several other components present in much lower amounts. Since only the heavy chain components of dynein obtained by Tris-EDTA or KCl extraction of Paramecium cilia have ATPase activity [12], it is unlikely that the intermediate and light chain components in the 30S and 14S dyneins shown in Figure 3 have ATPase activity, but this possibility cannot at present be excluded.

Effect of EHNA and of Vanadate on 14S and 30S Dynein ATPase Activities in the Presence and Absence of Calmodulin

The data so far presented suggest that two types of 14S dynein may be extracted from the cilia of Tetrahymena, one very sensitive to activation by calmodulin (depending on its location in the sucrose gradient) and one rather insensitive. The 30S dynein can also be isolated in two forms, one only slightly activated ($\approx$ 1.3-fold) by calmodulin, and the other activated about 1.6-fold or more and as much as six fold but this degree of activatability being quite unstable. Although their position in sucrose gradients both before and after chromatography on calmodulin-Sepharose 4B columns or DEAE-Sephacel columns [9] indicates that dyneins are the major components present in these fractions, one cannot rule out the possibility that some other

ATPase was also extracted and is responsible for the increase in activity caused by calmodulin. This possibility was also consistent with the finding [2] that some polypeptides besides the dynein heavy chains are photoaffinity labeled by photosensitive calmodulin derivatives. We therefore examined the effects of two inhibitors known to inhibit dynein ATPase activity by different mechanisms.

Table I shows the effect of EHNA on the ATPase activities of 14S and 30S dyneins obtained by a 5-min extraction of demembranated Tetrahymena cilia. The first point to notice is that 0.62 mM EHNA is a considerably stronger inhibitor of the 14S dynein ATPase than of the 30S dynein ATPase. This 30S dynein fraction was chosen because it had one of the highest CAR values ever obtained. It can be seen that addition of EHNA in the presence of calmodulin caused an *increase* in the inhibitory potency of EHNA. This was also observed for this sample of 14S dynein, chosen because it, too, was unusually strongly activated by calmodulin. The same experiments, with similar results, were also carried out with 14S and 30S dyneins obtained by a 20-hr extraction of the axonemal pellet that had been extracted for 5 min (Table I). These results are not consistent with the hypothesis that there was an appreciable amount of a nondynein ATPase present which was activated by calmodulin and insensitive to EHNA (see [3]).

Table II shows the effects of vanadate on 30S dynein ATPase activity obtained from a 5-min extraction. It can be seen that about the same inhibitions of 30S dynein and of 14S dynein ATPase activities were observed in the presence of calmodulin as in its absence. Similar results were obtained with the 14S and 30S dynein fractions obtained from 5-min to 20-hr extractions except that vanadate inhibited the ATPase activity in the presence of calmodulin somewhat more than it inhibited in the absence of calmodulin. In each case (ie, 14S and 30S dyneins from both long and brief extractions) concentrations of vanadate above 2 μM caused total inhibition of ATPase activity both in the presence and absence of added calmodulin (data not shown). Thus the results with both vanadate and with EHNA do not support the view that any ATPase activity other than that due to dynein is present in the 14S or 30S regions of sucrose gradients of material obtained by brief or by long extractions of demembranated axonemes.

DISCUSSION

When Tetrahymena cilia are extracted by a short (5 min) exposure to a buffer containing 0.5 M NaCl, the 14S and 30S dynein ATPases that are obtained by sedimentation on a sucrose density gradient differ from the dynein ATPases obtained by a subsequent extraction of the briefly extracted axonemal pellet for 20 hr. The 14S dynein ATPase obtained by the brief extraction is either not activated by the addition of calmodulin or is activated to a relatively small extent. Furthermore, even in the preparations in which activation was observed, it did not increase monotonically from the "heavy" 14S dynein fractions to the "light" 14S dynein fractions (ie, from the higher sucrose concentration of fraction 13 to the lower sucrose concentration of fraction 17 in Fig. 1) as had been reported earlier [7] for 14S dynein ATPase obtained by a single 20-hr extraction and as observed here for the 14S dynein obtained by the 5-min–20-hr extraction.

The 30S dynein ATPase obtained by a single 20-hr extraction of Tetrahymena cilia generally has a CAR value ranging from 1.2 to 1.4 [7]. That obtained from the

TABLE II. Effects of Vanadate on ATPase Activity of 30S and 14S Dyneins in the Presence and Absence of Added Calmodulin*

Vanadate	Calmodulin	ATPase (nmol/min·mg)	Inhibition (%)	CAR
I. 5-min extraction				
A. 30S dynein				
−	−	625	—	
+	−	123	80	
−	+	984	—	1.6
+	+	226	77	1.8
B. 14S dynein				
−	−	690	—	
+	−	264	62	
−	+	831	—	1.2
+	+	291	65	1.4
II. 5-min–20-hr extraction				
A. 30S dynein				
−	−	190	—	
+	−	45	72	
−	+	259	—	1.4
+	+	50	78	1.3
B. 14S dynein				
−	−	109	—	
+	−	48	55	
−	+	326	—	3.0
+	+	99	70	2.0

*Two different preparations of demembranated axonemes were extracted for 5 min and then reextracted for about 20 hr as described in Methods. The crude extracts were resolved by sucrose density gradient sedimentation, and individual fractions from the 30S and 14S dynein regions were assayed in the presence or absence of 0.75 μM ammonium vanadate or 20 μg/ml calmodulin, as indicated. The ATP concentration during the assay was 1.0 mM.

5-min–20-hr extraction has the same low CAR values (Fig. 1) except in one preparation where a value of about 1.6 was observed. The 30S dynein ATPase obtained by a brief extraction, however, has CAR values that usually lie in the range 1.6–1.8 and higher. In one preparation a sixfold activation was observed. Clearly, then, 30S dynein is also potentially sensitive to considerable activation by calmodulin. This is not unexpected, since it has a calmodulin binding site as indicated both by Ca^{++}-dependent binding to calmodulin-Sepharose 4B [1] and by the reaction of photoaffinity labeled calmodulin derivatives with the heavy chain(s) of the 30S dynein [2].

Although the protease inhibitors α_2-macroglobulin and leupeptin were present at various stages of the isolation and extraction procedures, some or all of the variability observed in the sensitivity to calmodulin may nevertheless be due to the action of proteases known to be released into the medium by Tetrahymena [13]. It is also possible, however, that some of the variability reflects the presence of varying states of the dyneins in cilia in different stages of their beating cycle. This seems especially likely for the 14S dynein, since it does not appear capable of binding to extracted axonemes or of conferring the ability to slide [14] and is, therefore, likely to play a regulatory role. Further work, however, will be required to decide whether the variability observed reflects the existence of different functional states or is merely

an artifact of uncontrolled proteolysis. Similarly, further work will be required to decide whether the loss of calmodulin activatability by 30S dynein ATPase is due to, for example, dissociation of a light chain or is entirely a reflection of proteolytic cleavage.

Despite the variability in response to calmodulin and the instability of this response in some cases, it was possible to show that sensitivity to calmodulin was retained in large part after purification of the dyneins by DEAE-Sephacel chromotography (Fig. 2) and by chromotography on calmodulin-Sepharose 4B columns [1,9]. Although retention of calmodulin activatability after these two differing purification methods suggests that the ATPase activity being studied was entirely due to dynein, it was nevertheless desirable to obtain further evidence ruling out the presence of some other ATPase that was activated by calmodulin and copurified with the 30S and 14S dynein fractions. The results obtained from inhibition studies with EHNA and with vanadate were consistent with the hypothesis that no ATPase activity other than dynein was present (Tables I, II). Recent reports that 21S dynein ATPase from both flagellar and cytoplasmic dynein from the sea urchin are activated by calmodulin also support the view that some dyneins are intrinsically activatable by calmodulin [15]. The present results suggest that dynein may exist in more than one form. Brief extraction of axonemes with a high ionic strength buffer (or long extraction with a low ionic strength Tris-EDTA buffer [1]) yields a 14S dynein that is usually insensitive to activation by added calmodulin, whereas long extractions yield a 14S dynein ATPase that is heterogeneous in its ability to be activated by added calmodulin, the "light" 14S dynein fraction having CAR ratios of up to 11. Several explanations might account for these findings. First, it is possible that there are two different kinds of 14S dynein, eg, one found on the inner arms and one on the outer arms. Second, 14S dynein may exist in two (or more) different states, eg, phosphorylated vs nonphosphorylated. Third, the 14S dynein that is obtained by brief extraction might already have been damaged by proteolytic enzymes during the deciliation procedures. Similar possibilities may be envisaged to account for the higher sensitivity of the 5-min-extracted 30S dynein to added calmodulin than that of the 20-hr-extracted material, except that if proteolysis were the cause of the difference, the proteolysis would presumably have had to occur not during the deciliation procedure but rather during the extraction. Further studies will be required to understand the reasons why each of the dyneins may be obtained with varying degrees of sensitivity to added calmodulin.

The finding that addition of calmodulin increased the inhibitory effect of EHNA and, to a lesser extent, that of vanadate on both 14S and 30S dynein ATPase activities suggests that the interaction of Ca^{++}-calmodulin with dynein causes a conformation change that results in greater susceptibility to inhibition of ATPase activity by these two widely differing inhibitors. It also appears that 14S dynein ATPase, which is more sensitive to activation by calmodulin than is 30S dynein ATPase, is more sensitive to inhibition by EHNA. The mechanistic implications of these kinetic findings remain to be determined.

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REFERENCES

1. Blum JJ, Hayes A, Jamieson GA Jr, Vanaman TC: J Cell Biol 87:386, 1980.
2. McCartney JE, Klevit RE, Blum JJ, Vanaman TC: In "Calcium Binding Proteins in Health and Disease." Amsterdam: Elsevier/North Holland Biomedical Press, Inc., 1983 (in press).
3. Penningroth SM, Cheung A, Bouchard P, Gagnon C, Bardin CW: Biochem Biophys Res Commun 104:234, 1982.
4. Gibbons IR, Cosson MP, Evans JA, Gibbons BH, Houck B, Martinson KH, Sale WS, Tang W-JY: Proc Natl Acad Sci USA 75:2220, 1978.
5. Kobayashi T, Martensen T, Nath J, Flavin M: Biochem Biophys Res Commun 81:1313, 1978.
6. Jamieson GA Jr, Vanaman TC, Blum JJ: Proc Natl Acad Sci USA 76:6471, 1979.
7. Blum JJ, Hayes A, Vanaman TC, Schachat FH: J Cell Biochem 19:45, 1982.
8. Blum JJ, Hayes A: J Supramol Struct 11:117, 1979.
9. Blum JJ, Hayes A, McCartney JE, Schachat FH: J Submicrosc Cytol 15:237, 1983.
10. Johnson KA, Porter ME: Cell Motil (Suppl) 1:101, 1982.
11. Johnson KA, Wall JS: J Cell Biol 96:669, 1983.
12. Doughty MJ, Kaneshiro ES: J Protozool 30:565, 1983.
13. Blum JJ: J Cell Physiol 86:131, 1975.
14. Gibbons IR: J Cell Biol 26:707, 1965.
15. Hisanaga S, Pratt MM: J Cell Biol 95:322a, 1982.