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Presence of calmodulin in *Tetrahymena*

(cilia/eukaryotes)

GORDON A. JAMIESON, JR., THOMAS C. VANAMAN, AND JACOB J. BLUM

Departments of Microbiology and Immunology and of Physiology, Duke University Medical Center, Durham, North Carolina 27710

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ABSTRACT **Ca-dependent affinity chromatography on phenothiazine-Sepharose 4B has been used to isolate a pure protein from the ciliate *Tetrahymena pyriformis*. This protein has been identified as calmodulin by demonstrating three of the Ca-dependent activities attributed to calmodulins. *Tetrahymena* calmodulin also has physicochemical properties similar to those of the previously characterized mammalian, coelenterate, and plant proteins, except for a lower molecular weight (15,000) and slightly different CNBr fragments compared to bovine brain calmodulin. Calmodulin is a constituent of demembranated *Tetrahymena* cilia from which it can be extracted with the crude dynein fraction. Sucrose density gradient fractionation indicated its presence in fractions containing the 14S dynein ATPase. It is concluded that the essential properties of calmodulin have been highly conserved during much of eukaryotic evolution, and it is suggested that calmodulin plays a role in the control of ciliary motility in *Tetrahymena*.**

Numerous enzyme activities in eukaryotic cells, particularly those involved in movement-associated processes, respond to stimulus-induced fluxes in the free intracellular concentration of Ca^{2+}. A single protein, termed calmodulin, is one of the primary intracellular receptors for these Ca^{2+} signals. Calmodulin activates the following enzyme systems in a Ca-dependent manner: brain 3′:5′-cyclic-nucleotide phosphodiesterase (PDEase; 3′:5′-cyclic-nucleotide 5′-nucleotidohydralase, EC 3.1.4.17) (1, 2); brain adenylate cyclase (3, 4); erythrocyte Ca^{2+}, Mg^{2+}-ATPase (5, 6); myosin light chain kinase (7); muscle phosphorylase kinase (8, 9); NAD kinase in plants (10); brain cytosol membrane protein kinase (11); and synaptosomal kinases which may be involved in neurosecretion (12). A role for calmodulin in regulating microtubule assembly/disassembly has also been suggested (13). It is well established by various criteria that calmodulin is present in representative species of both the plant (14) and animal (15–17) kingdoms. Preliminary evidence, consisting primarily of Ca-dependent enzyme activation by partially purified preparations, indicates that a similar protein may also be present in such protists as *Amoeba*, *Physarum*, and *Euglena* (18). Calmodulin has not been isolated from any protists, however, and consequently no data concerning its detailed physical and chemical properties are available. Detailed analyses of calmodulin from bovine brain (19, 20), uterine smooth muscle (21), and rat testes (22) have shown little variation in structure. Indeed, calmodulin from the coelenterate *Renilla reniformis* differs from that of the bovine brain at only five positions clustered at the amino and carboxyl termini (23). This highly conserved structure is the earliest evolutionary ancestor of the family of four-domain Ca-binding proteins thus far described (24), in accord with its central role as a ubiquitous Ca-dependent regulator.

We report here the detailed characterization of calmodulin purified from the eukaryotic ciliate *Tetrahymena pyriformis* by Ca-dependent affinity chromatography on an immobilized phenothiazine conjugate (25, 26) and that substantial amounts of calmodulin are present in the purified cilia of *Tetrahymena*, associated in part with the 14S dynein fraction.

MATERIALS AND METHODS

Materials. 10-Chloro-(3-aminopropyl)phenothiazine (CAPP), a generous gift from Carl Kaiser (Smith, Kline & French) was coupled to Sepharose 4B, and the resulting CAPP-Sepharose 4B conjugate was prepared for use as described (25). Sephadex gel filtration media were prepared for use as described by Pharmacia. Sodium dodecyl sulfate ($NaDodSO_4$), acrylamide, and bisacrylamide were used as supplied by Gallard-Schlesinger/BDH, Carle Place, NY; *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and 2-mercaptoethanol as supplied by Eastman; and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), cyclic AMP (cAMP), 5′-AMP, chlorpromazine, and Coomassie brilliant blue R as supplied by Sigma. Urea was deionized immediately before use. Reagents for protein chemical procedures were as described (20). Bovine brain calmodulin and skeletal muscle troponin subunits were prepared according to Watterson *et al.* (27) and Perry and Cole (28), respectively. Protein M_r standards were from Bio-Rad and contained lysozyme (14,300), soybean trypsin inhibitor (21,000), carbonic anhydrase (30,000), ovalbumin (43,000), bovine serum albumin (68,000), and phosphorylase B (94,000). All other reagents were laboratory grade.

Cilia. *T. pyriformis*, strain HSM, was grown in proteose-peptone medium (29). Cilia were prepared by a procedure that involved shaking the cells in the absence and then the presence of $CaCl_2$ (30). The cilia were stored in 50% (vol/vol) glycerol at −20°C until used and, when specified, were demembranated by stirring for 5 min at 0°C with 0.05% Triton X-100. Methods for preparation of crude dynein by extraction with 0.1 mM EDTA/1 mM Tris·HCl, pH 8.2, sucrose density gradient resolution of the crude dynein fraction (29), and the methods for assay of ATPase activity and the turbidity response (ΔA_{350}) of demembranated axonemes (31, 32) have been reported.

Homogenate. Calmodulin was isolated (see *Results*) from crude extracts prepared as follows. Twelve liters of cells (≈3.5 × 10^9 cells) grown in proteose-peptone medium supplemented with glucose (1 g/liter) were chilled, collected by continuous flow centrifugation (1200 × *g*, 0°C), and washed once with 0.9% NaCl at 0°C. The cells were then centrifuged (1400 × *g*, 3 min, 0°C), the supernatant was discarded, and the pellet was stored at −60°C until used. All subsequent operations were at 4°C. After thawing, the cells were homogenized in a Sorvall Omnimixer in 2 vol of homogenization buffer (1 mM EDTA/1 mM 2-mercaptoethanol/20 m*M* TES·NaOH, pH 7.0). The

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Abbreviations: CAPP, 10-chloro-(3-aminopropyl)phenothiazine; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; EGTA, ethylene glycol bis(μ-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; PDEase, 3′:5′-cyclic-nucleotide phosphodiesterase (EC 3.1.4.17); TnI, troponin I; TnC, troponin C; cAMP, adenosine 3′,5′-cyclic monophosphate.

homogenate was centrifuged for 1 hr at 100,000 × *g* and the supernatant was decanted and saved. The pellet was rehomogenized in an equal volume of homogenization buffer and centrifuged as above. The supernatants were pooled and brought to 50% $(NH_4)_2SO_4$ saturation (pH was maintained at 7.0). The pellet obtained after centrifugation (100,000 × *g*, 1 hr) was discarded and the supernatant was brought to pH 4.0 by slow addition of 0.5 M H_2SO_4 in 50% $(NH_4)_2SO_4$. The material precipitated by this procedure was collected by centrifugation (100,000 × *g*, 1 hr) and dissolved in a small volume of homogenization buffer, and the pH was adjusted to 7.0. This solution was made 10 mM in $CaCl_2$ and then NaCl or homogenization buffer was added to yield an osmolarity equivalent to that of the CAPP-Sepharose 4B column buffer (300 mM NaCl/1 mM $CaCl_2$/1 mM 2-mercaptoethanol/20 mM TES·NaOH, pH 7.0).

Analytical Procedures. Electrophoretic analyses were performed by standard methods using procedures and gel compositions given in the figure legends. Amino acid analyses and CNBr digestion were performed as described by Watterson *et al.* (20). The concentration of calmodulin in crude homogenates was determined by spectrophotometric assay of the Coomassie blue stain eluted with 50% (vol/vol) pyridine from the gel slice containing the material migrating as calmodulin; quantitation was by comparison to known concentrations of bovine brain calmodulin run simultaneously in the same gel slabs (33).

Protein concentrations were determined by the method of Lowry *et al.* (34) except for purified calmodulin and rabbit skeletal troponin C (TnC) and troponin I (TnI) protein stocks, for which concentration was determined by amino acid analysis after acid hydrolysis, and for analysis of fractions from density gradients, for which concentration was assayed by a fluorometric procedure (32).

Assays for Activity of Brain PDEase. Activity of bovine brain PDEase was measured by resolving 5′-AMP from cAMP on a Whatman Partisil 10-SAX ion exchange column in a Hewlett–Packard 1084B high-pressure liquid chromatograph as described by Watterson *et al.* (35) except that columns were operated at 37°C. Production of 5′-AMP was linear with time under the conditions of assay described in Table 1.

RESULTS

Purification of *Tetrahymena* Calmodulin. One of the Ca-dependent properties common to calmodulins from both plant and animal species is their ability to bind to phenothiazines such as chlorpromazine. The procedures for rapid purification of mammalian calmodulin (25) were used to purify *Tetrahymena* calmodulin. Because of its high viscosity, crude *Tetrahymena* homogenate supernatant could not be applied directly to CAPP-Sepharose 4B. Therefore, an initial ammonium sulfate pH 4 pellet fraction was prepared by using conditions originally described for the purification of bovine brain calmodulin (27). Subsequent affinity chromatography of this pellet fraction on CAPP-Sepharose 4B gave the elution profile shown in Fig. 1. Polyacrylamide gel electrophoresis of the column fractions (Fig. 1 *Inset*) showed almost quantitative retention of the calmodulin in the presence of Ca^{2+} and subsequent elution as a nearly pure protein upon addition of excess ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) to the elution buffer. Minor contaminants present in the fractions, pooled as indicated in Fig. 1, were removed by Sephadex G-100 gel filtration to yield a product that was homogenous by polyacrylamide gel electrophoresis under both denaturing and nondenaturing conditions (see Figs. 2 and 3). The yield of pure calmodulin was about 0.5 mg/30 ml of packed cells.

Properties of Purified *Tetrahymena* Calmodulin. A widely

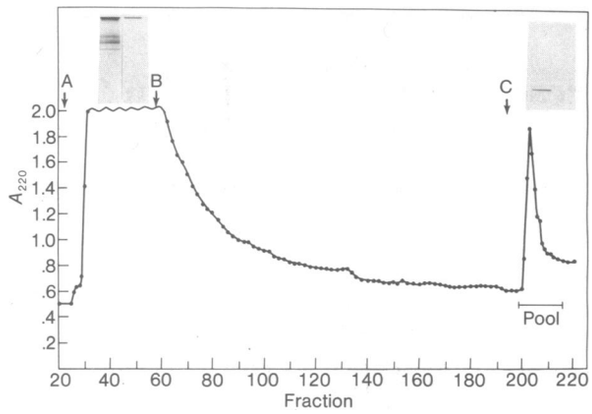

FIG. 1. CAPP-Sepharose affinity chromatography. The redissolved ammonium sulfate fraction (60 ml) was applied (arrow A) to a column (1.5 × 10 cm) of CAPP-Sepharose 4B, washed exhaustively with column buffer, and then eluted. Arrows: A, 300 mM NaCl/5 mM $CaCl_2$; B, 300 mM NaCl/1 mM $CaCl_2$; C, 300 mM NaCl/10 mM EGTA. Fractions (2.5 ml) were collected at 4°C and assayed for absorbance at 220 nm. (*Insets*) Aliquots (50 μl) of selected fractions were analyzed on 10% polyacrylamide/alkaline urea gels (33).

used functional test for calmodulin is its ability to activate preparations of "activator-depleted" brain PDEase (1, 2). Table 1 presents the results of assays with such a PDEase preparation performed in the presence or absence of purified *Tetrahymena* calmodulin or bovine brain calmodulin with either EGTA or calcium added. The degree of activation of PDEase by 0.5 and 2.0 μg of calmodulin was the same for the bovine brain and *Tetrahymena* calmodulins and in both cases was dependent upon the presence of Ca^{2+}.

Another general property of calmodulins is their ability to form Ca-dependent complexes with the TnI (inhibitory) and tropomyosin-binding subunits of skeletal muscle troponin (36), a consequence of the structural relatedness of calmodulin and TnC, the Ca-binding subunit of troponin (19, 20). *Tetrahymena* calmodulin formed a complex of intermediate mobility with rabbit skeletal muscle TnI in the presence of Ca^{2+} as was also observed for bovine brain calmodulin and TnC (see arrow, Fig. 2 *Upper*). Similar complexes were not observed in the presence of excess EGTA (see arrow, Fig. 2 *Lower*). In this discontinuous gel system, both *Tetrahymena* and the bovine brain calmodulins migrated appreciably farther than TnC in the absence of Ca^{2+} but migrated a distance similar to that of TnC when Ca^{2+} was present.

Physicochemical characterization of the *Tetrahymena* protein further confirmed its identification as a calmodulin. *Tetrahymena* calmodulin comigrated with the bovine brain protein on nondenaturing gels both in the presence and absence of Ca^{2+} (Fig. 2) and had an almost identical mobility on alkaline

Table 1. Activity of bovine brain PDEase under various conditions

Additions to assay mixture*	PDEase activity,† nmol AMP/mg enzyme per min
1. None	22.5
2. Bovine brain calmodulin (0.5 μg)	149.0
3. *Tetrahymena* calmodulin (0.5 μg)	143.0
4. Same as 1 plus 2 mM EGTA	2.7
5. Same as 2 plus 2 mM EGTA	6.4
6. Same as 3 plus 2 mM EGTA	4.6
7. Bovine brain calmodulin (2.0 μg)	162.0
8. *Tetrahymena* calmodulin (2.0 μg)	163.0

* Standard assay mixture: 2 mM cAMP, 20 mM Tris·HCl (pH 8.0), 1 mM Ca^{2+}, 0.4 mM Mn^{2+}, and 50 μg of brain PDEase.
† Incubation was for 30 min at 30°C.

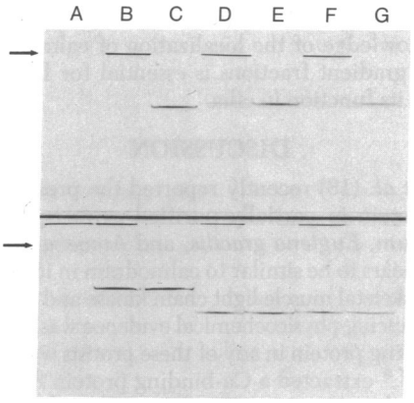

FIG. 2. Formation of Ca-dependent *Tetrahymena* calmodulin–rabbit skeletal muscle TnI complex—demonstrated by discontinuous polyacrylamide gel electrophoresis on 12.5% acrylamide gels as described (27), except that EDTA was replaced with either 1 mM Ca^{2+} (*Upper*) or 1 mM EGTA (*Lower*) in the upper running buffer. Identical samples (containing 0.3 nmol of each protein) were loaded in the slots across both gels except that for *Upper* they contained 25 mM Ca^{2+} and for *Lower* they contained 25 mM EGTA. Lanes: A, TnI alone; B, TnI + TnC; C, TnC alone; D, TnI + *Tetrahymena* calmodulin; E, *Tetrahymena* calmodulin alone; F, TnI + bovine brain calmodulin; G, bovine brain calmodulin alone.

urea gels but was resolved from the bovine protein when run as a mixture (data not shown). $NaDodSO_4$/polyacrylamide gel electrophoresis using discontinuous buffer systems (Fig. 3) indicated that *Tetrahymena* calmodulin is slightly smaller than the bovine brain protein [M_r, 16,680 (20)]. Both bovine brain calmodulin and rabbit muscle TnC gave anomalously high M_rs compared to the set of standard proteins. Based on the mobilities of the two other Ca-binding proteins, we estimate the M_r of the *Tetrahymena* calmodulin to be 15,000.

Another major characteristic that serves to identify calmodulins is the presence of a single trimethyllysyl residue and the absence of tryptophan and cysteine (27). The amino acid composition of *T. pyriformis* calmodulin is shown in Table 2 together with the compositions of calmodulins from bovine brain (20) and the coelenterate *R. reniformis* (17) determined by sequence analyses. The protozoan calmodulin differs more from the brain protein and the coelenterate protein than the metazoan proteins do from each other. However, the *Tetrahymena* protein lacks tryptophan and cysteine, contains trimethyllysine (detected but not quantified), and is clearly similar in overall amino acid composition to these more extensively characterized calmodulins.

Fig. 4 shows the pattern of Coomassie blue-staining bands obtained when CNBr digests of bovine brain and *Tetrahymena* calmodulins and of rabbit skeletal muscle TnC were analyzed on alkaline urea gels. One major band in the bovine brain cal-

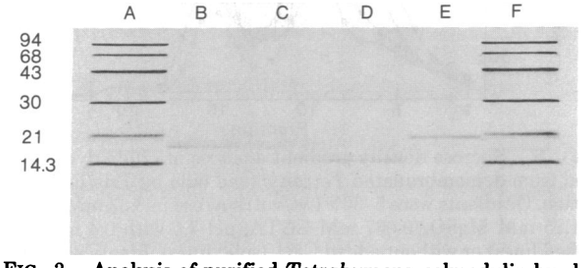

FIG. 3. Analysis of purified *Tetrahymena* calmodulin by electrophoresis was performed on $NaDodSO_4$/15% polyacrylamide discontinuous gels (37). Lanes: A and F, 4 μg each of six M_r standards; B, 5 μg of bovine brain calmodulin; C, 2.5 μg each of *Tetrahymena* and bovine brain calmodulins; D, 5 μg of *Tetrahymena* calmodulin; E, 5 μg of rabbit skeletal muscle troponin.

Table 2. Amino acid composition of calmodulins from various sources

Amino acid	Residues/molecule: Bovine brain*	Residues/molecule: *R. reniformis*†	mol/16,700 g *T. pyriformis*‡
Lysine	7	8	7.72
Trimethyllysine	1	1	§
Histidine	1	1	2.3
Arginine	6	6	5.4
Aspartic acid	23	23	23.5
Threonine	12	12	10.6
Serine	4	5	4.7
Glutamic acid	27	25	26.5
Proline	2	2	2.3
Glycine	11	11	12.1
Alanine	11	10	10.7
Valine	7	7	6.2
Methionine	9	9	8.1
Isoleucine	8	8	9.2
Leucine	9	9	13.6
Tyrosine	2	1	1.0
Phenylalanine	8	9	8.5
1/2 Cystine	0	0	0¶
Tryptophan	0	0	0‖

* From refs. 19 and 20.
† From ref. 17 and unpublished data.
‡ Analysis after 3–24 hr of hydrolysis.
§ Trimethyllysine was detected as a shoulder on the lysine peak by a double-column method and is reported together with lysine.
¶ Determined and quantified by analysis of performic acid-oxidized material.
‖ By spectral analysis, $\epsilon_{M,276\ nm} = 3036$.

modulin digest was absent from that of the *Tetrahymena* protein, which in turn had a major band migrating faster than any of the components derived from the bovine brain protein. The remainder of the major bands were grossly similar in mobility but minor differences existed which may reflect additional structural differences. The CNBr digest of *Renilla* calmodulin was indistinguishable from that shown for the bovine brain protein (data not shown), indicating again that the two metazoan calmodulins are more similar to each other than to the protozoan calmodulin. Interestingly, the *Tetrahymena* calmodulin digest resembled that of rabbit skeletal muscle TnC more than did the digests of the metazoan calmodulins.

Presence of Calmodulin in Cilia of *Tetrahymena*. Because calmodulin and TnC are involved in the control of smooth muscle and skeletal muscle contractility, respectively, it seemed plausible that calmodulin in *Tetrahymena* might be involved in the Ca-dependent control of ciliary motility (38–40). Therefore, a preparation of cilia from approximately 4×10^9 cells was extracted in a buffered urea solution and analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 5). Considerable amounts of calmodulin were present in the cilia. Much of this ciliary calmodulin could be extracted with the crude dynein fraction obtained by Tris/EDTA extraction of demembranated cilia. To ensure that the protein observed in extracts of cilia and in crude dynein was actually calmodulin, a crude dynein extract of demembranated cilia was subjected to Ca-dependent affinity chromatographic procedures described above. The elution profile obtained from CAPP-Sepharose 4B and the electrophoresis of appropriate column fractions yielded results identical to those in Fig. 1.

To determine whether or not calmodulin might be associated with either 14S or 30S dynein ATPase, crude dyneins were resolved by sucrose density gradient centrifugation (41) with gradients prepared with EGTA or with EGTA plus excess Ca^{2+} and assayed for calmodulin by gel electrophoresis. The ATPase

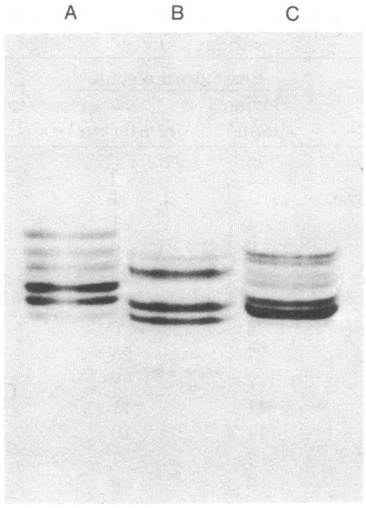

FIG. 4. Gel electrophoresis of calmodulin and TnC CNBr fragments. Dried CNBr digests of 50 μg of protein were dissolved in 5 M urea/10 mM Tris/50 mM glycine, pH 8.2, and resolved by electrophoresis on a 15% polyacrylamide/alkaline urea slab gel (33). Lanes: A, bovine brain calmodulin; B, *Tetrahymena* calmodulin; C, rabbit skeletal muscle TnC.

activity profile of the gradient with very low free Ca^{2+} was identical to profiles previously described (41), with a peak of ATPase activity corresponding to 14S dynein at fraction 15 (Fig. 6). When the gradients (and the crude dynein) contained approximately 0.33 mM Ca^{2+}, the 30S dynein peak shifted one fraction (≈1.2 ml) toward the denser region of the gradient, with a corresponding shift in protein distribution. The distribution of 14S dynein did not appear to change, but its ATPase activity was about 30% lower in the Ca-containing gradients than in the Ca-free gradient.

The upper portion of Fig. 6 shows the results obtained when 100-μl aliquots of each fraction of both gradients were subjected to electrophoresis and the Coomassie blue-stained calmodulin bands were quantitated by elution of dye and measurement of absorbance of the eluted material at 595 nm. In the Ca-free gradient, most of the calmodulin was found in fractions 19–22, as might be expected for a low M_r protein after centrifugation for 20 hr at 60,000 × *g* but some of the calmodulin was clearly present in fractions containing the 14S dynein. In the gradient containing about 0.33 mM free Ca^{2+}, much less calmodulin was present in fractions 19–22, indicating that it might have shifted into the faster-sedimenting region of the gradient. The exact location of this calmodulin was not clear from these studies. Clearly, knowledge of the localization of calmodulin in Ca-containing gradient fractions is essential for further understanding of its function in cilia.

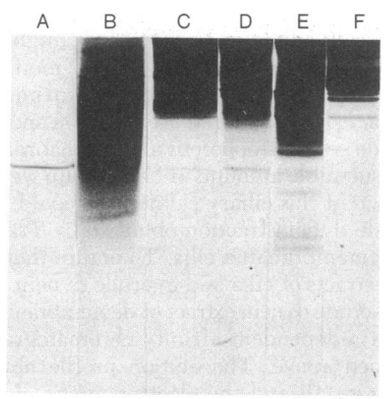

FIG. 5. Calmodulin in cilia and ciliary subfractions. Samples were analyzed by electrophoresis on 10% polyacrylamide/alkaline urea slab gels (33). Lanes: A, 1 μg of bovine brain calmodulin; B, whole *Tetrahymena* extract (logarithmic phase cells), 600 μg of protein; C, whole cilia extract, 250 μg of protein; D, cilia dynein fraction, 120 μg of protein; E, cilia axonemal pellet, 200 μg of protein; F, pellet II (twice-extracted axonemes), 85 μg of protein.

DISCUSSION

Kuznicki *et al.* (18) recently reported the presence of a Ca-binding protein in partially purified extracts of *Physarum polycephalum*, *Euglena gracilis*, and *Amoeba proteus*. This protein appears to be similar to calmodulin in its ability to activate both skeletal muscle light chain kinase and brain PDEase, but no convincing physicochemical evidence was presented that the Ca-binding protein in any of these protists was calmodulin. Nagao *et al.** extracted a Ca-binding protein from *Tetrahymena* [by heat treatment, precipitation with 60–100% $(NH_4)_2SO_4$, and gel electrophoresis] which could enhance the activity of membrane-bound guanylate cyclase about 20-fold in the presence of Ca^{2+}. Because an effect of calmodulin on guanylate cyclase in metazoa or plants has not been reported previously, it is not clear whether the protein isolated by Nagao *et al.** is the same as the *Tetrahymena* calmodulin that we have purified to homogeneity. Recently, it has been shown (J. Chafouleas, A. R. Means, and G. A. Thompson, Jr., personal communication) that an antibody prepared against rat testis calmodulin crossreacts in a radioimmunoassay procedure with a protein present in homogenates of *Tetrahymena* and in isolated *Tetrahymena* cilia—presumably the calmodulin that we have purified and characterized. The same antibody has also been used for immunofluorescent localization of crossreacting antigen in *Paramecium tetraurelia* (42).

Calmodulin is present in considerable amounts in *T. pyriformis*, as evidenced by the yield of pure protein (0.5 mg/30

* Nagao, S., Suzuki, Y., Watanabe, Y. & Nozawa, Y. (1979) *XI International Congress of Biochemistry*, p. 390 (abstr.).

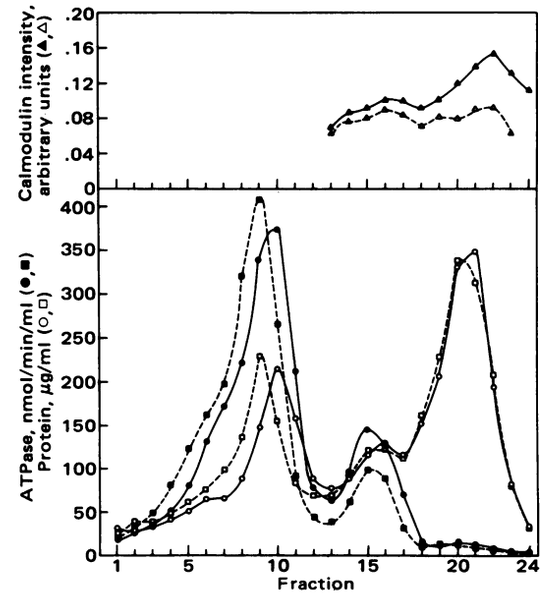

FIG. 6. Sucrose density gradient analysis of crude dynein prepared from demembranated *Tetrahymena* cilia by Tris/EDTA extraction. Gradients were 5–30% (wt/vol) sucrose in 8.33 mM imidazole/1.25 mM $MgSO_4$/0.067 mM EGTA, pH 7.5 with 0.4 mM Ca^{2+} (dashed lines) or without added Ca^{2+} (solid lines). Identical aliquots of crude dynein were placed on top of each gradient except for the addition of 0.4 mM Ca^{2+} to the crude dynein placed on the Ca-containing gradient. Fractions (40 drops) were collected and assayed for ATPase activity (●,■) and for protein content (○,□). In addition, 100 μl of each fraction was assayed by electrophoresis on alkaline urea gels and extraction of the Coomassie blue-stained bands (△,▲).

g of packed cells) obtained here. This amount represents approximately one-fifth of that isolatable from an equal weight of bovine brain by these procedures (25). Preliminary estimates by polyacrylamide gel electrophoresis indicate that 1% of the total urea-soluble protein in *Tetrahymena* may be calmodulin. This value is in the range of values observed (43) for rapidly growing cultured chicken embryo fibroblasts. The physicochemical properties reported here for *Tetrahymena* calmodulin resemble those of the vertebrate and invertebrate proteins. However, some differences in the amino compositions, CNBr peptide maps, and electrophoretic properties of metazoan and *Tetrahymena* calmodulins were evident.

Although no calmodulin-regulated enzyme systems have been identified in *Tetrahymena*, a large body of evidence implicates Ca^{2+} in the control of ciliary motility in general (38, 39) and in *Tetrahymena* in particular (31). Therefore, it seemed likely that calmodulin might comprise part of the Ca-control system in cilia. Early studies on the effects of chlorpromazine on *Tetrahymena* provide some evidence to support this hypothesis. To date, growth (44, 45), motility (46), glucose utilization (45), and phosphate uptake (47) of this ciliate have been reported to be inhibited by low concentrations of chlorpromazine and other phenothiazines. More direct evidence comes from the demonstration here by gel electrophoresis that calmodulin is present in *Tetrahymena* cilia and may be associated with 14S dynein. Preliminary data (unpublished results) indicate that chlorpromazine inhibits the ATPase activity of 14S dynein, 30S dynein, and demembranated axonemes and also inhibits the turbidity response of axonemes, further suggesting that calmodulin may play a direct role in ciliary motility.

It seems premature to conclude that calmodulin plays a general role in the control of flagellar motility in view of the report (48) that it was undetectable in the flagellar segments of sperm obtained from sea urchins and several vertebrate species. However, it is well documented (38, 39) that ciliates such as *Paramecium* and *Tetrahymena* contain a Ca-dependent system in their cilia which controls the orientation of the beat direction, is at least partially separate from sliding and bend propagation. The exact role of calmodulin in providing Ca-dependent regulation of ciliary function and other cellular processes in the ciliates remains to be established.

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