Calmodulin Interaction with Cytoplasmic and Flagellar Dynein: Calcium-Dependent Binding and Stimulation of Adenosinetriphosphatase Activity†

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ABSTRACT: Hisanaga and Sakai [Hisanaga, S., & Sakai, H. (1983) *J. Biochem. (Tokyo) 93*, 87–98] demonstrated that cytoplasmic dynein could be purified, in part, by chromatography on a calmodulin–Sepharose 4B affinity column and that the adenosinetriphosphatase (ATPase) activity of the enzyme was stimulated by Ca^{2+}–calmodulin. In the present study, we have investigated, in detail, the interaction of cytoplasmic and flagellar dynein from the sea urchin *Hemicentrotus pulcherrimus* with calmodulin (CaM) isolated from porcine brain or sea urchin egg. The dynein Mg^{2+}-ATPase activity is stimulated 3–8-fold by calmodulin from either source. The stimulation is dependent on calcium ions and is inhibited by trifluoroperazine. CaM stimulation is sensitive to physiologically regulatory calcium ion concentrations around 1 μM. Activation is also sensitive to pH and occurs maximally at physiological pH near 7.0. Calmodulin binds directly to cytoplasmic dynein as judged by cosedimentation in a sucrose density gradient. The binding and enzymatic stimulation occur at calmodulin:dynein ratios of 150:1 to 300:1, which are consistent with estimates of in vivo ratios. Cytoplasmic and flagellar dynein ATPase activities are also stimulated by Triton X-100, a nonionic detergent, and by limited protolysis with trypsin. Both of these treatments abolish further activation by calmodulin. The possibility of a trypsin-labile, CaM binding subunit of the enzyme is discussed. In addition, since both CaM and dynein are localized in the mitotic apparatus, we suggest that CaM may regulate possible mitotic dynein activity.

Calcium ions (Ca^{2+}) have been shown in recent years to play a regulatory role in diverse cellular processes (Berridge, 1975). Microtubule-associated motility is one of these processes, most clearly in the case of axonemal movement (Naitoh & Eckert, 1974; Gibbons, 1981). Certain aspects of both ciliary and flagellar activity appear to be under calcium control. Changes in internal calcium concentration are responsible for ciliary reversal during the avoiding response in *Paramecium* (Naitoh & Kaneko, 1973), for ciliary arrest in gill cilia of freshwater mussels (Walter & Satir, 1978; Satir, 1975) and saltwater mussels (Tsuchiya, 1977), and for alterations in flagellar wave form in *Chlamydomonas* during a phototactic response (Bessen et al., 1980). In addition, calcium is required for maintaining quiescence in sea urchin sperm flagella (Gibbons & Gibbons, 1980).

In many cases, calcium regulation of a cellular event is mediated by the protein calmodulin [or calcium-dependent regulatory protein (Cheung, 1980)]. Calmodulin (CaM) is a component of ciliary axonemes, although there is disagreement as to its ultrastructural location and precise function (Reed & Satir, 1980; Satir et al., 1980; Gitelman & Witman, 1980; Walter & Schultz, 1981; Stommel et al., 1982; Ohnishi et al., 1982). Blum et al. (1980) reported that the adenosinetriphosphatase (ATPase) activity of dynein, isolated from *Tetrahymena* cilia, could be activated by Ca^{2+}–calmodulin. Thus, the suggestion has been made that calcium effects on ciliary and flagellar activity may be mediated by calmodulin (Satir, 1980), possibly through a change in dynein ATPase activity.

Nonaxonemal microtubule-based motility may also be subject to calcium regulation. Chromosome movement within the mitotic apparatus (MA) is a particularly well-studied system, and there are considerable data concerning the effects of calcium on spindle microtubules. Microinjection of micromolar calcium (using calcium–EGTA buffers) into dividing sea urchin blastomeres causes a transient, local reduction in spindle birefringence (Kiehart et al., 1981). Micromolar levels of calcium have also been shown to disassemble spindle microtubules in an isolated mitotic apparatus (Salmon & Segall, 1980) and to depolymerize purified sea urchin microtubules (Nishida & Kumagai, 1980; Keller et al., 1980). It seems unlikely that these effects are mediated by calmodulin, for, although CaM has been localized in the MA (Anderson et al., 1978; Welsh et al., 1979; Hamaguchi & Iwasa, 1980), it has been demonstrated that this protein has no effect on the calcium sensitivity of whole sea urchin egg tubulin (Nishida & Kumagai, 1980) or on mitotic tubulin, specifically (Keller et al., 1980).

Dynein has been localized in mitotic spindles by immunofluorescence (Mohri et al., 1976) and by the identification of dynein-like ATPase in isolated MA (Pratt et al., 1980). In addition, there is some indication that this enzyme may function as a force generator for chromosome movement. Sakai et al. (1976) reported that chromosome movement in the glycerol isolated mitotic apparatus (from sea urchin eggs) was induced by ATP but not other nucleotides and was inhibited by anti-dynein serum. Cande & Wolniak (1978) showed that chromosome movement in lysed cell models (mammalian) was stopped by a dynein ATPase inhibitor, vanadate anion. Thus, since both CaM and dynein are found in the MA, we would like to know whether CaM might reg-

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0006-2960/84/0423-3032$01.50/0

ulate the activity of a mitotic dynein.

Hisanaga & Sakai (1983) have recently reported a purification procedure for sea urchin egg cytoplasmic dynein that includes chromatography on a CaM–Sepharose 4B affinity column. In addition, they showed that the purified cytoplasmic dynein ATPase activity was activated by Ca^{2+}–calmodulin. The present study reports a detailed investigation of the interaction of cytoplasmic dynein and calmodulin, as well as the calmodulin-mediated stimulation of flagellar dynein ATPase. We find that CaM binds directly to purified cytoplasmic dynein in a calcium-dependent manner. An investigation of the effects of calcium ion concentration and pH on the interaction demonstrates that the maximal enzymatic stimulation occurs under physiological conditions. In addition, we report that cytoplasmic dynein ATPase is stimulated by Triton X-100, indicating that the enzyme is isolated in a latent form (Gibbons & Fronk, 1979). Finally, we discuss some functional implications of the CaM–dynein interaction, especially with respect to dynein in the mitotic apparatus.

Experimental Procedures

Preparation of Dynein and Calmodulin. Cytoplasmic dynein was purified from unfertilized eggs of the sea urchin *Hemicentrotus pulcherrimus* by the procedure described in a previous paper (Hisanaga & Sakai, 1983). The final enzyme preparation consisted of a single high molecular weight polypeptide, comprising 80% of the total protein, and several lower molecular weight polypeptides [as assayed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis]. The specific Mg^{2+}-ATPase activity of the cytoplasmic egg dynein was 0.25–0.5 μmol of P_i mg^{-1} min^{-1}, depending on the particular preparation.

Flagellar dynein was extracted from flagellar axonemes, which were prepared from sperm of *H. pulcherrimus* by the method of Gibbons & Fronk (1972). The 21S form of the dynein, purified by sucrose density gradient centrifugation, was used for all experiments.

Porcine brain calmodulin was purified by the method of Teo et al. (1973).

Sea urchin egg calmodulin, which was prepared from unfertilized eggs of *H. pulcherrimus* by essentially the same method as that for brain calmodulin purification (Nishida et al., 1980), was generously given by Dr. E. Nishida and Dr. H. Kumagai.

Measurement of ATPase Activity. Assays of ATPase activity were carried out at 25 °C in a solution containing 1 mM ATP, 2 mM $MgCl_2$, 30 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, or 30 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.8, and indicated concentrations of $CaCl_2$ or ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and calmodulin. The reaction was started by adding ATP and stopped by adding cold 15% trichloroacetic acid. The protein precipitate was removed by centrifugation, and liberated inorganic phosphate in the supernatant was measured by the method of Fiske & SubbaRow (1925).

Measurement of Calmodulin Activity. Calmodulin activity was assayed on the basis of specific stimulation of Ca^{2+}-dependent brain phosphodiesterase. The assays were carried out as described by Nishida et al. (1980).

Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of SDS was performed according to the method of Laemmli (1974) using acrylamide gradient gels.

Others. Protein concentration was determined by the method of Lowry et al. (1982), using bovine serum albumin as a standard, or by absorbance at 280 nm, with an estimated extinction coefficient for dynein of $E_{280}^{1mg/mL} = 1$.

Free calcium concentrations in $CaCl_2$–EGTA buffers were determined according to Nishida et al. (1979). The pH of the ATPase assay solution was not affected by the addition of EGTA or calcium.

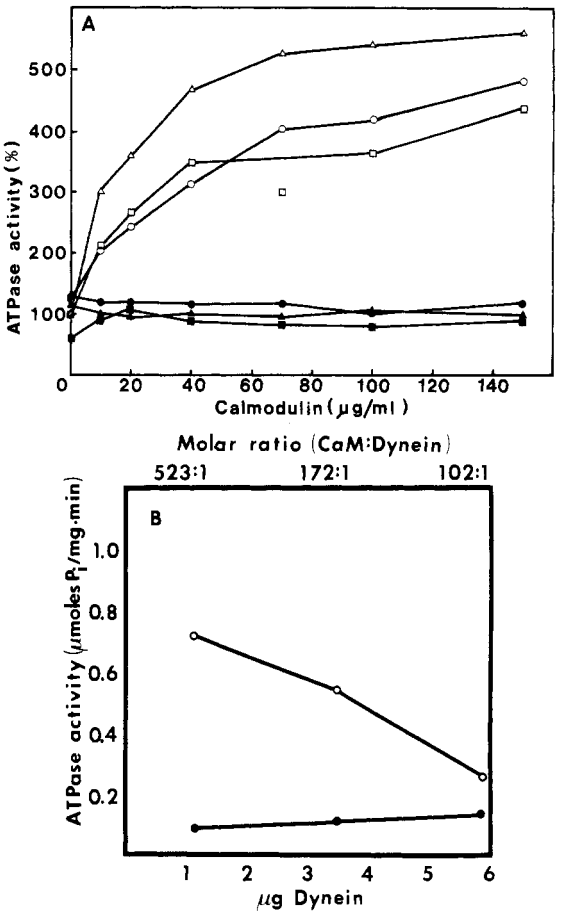

FIGURE 1: Activation of dynein ATPase by calmodulin. (A) Mg^{2+}-ATPase activity was determined by using 12 μg/mL cytoplasmic dynein or 8.8 μg/mL flagellar 21S dynein with various concentrations of calmodulin. (○ and ●) Cytoplasmic dynein and egg calmodulin; (□ and ■) cytoplasmic dynein and brain calmodulin; (△ and ▲) flagellar 21S dynein and brain calmodulin. (B) Mg^{2+}-ATPase activity was determined by using 100 μg/mL brain CaM and varying amounts of cytoplasmic dynein. Molar ratios were calculated by using molecular weights of 18000 for CaM and 550000 for cytoplasmic dynein. (Open symbols) ATPase activity in the presence of 0.2 mM $CaCl_2$; (closed symbols) ATPase activity in the presence of 0.2 mM EGTA.

Results

Activation of Dynein ATPase by Calmodulin. Cytoplasmic dynein purified from unfertilized sea urchin egg, by the method of Hisanaga & Sakai (1983), is composed primarily of a single polypeptide of molecular weight over 300000. The cytoplasmic dynein ATPase activity is stimulated by Ca^{2+}–calmodulin using both brain CaM and sea urchin egg CaM (Figure 1A). At pH 6.8 (MES buffer), cytoplasmic dynein Mg^{2+}-ATPase activity is stimulated by increasing concentrations of calmodulin. Typically, the activation is 3–8-fold and remains constant over 4–5 days (the maximum length of storage, on ice, tested).

Sea urchin sperm flagellar 21S dynein is also activated by brain CaM (Figure 1A). Again, the maximal activation varies between preparations from 4- to 10-fold. This is comparable to the 3- and 10-fold activation reported for *Tetrahymena* ciliary 30S and 14S dynein, respectively (Blum et al., 1980).

For both egg and flagellar dynein, maximal activation is seen at approximately 70 μg/mL calmodulin (4 μM). Since the molecular weight of purified cytoplasmic dynein (5.5×10^5, estimated from sucrose density gradient centrifugation and gel filtration) is around 30 times that of CaM (1.8×10^4; Klee et al., 1980), this represents a molar ratio of nearly 190 CaM:1 dynein.

To determine whether the dynein ATPase stimulation is dependent on the absolute CaM concentration, or on the ratio of CaM:dynein, assays were conducted in which the concentration of CaM was held constant and the concentration of dynein was varied. As shown in Figure 1B, the amount of stimulation was proportional to the CaM:dynein ratio, and approximately 5-fold stimulation was seen at a molar ratio of 172:1, comparable to the results above.

The activation does not appear to be a nonspecific effect of either the calcium or the calmodulin, since both are required. In addition, as previously shown by Hisanaga & Sakai (1983), the Ca^{2+}–calmodulin stimulation of Mg^{2+}-ATPase activity is specifically inhibited by trifluoroperazine (TFP), a CaM binding antagonist. At 100 μg/mL CaM (5.9 μM) and 0.2 mM $CaCl_2$, dynein stimulation is reduced 50% by 12 μM TFP (data not shown). At 20 μM TFP, the stimulation is totally reversed and the dynein Mg^{2+}-ATPase activity returns to control levels (minus calcium or CaM). This is consistent with a 2:1 stoichiometry of TFP:CaM and a dissociation constant of 1 μM (Levin & Weiss, 1976). Finally, the activation does not seem to be a consequence of increased protein concentration in the assay since (1) CaM in the presence of EGTA does not stimulate the enzymatic activity and (2) bovine serum albumin, added in the place of CaM at the same or higher concentrations, does not stimulate dynein ATPase, whether or not calcium ions are present (data not shown).

Calcium can substitute for magnesium in activating dynein ATPase, though it is usually supposed that Mg^{2+}-ATP is the preferred substrate in vivo. It is typical for dynein ATPase to exhibit a Ca^{2+}-ATPase:Mg^{2+}-ATPase ratio of 0.5 to 1.0. Calmodulin also stimulates cytoplasmic dynein Ca^{2+}-ATPase activity, and the level of CaM required for maximal activation (70 μg/mL) is the same as that observed for stimulation for Mg^{2+}-ATPase (data not shown).

Binding of Calmodulin to Cytoplasmic Dynein. Although egg dynein is retained by a calmodulin–Sepharose 4B column in the presence of 2 mM $CaCl_2$ and eluted with 2 mM EGTA (Hisanaga & Sakai, 1983), many proteins in addition to dynein bind to the column in a calcium-dependent manner. There is a possibility, then, that the dynein does not bind directly to CaM but rather is retained nonspecifically or through association with another protein in the eluted fraction.

To establish that cytoplasmic dynein binds directly to calmodulin and that the binding is dependent on calcium ions, the interaction of purified proteins was studied by using sucrose density gradient centrifugation (SDGC). A mixture of purified cytoplasmic dynein [after hydroxyapatite and CaM–4B chromatography and SDGC; see Hisanaga & Sakai (1983)] and calmodulin was centrifuged on a 5–20% linear sucrose gradient in either the presence or absence of calcium. The results are shown in Figure 2. In the absence of calcium (1 mM EGTA), CaM activity (as assayed by activation of brain phosphodiesterase) was found in the expected position near the top of the gradient; dynein ATPase migrated to a position near the bottom of the gradient, as expected for a protein of 12 S. In the presence of calcium (1 mM $CaCl_2$), a portion of the calmodulin sedimented with the dynein. The shifting of the calmodulin activity by one fraction, with respect to the

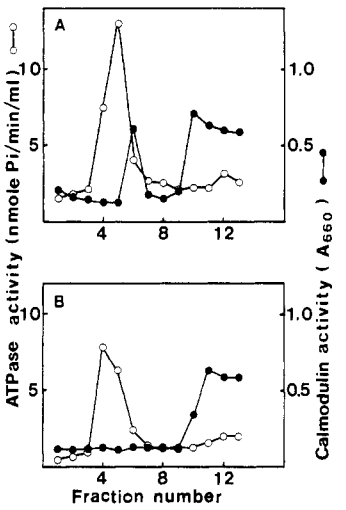

FIGURE 2: Binding of calmodulin to cytoplasmic dynein in the presence and absence of calcium. A mixture (0.3-mL total) of cytoplasmic dynein and egg calmodulin was centrifuged on a 12.5-mL 5–20% sucrose gradient at 180000*g* for 16 h in a Hitachi RPS 40T rotor. Migration is from right to left. Dynein Mg^{2+}-ATPase activity (○) was assayed under standard reaction conditions (see Experimental Procedures). CaM activity (●) was assayed by measuring the activation of calmodulin-dependent phosphodiesterase under conditions described under Experimental Procedures. (A) Centrifugation in the presence of 1 mM $CaCl_2$; (B) centrifugation in the presence of 1 mM EGTA.

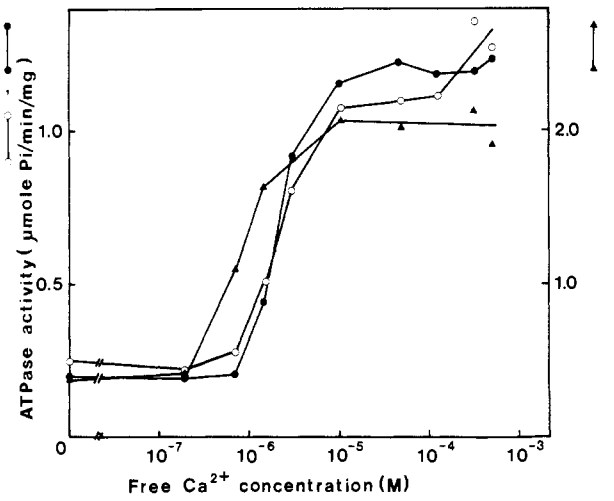

FIGURE 3: Calcium dependence of calmodulin stimulation of dynein ATPase. Mg^{2+}-ATPase activity was determined by using 12 μg/mL cytoplasmic dynein and 100 μg/mL brain calmodulin (○) or 100 μg/mL egg calmodulin (●) and 8.8 μg/mL flagellar 21S dynein and 100 μg/mL brain calmodulin (▲). Free calcium concentrations, in calcium–EGTA buffers, were determined according to Nishida et al. (1980).

dynein ATPase peak, may be due to some dissociation of the two proteins during the 16–18 h of centrifugation. When calmodulin alone was centrifuged on an identical gradient, it was always found in the fractions at the top regardless of the presence of calcium.

Calmodulin purified from sea urchin eggs was used for these experiments. The calmodulin used in the affinity column was prepared from porcine brain. Thus, cytoplasmic dynein is activated by and binds to both types of calmodulin.

Calcium Dependence of Calmodulin Stimulation of Dynein ATPase. In the experiments reported above, an excess of calcium was used to ensure the formation of the Ca^{2+}–calmodulin complex. If the interaction of CaM and dynein is functionally relevant, however, it should be regulated by physiologically low calcium concentrations (in the micromolar

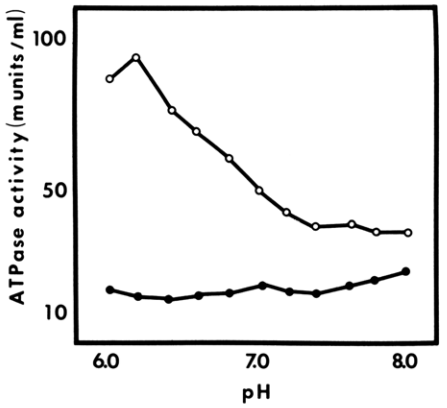

FIGURE 4: pH dependence of calmodulin stimulation of cytoplasmic dynein ATPase. Mg^{2+}-ATPase activity was determined by using 12 μg/mL cytoplasmic dynein and 100 μg/mL egg calmodulin in the presence of 0.2 mM $CaCl_2$ (○) or 0.2 mM EGTA (●). Different buffers were used in different pH ranges as follows: MES (pH 6.2–6.83), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.02–8.01), Tris (pH 8.1–9.3), and (cyclohexylamino)propanesulfonic acid (pH 10.0–11.0).

range). We, therefore, examined the stimulation of dynein Mg^{2+}-ATPase by CaM at a wide range of free calcium concentrations, using calcium–EGTA buffers. The results are shown in Figure 3.

In the case of cytoplasmic dynein, 50% of full stimulation occurs at 3–4 μM calcium, and brain and egg CaM exhibit nearly identical calcium-dependence profiles for egg dynein activation (Figure 3). Calmodulin stimulation of flagellar 21S dynein is slightly more sensitive to free calcium concentration.

pH Dependence of CaM Stimulation of Dynein ATPase. Dynein ATPase from a variety of axonemal sources exhibits a broad pH-dependence profile with slight maxima at pH 6.0 and 8.0. Typically, dynein ATPase is assayed at pH 8.0, following the work of Gibbons & Fronk (1972). Since the physiological pH of sea urchin egg cytoplasm is at least 1 unit nearer to neutrality (Johnson & Epel, 1981), we examined the pH dependence of cytoplasmic dynein–CaM interaction.

Figure 4 shows that the activation is greatest below pH 7.0. Only 2-fold stimulation is seen at pH 8.0. Ca^{2+}–calmodulin has little effect on dynein ATPase measured between pH 9.0 and pH 10.0, where dynein exhibits a peak of ATPase activity (data not shown).

Ca^{2+}–calmodulin stimulation of flagellar dynein Mg^{2+}-ATPase is also greater at pH 6.8 than at pH 8.0 (8-fold vs. 2-fold; data not shown).

Activation of Cytoplasmic Dynein by Triton X-100. Ca^{2+}–calmodulin activation of many enzymes is brought about by the interaction of hydrophobic sites on the complex with similar sites on the enzyme (LaPorte et al., 1980). To examine both the specificity and the possible mechanism of the CaM stimulation of cytoplasmic dynein activity, we assayed the effects of the nonionic detergent Triton X-100, a nonspecific hydrophobic perturbant, on dynein Mg^{2+}-ATPase activity. Treatment of cytoplasmic dynein with 0.05–0.1% Triton X-100 for 5 min at 4 °C just prior to enzyme assay increased the measured enzyme activity approximately 3.5-fold. This is less than the 10-fold stimulation seen for sperm flagellar latent activity dynein (LAD) (Gibbons & Fronk, 1979). At concentrations greater than 0.1% Triton, the enzyme activation drops off. The Triton activation of cytoplasmic dynein is independent of both calcium and calmodulin.

In every case, 0.2 mM $CaCl_2$ and 100 μg/mL CaM (no Triton) stimulate cytoplasmic dynein Mg^{2+}-ATPase to a greater extent than Triton X-100. Triton does not act synergistically with calmodulin to additionally stimulate ATPase; instead, increasing concentrations of Triton reduce CaM stimulation of cytoplasmic dynein to the level of activity seen with Triton alone (data not shown).

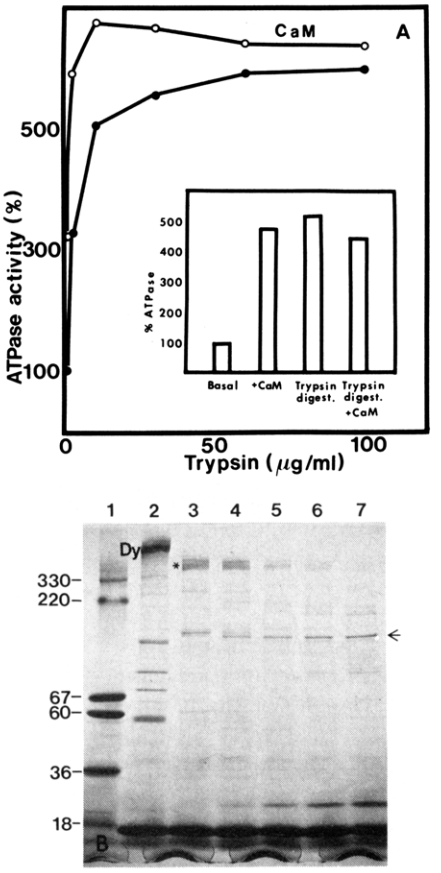

FIGURE 5: Effects of trypsin treatment and calmodulin on dynein ATPase. Flagellar 21S dynein (25.6 μg/mL) was incubated with various concentrations of trypsin for 70 min at 25 °C and pH 8.0. Trypsin digestion was stopped by adding soybean trypsin inhibitor (SBTI, 0.4 mg/mL final concentration). Cytoplasmic dynein (7.5 mg/mL) was incubated with an equal amount of trypsin at 25 °C for 30 min at pH 7.5. The reaction was stopped with SBTI as above. (A) Mg^{2+}-ATPase activity of trypsin-treated flagellar dynein in the presence (○) and absence (●) of 100 μg/mL brain calmodulin and 0.2 mM $CaCl_2$. (B) SDS–polyacrylamide gel electrophoresis of trypsin-treated flagellar dynein. Dynein (10 μg) was electrophoresed according to the method of Laemmli (1974) using a 5–12.5% polyacrylamide gradient. (Lane 1) Standard proteins (thyroglobulin, ferritin, albumin, catalase, lactate dehydrogenase; molecular weight times 10^{-3}); (lanes 2–7) flagellar dynein digested with 0, 2, 10, 30, 60, and 100 μg/mL trypsin, respectively. Native dynein heavy chains (Dy) and two cleavage products (*, arrow) are indicated. See the text for details.

Activation of Dynein ATPase by Trypsin. A brief digestion with trypsin of many calmodulin-regulated enzymes, including myosin light chain kinase (Adelstein et al., 1980) and muscle phosphorylase kinase (Cohen et al., 1980), results in a stimulation of the respective enzymatic activities and concomitantly renders them insensitive to CaM (Klee et al., 1980). Figure 5A demonstrates that CaM activation of cytoplasmic and flagellar 21S dynein ATPase is similarly sensitive to trypsin.

Trypsin digestion of dynein causes an increase in basal ATPase activity similar to that seen with Ca^{2+}-calmodulin. When CaM is added to the digested dynein, very little further stimulation of Mg^{2+}-ATPase activity is measured. As the trypsin concentration is increased, both basal and CaM-stimulated ATPase activity is increased, but the ratio of CaM activated to basal Mg^{2+}-ATPase decreases from 3.2 to 1.1, reflecting a loss of Ca^{2+}–calmodulin stimulation.

Figure 5B depicts the electrophoretic profile of trypsin-digested flagellar dynein polypeptides. The rapid increase in ATPase activity seems to be attributable to the breakdown of dynein A to lower molecular weight polypeptides, marked by an asterisk in Figure 5B. These may correspond to the A^{275T} fragments designated by Bell & Gibbons (1982). The decrease in CaM sensitivity is correlated with the appearance of a smaller polypeptide (Figure 5B, arrow) produced by further incubation with trypsin.

Discussion

In an extension of an earlier report by Hisanaga & Sakai (1983), we have demonstrated that (1) the Mg^{2+}-ATPase activity of purified cytoplasmic dynein or flagellar dynein is stimulated by calmodulin isolated from either porcine brain or sea urchin egg, (2) Ca^{2+}–calmodulin binds directly to purified cytoplasmic dynein, (3) binding and enzymatic stimulation occur at in vivo pH and calcium ion concentrations and in vivo levels of calmodulin and dynein, and (4) cytoplasmic dynein can also be stimulated by Triton X-100 or brief trypsin treatment. These data indicate that the cytoplasmic enzyme exists in a latent form and that CaM may play a role in modulating the enzyme activity.

One important question is whether the activation by Ca^{2+}–calmodulin is physiologically relevant. Some of the data are consistent with an in vivo role for calmodulin in cytoplasmic dynein regulation. First, the difference between basal and CaM-stimulated Mg^{2+}-ATPase activity is greatest near pH 7.0, which is physiological. Measurements of the pH of sea urchin egg cytoplasm range between pH 6.8 and pH 7.2, depending on the species and whether the eggs are fertilized (Johnson & Epel, 1981). In addition, the activation is sensitive to low, regulatory concentrations of calcium ions, around 1–5 μM.

On the other hand, the CaM concentration (70 μg/mL) that provides maximal stimulation of cytoplasmic dynein (12 μg/mL) constitutes a molar ratio of approximately 190:1 CaM:dynein, using molecular weights of 18 000 and 550 000 for CaM and dynein, respectively. This value is extraordinarily high but appears more reasonable when three important points are considered. First, the estimated concentration of CaM in the sea urchin egg is 100–150 μg/mL (Head et al., 1978), and the CaM concentrations in the ATPase assay that provide greater than 3-fold stimulation (30–150 μg/mL) are consistently at or well below this level. Second, from the yield of purified cytoplasmic dynein reported by Hisanaga & Sakai (1983), 0.01% of total egg protein, a minimum concentration of the enzyme in the eggs of *H. pulcherrimus* can be calculated to be 10–15 μg/mL. A reasonable upper estimate for the dynein concentration, assuming 50% loss during purification, is 20–30 μg/mL. The ATPase assays contained 5–15 μg/mL cytoplasmic dynein, again at or below in vivo concentrations. Third, if these concentrations are converted to molarities, with the molecular weights above, estimated in vivo molar ratios of CaM:dynein are between 300:1 and 150:1, equaling those used in the assay system (see Figure 1B).

It should also be noted that these are not binding ratios but only measurements of the molar ratios of proteins as present in the egg or mixed in the assay system. Sucrose density gradient cosedimentation using purified proteins clearly demonstrates that Ca^{2+}–calmodulin can bind directly to cytoplasmic dynein and that the binding is dependent on calcium ions. Absolute molar binding ratios (CaM:dynein), however, cannot be calculated from these experiments. It is most reasonable to predict that the binding ratio is considerably lower than the mixing ratio above. The data are consistent with this idea (Figure 2) since not all of the CaM activity cosediments with the cytoplasmic dynein.

The fact that Triton X-100 can also stimulate the enzyme and that the detergent can perturb CaM stimulation may indicate that CaM–dynein binding is hydrophobic as is typical for CaM–enzyme interactions (LaPorte et al., 1980). It is unclear whether CaM and Triton bind to the same site on the enzyme, in which case Triton binding is favored but CaM binding is more effective, or whether Triton perturbs CaM binding allosterically.

Another important question is the the mechanism of CaM stimulation. In the system described here, where purified cytoplasmic dynein and CaM only are present in the assay, we assume that CaM binding to the enzyme affects the enzymatic stimulation directly. One possibility is that the enzyme contains a regulatory subunit that allosterically modulates enzyme activity. The model proposes that the enzyme activity is kept low until the regulatory subunit interacts via hydrophobic binding with CaM or a suitable substitute, for example Triton X-100. The enzyme activation and loss of CaM sensitivity after trypsin digestion may reflect cleavage of this regulatory portion of the molecule and the consequent generation of an irreversibly stimulated level of ATPase activity. A similar mechanism has been proposed by Adelstein et al. (1980) for myosin light chain kinase.

In many cases, Ca^{2+}–calmodulin regulation of a particular enzyme activity is modulated through another enzyme, usually a kinase. Typically, the kinase binds to and is activated by calmodulin, and the activity of the enzyme in question is controlled by a phosphorylation carried out by the kinase. Examples include smooth muscle myosin (Adelstein et al., 1980) and skeletal muscle phosphorylase (Cohen et al., 1980). CaM regulation of dynein ATPase could conceivably be through a similar system, and the enzyme may not directly bind CaM in vivo. In preliminary experiments, however, we have been unable to detect phosphorylation of any dynein polypeptides, using enzyme preparations and conditions where Ca^{2+}–calmodulin activation is seen.

Finally, we would like to suggest that calmodulin may regulate dynein ATPase in the mitotic apparatus (MA). In addition to reports that the calcium sensitivity of cytoplasmic tubulin/microtubules from sea urchin eggs is *not* affected by CaM (Nishida & Kumagai, 1980; Keller et al., 1982), Nagle & Egrie (1980) have shown that the activity of the mitotic Ca^{2+}-ATPase, postulated to have a role in regulating calcium ion concentration in the MA (Petzelt et al., 1980), is not modulated by CaM. Cytoplasmic dynein, then, is the only component of the MA that shows a sensitivity to Ca^{2+}–calmodulin. It is possible that the high molar ratios required for CaM regulation of dynein preclude some necessary precision in the system, but alternatively, they could suggest a rationale for the concentration of CaM in the MA. In addition, it may be particularly significant that micromolar levels of calcium ions are regulatory for cytoplasmic dynein–CaM interaction, mitotic microtubule disassembly (Nishida & Kumagai, 1980; Keller et al., 1982; Salmon & Segall, 1980), and shortening of chromosomal spindle fibers in the isolated mitotic apparatus (Salmon & Segall, 1980).

Acknowledgments

We are grateful to Dr. H. Sakai for providing laboratory space and supplies and valuable guidance during this project. We also thank Drs. E. Nishida and H. Kumagai for providing calmodulin and helpful discussions. In addition, we thank Dr. Ray Stephens and Elijah Stommel for their critical reading of the manuscript. We also thank the editor and reviewers

for suggesting improvements in the interpretation of the data.

Registry No. ATPase, 9000-83-3; calcium, 7440-70-2.

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